frontiers | Research Topics

New perspectives in the treatment of myasthenia gravis

Edited by
Elena Cortés-Vicente, Francesco Saccà, Pushpa Narayanaswami and Bettina Schreiner

Published in
Frontiers in Neurology

ISSN 1664-8714
ISBN 978-2-8325-5064-9
DOI 10.3389/978-2-8325-5064-9

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

New perspectives in the treatment of myasthenia gravis

Topic editors

Elena Cortés-Vicente — Unitat de Malalties Neuromusculars, Hospital Santa Creu i Sant Pau, Spain

Francesco Saccà — University of Naples Federico II, Italy

Pushpa Narayanaswami — Beth Israel Deaconess Medical Center, Harvard Medical School, United States

Bettina Schreiner — University of Zurich, Switzerland

Citation

Cortés-Vicente, E., Saccà, F., Narayanaswami, P., Schreiner, B., eds. (2024). *New perspectives in the treatment of myasthenia gravis*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-5064-9

Table of contents

frontiers | Frontiers in Neurology

TYPE Editorial
PUBLISHED 11 June 2024
DOI 10.3389/fneur.2024.1418631

OPEN ACCESS

EDITED AND REVIEWED BY
Carlo Provenzano,
Catholic University of the Sacred Heart, Italy

*CORRESPONDENCE
Elena Cortés-Vicente
ecortes@santpau.cat

RECEIVED 16 April 2024
ACCEPTED 02 May 2024
PUBLISHED 11 June 2024

CITATION
Cortés-Vicente E, Schreiner B, Saccà F and Narayanaswami P (2024) Editorial: New perspectives in the treatment of myasthenia gravis. *Front. Neurol.* 15:1418631. doi: 10.3389/fneur.2024.1418631

Editorial: New perspectives in the treatment of myasthenia gravis

Elena Cortés-Vicente[1]*, Bettina Schreiner[2], Francesco Saccà[3] and Pushpa Narayanaswami[4]

[1]Unitat de Malalties Neuromusculars, Hospital Santa Creu i Sant Pau, Barcelona, Spain, [2]Department of Neurology, University Hospital Zurich, Zürich, Switzerland, [3]Department of Neurosciences, Reproductive and Odontostomatological Sciences, School of Medicine and Surgery, University of Naples Federico II, Naples, Campania, Italy, [4]Beth Israel Deaconess Medical Center/Harvard Medical School, Boston, MA, United States

KEYWORDS

myasthenia gravis, treatment strategy, new treatments, thymectomy, rituximab, complement inhibitors, neonatal Fc receptor antagonist

Editorial on the Research Topic
New perspectives in the treatment of myasthenia gravis

Myasthenia gravis (MG) is a chronic autoimmune disorder characterized by fatigable muscle weakness. The disease is very heterogeneous in terms of clinical manifestations depending on the muscle group involved, which includes the ocular, bulbar, limb and respiratory muscles. It is also complex from an immunological point of view. Anti-acetylcholine receptor antibodies are the most common, but anti-MuSK, anti-LRP4 or even seronegative patients can also be found. In approximately 5% of patients, no antibodies are detected (seronegative MG). Thymic abnormalities are frequent, mainly hyperplasia or thymoma, but there are patients with a normal thymus. Treatment includes acetylcholinesterase inhibitors, immunosuppressive and immunomodulatory drugs and thymectomy. Early diagnosis and appropriate management are essential to optimizing outcomes and quality of life for individuals with this condition.

In recent years our understanding of the physiopathology of the disease has led to new treatment strategies. The anti-CD20 monoclonal antibody rituximab was the first treatment used in the early 2000. In 2017, the first complement inhibitor, which prevents antibody-mediated complement activation and damage at the neuromuscular junction was approved for clinical use. Subsequently, these were followed by neonatal Fc receptor (FcRn) antagonists, which induce the catabolism of IgG (including pathogenic MG autoantibodies) by lysosomal degradation and reduce its extracellular concentration. This Research Topic aims to provide an overview of these emerging treatment strategies for MG. The authors discuss the underlying pathophysiological aspects of these agents. Clinical trials and observational studies are reviewed, describing the efficacy outcomes and safety profile of these drugs. These drugs are a new hope for MG patients, especially for drug-refractory patients. In parallel, a case report showing a refractory patient in myasthenic crisis and her response to efgartigimod is included. Myasthenic crisis patients are not usually included in clinical trials, and information about treatments that are useful to this subset of patients is important.

This Research Topic includes original research. Two articles studied the current treatment strategies to improve MG. The first described the therapeutic and prognostic features of MG patients followed in a tertiary neuromuscular disease center in Turkey.

Interestingly, they found that the use of corticosteroids was more common in patients younger than 50 years, and the use of non-steroidal immunosuppressant drugs was more common over the age of 50. In keeping with this, the second study evaluates treatment modalities for early-onset (<50 years of age) and late-onset MG (≥50 years of age). Although similar strategies and treatment-related adverse events were found, corticosteroid-related adverse events appeared to differ between groups, with hypertension, hypercholesterolemia, diabetes mellitus and malignancies being more common in late-onset MG patients. As MG can occur at any age, these findings may help in the selection of treatment options for our patients.

Thymectomy has been used to treat MG patients both with and without thymoma. However, new, limited surgical techniques have increased in popularity in recent years. A study showing the benefits of video-assisted thoracoscopic thymectomy (VATS) is included in this Research Topic. Patients undergoing VATS thymectomy had lower rates of intra-surgery and post-surgery complications, reduced morbidity, a shorter postoperative hospital stay, and a favorable impact on MG symptoms, both immediately post-surgery and in the long term, in addition to lower rates of local and distant thymoma recurrence when compared to patients undergoing sternotomy thymectomy. This work supports VATS thymectomy as the recommended surgical technique for thymic resection.

Another topic of great interest to neurologists treating MG patients is myasthenic crisis. This Research Topic includes a pilot study that used routine, cost-effective and widely available laboratory parameters related to inflammation and hemograms to identify potential risk factors for myasthenic crises. The results of this study provide proof of the concept that elevated basophil, neutrophil, leukocyte, and platelet counts may be associated with a higher risk of developing myasthenic crisis in MG patients. These potential biomarkers, together with clinical data, may help to individualize treatment strategies.

Finally, a bibliometric analysis of the characteristics of MG publications over the last 20 years was included by mapping the scholarly contributions of various countries or regions, institutions, journals, and authors in the field of MG. The close collaboration between countries and institutions was remarkable. This study also explored future trends and prospective directions, emphasizing individualized treatment based on subtypes, novel immunotherapeutic approaches, and thymectomy, all of which are featured topics in the current Research Topic.

Overall, this Research Topic aims to improve knowledge of current and emerging therapies for the treatment of MG, while stimulating research into unanswered questions. The new drugs are likely to change the current strategy for treating MG patients. Moreover, our increased knowledge of physiopathology will improve the selection of therapies for each subgroup of MG patients. When, how, and who to treat with each therapy is the burning question- we are closer to finding the answer.

Author contributions

EC-V: Writing—original draft, Writing—review & editing. BS: Writing—review & editing. FS: Writing—review & editing. PN: Writing—review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Conflict of interest

Unrelated to this study, PN received research support from AHRQ, PCORI, Alexion/AstraZeneca Rare Disease, Momenta/Janssen, and Ra/UCB; involved in Advisory boards/Consultations with Alexion/Astra Zeneca Rare disease, Amgen, Argenx, CVS, Dianthus, GSK, ImmuneAbs, Janssen, Novartis, UCB; Data Monitoring Committee Chair for Sanofi, Argenx and received royalties from Springer Nature.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

frontiers | Frontiers in Neurology

TYPE Original Research
PUBLISHED 03 August 2023
DOI 10.3389/fneur.2023.1176636

OPEN ACCESS

EDITED BY
Francesco Saccà,
University of Naples Federico II, Italy

REVIEWED BY
Nakul Katyal,
Stanford Healthcare, United States
Hacer Erdem Tilki,
Ondokuz Mayıs University, Türkiye
Deniz Tuncel Berktaş,
Kahramanmaras Sütçü Imam University, Türkiye

*CORRESPONDENCE
Aylin Yaman
✉ yaman.aylin@yahoo.com

RECEIVED 28 February 2023
ACCEPTED 23 July 2023
PUBLISHED 03 August 2023

CITATION
Yaman A and Kurtuluş Aydın F (2023) Therapeutic and prognostic features in myasthenia gravis patients followed in a tertiary neuromuscular diseases center in Turkey. *Front. Neurol.* 14:1176636. doi: 10.3389/fneur.2023.1176636

Therapeutic and prognostic features in myasthenia gravis patients followed in a tertiary neuromuscular diseases center in Turkey

Aylin Yaman[1]* and Fatma Kurtuluş Aydın[1,2]

[1]Neurology Department, Antalya Training and Research Hospital, Antalya, Türkiye, [2]Ankara Etlik City Hospital, Ankara, Türkiye

Introduction: In this study, we aim to evaluate the treatment responses and prognostic characteristics of Myasthenia Gravis (MG) patients followed in a tertiary neuromuscular diseases center in Turkey.

Methods: One hundred seventy four MG patients (between years 2011 and 2022) in Antalya, Turkey were diagnosed, and evaluated on a classification of MG was based on Myasthenia. Gravis Foundation of America (MGFA) clinical classification. Exclusion of other possible diseases in the differential diagnosis and support by beneficial response to treatment with acetylcholinesterase inhibitors were also taken into consideration.

Results: Mean age of participants was 54.86 (SD = 14.856; min-max = 22–84). Ninety (51.7%) were female. MG was more common in women under the age of 65 (58%) and in men over the age of 65 (64%). Generalized MG was seen in 75.3% of the patients. Anti-AChR positivities were detected in 52.3%, Anti-MuSK positivity in 4.6%, and seronegativity in 22.4%. Thymoma was detected in nearly 9.8% and thymectomy was performed in 28.7 percent. Most of the patients (57.5%) were using corticosteroids. Azathioprine was used by 39% and mycophenolate mofetil by 10.3% of patients. Mortality was higher and disease was more severe in late-onset (>50 years) MG patients (especially in the COVID-19 pandemic). Eight patients (four women, four men, mean age 75.5 years) died during follow-up. None of them died due to myasthenic worsening, two died due to malignancy and two due to infection. During the COVID pandemic, 16 patients (9.2%) had COVID infection. Four patients died due to COVID-19 infection, these four patients had serious comorbidities, and three of them were elderly (>75 years).

Conclusion: In conclusion, MG is more common in women between the ages of 20–40 and in men over the age of 65. The use of corticosteroids was more common under the age of 50, and the use of non-steroidal immunosuppressant agents was more common over the age of 50. Thymectomy is still an important supportive treatment approach in anti-AChR positive and seronegative generalized patients under 50 years of age. IVIG and plasmapheresis are effective treatments during acute exacerbations and bridging periods of treatments. Specific treatments are needed especially for resistant group of patients.

KEYWORDS

myasthenia gravis, treatment, autoantibodies, thymectomy, COVID-19, Turkey, electromyography

Introduction

Autoimmune Myasthenia gravis (MG) is the most common disease affecting the neuromuscular junction in skeletal muscles (1), which is an antibody-mediated disease. The main finding of the disease is fluctuating muscle weakness, especially in ocular, bulbar, respiratory and extremity muscles (2). The number of patients diagnosed with MG is increasing all over the world, and its prevalence is approximately 12.4 per 100,000 (3). MG is more common in women aged 20–40 years, and more common in men over 50 years of age.

Autoantibodies against acetylcholine receptors (AChR) are present in most patients, while autoantibodies against muscle specific kinase (MuSK), low-density lipoprotein receptor-related protein 4 (Lrp4), and agrin can be found less frequently.

MG may be classsified based on clinical manifestations (ocular or generalized), age of onset (before 50 years and after 50 years), antibody status (anti-AChR, anti-MuSK, anti-LRP4, seronegative) and thymus pathology (normal/atrophic thymus pathology, thymic hyperplasia and paraneoplastic occurrence associated with thymoma).

In generalized MG, anti-AChR antibody positivity is 80%–85% and anti-MuSK antibody positivity is around 4%–5% (2).

Presence of thymoma is seen in 10%–15% of cases. It is thought that the patients who are seronegative for both antibodies, who do not have thymoma have a milder clinical picture, and those who are associated with thymoma, who have a late onset (>50 years) and who are anti-MuSK antibody positive have a more severe clinical picture (3). Anti-MuSK positive disease is more frequently seen in young women, and it progresses more severely and more frequently with bulbar involvement (4). It is estimated that up to 60% of ocular-onset cases will evolve to the generalized form within the first 2 years. Anti-AChR antibody is positive in approximately 50% of cases with ocular MG, anti-MuSK antibody positivity is much rarer (3%–4%). Ocular MG patients who are seronegative for both antibodies are much less likely to develop the generalized form than those who are antibody positive. There are studies that have found that late-onset ocular MG cases are more likely to be generalized (5).

MG has turned historically from a disease with a poor prognosis to a well-managed and treatable disease in recent years, with the widespread use of immunosuppressive and immunomodulatory therapies, the use of thymectomy in appropriate patients, and close patient follow-up. However, there are cases that are resistant to treatment, albeit at a low rate. By the 2000s, the mortality of the disease has decreased significantly (3%–4%).

Respiratory failure seen with myasthenic crisis is the leading cause of death (6, 7).

In this study, we aimed to reveal the treatment responses and prognostic characteristics of MG patients followed in a tertiary neuromuscular diseases center in Turkey.

Materials and methods

This cohort includes all the autoimmune MG patients who had been followed-up in tertiary neuromuscular diseases center in Ministry of Health Antalya Education and Research Hospital between the years 2011 and 2022 in Antalya, Turkey.

One hundred seventy four patients were included to the study. Diagnosis was based on the muscle weakness or fatigue confirmed in examination, antibody testing, electrophysiological tests consistent with neuromuscular transmission impairment, chest images to detect the thymic pathologies.

EMG tests included repetitive nerve stimulations and jitter measurements.

Exclusion of other possible diseases in the differential diagnosis and support by beneficial response to treatment with acetylcholinesterase inhibitors were also taken into consideration.

Myasthenia Gravis Foundation of America (MGFA) clinical classification was used for the documentation of clinical severity at the time of application. MGFA-Post Intervention Status (PIS) was used as an outcome measure (8).

The adverse effects of corticosteroids (CS), which are the most commonly used agents in the treatment of MG, were defined as "mild" and "serious". Mild adverse effects were accepted as effects like mild weight gain and glucose intolerance; severe adverse effects were accepted as severe weight gain, cushingoid appearance, vertebral fracture, cataract formation, glaucoma, femoral head avascular necrosis, osteoporosis, overt diabetes, neuropsychiatric disturbances etc. In maintenance use, low dose was accepted as below 20 mg/day for methyl-prednisolone and below 25 mg/day for prednisolone.

Refractory MG is defined as 'PIS is unchanged or worse after CS and at least 2 other immunosuppressive agents, used in adequate doses for an adequate duration, with persistent symptoms or side effects that limit functioning, as defined by patient and physician' (9).

Ethics committee approval was obtained. It was obtained from the ethics committee of Antalya Training and Research Hospital.

Statistical analysis was performed as follows: non-parametric tests were used for data showing ordinal and quantitative distribution, and parametric tests were used for data showing continuous distribution. Alpha was accepted as 0.05 as the significance level.

Results

The mean age of participants was 54.86 (SD = 14.856; min-max = 22–84; n = 174). Ninety (51.7%) were female and 84 (48.3%) were male. Ninety nine (56.9%) of the patients were above 50 and 43 (24.7%) of them were above 65 years of age. MG was more common in women under 65 years of age (n = 76; 58%) and in men over 65 years of age (n = 29; 64%) ($p < 0.05$). The mean duration of their diseases was 7.21 (SD = 7.055; min-max = 1–37) years. The mean follow-up duration of patients was 4.84 (SD = 2.748; min-max = 1–11) years, median follow-up was 5 years. Patients under 65 years of age had longer duration of illness (mean = 7.93; SD = 7.604; n = 131 vs. mean = 5.02: SD 4.421; n = 43) ($p < 0.05$). Sole ocular involvement was evident in 43 (24.7%) and generalized involvement in 131 (75.3%) patients. Anti-AChR positivity was detected in 91 (52.3%), Anti-MuSK positivity in 8 (4.6%), seronegativity in 39 (22.4%) patients. Since the anti-MuSK antibody level cannot be measured in the hospital laboratory and examination outside the hospital is not covered by the general insurance, 35 (20%) of patients were found to be anti-AChR antibody negative and anti-MuSK antibody status of these patients was not known. These patients were excluded from the analysis when determining the disease characteristics and outcome of anti-AChR positive vs. anti-MuSK positive vs. seronegative patients. Anti-AChR antibody (n = 58; 64%) was more common at the age of 50 and over, and anti-MuSK positivity was more common in women

($n = 7$; 88%) under the age of 50 ($n = 7$; 88%). Anti-titin antibody was positive in one patient (Anti-titin antibody was not investigated in the rest of the patients). No paraneoplastic etiology was found in this patient. In generalized form, anti-AChR antibody ($n = 73$; 57% vs. $n = 18$; 42%) was higher than anti-MuSK ($n = 8$; 6% vs.0) and seronegativity ($n = 11$; 26% vs. $n = 28$; 22%) was found to be lower ($p < 0.05$), when compared to ocular form. Under 50 years of age (clinical ocular, $n = 16$; 21%; clinical generalized, $n = 59$; 79%) and above (clinical ocular, $n = 27$; 28%; clinical generalized, $n = 72$; 72%) in terms of clinical features, there was no difference between groups ($p > 0.05$). Seventeen (9.8%) patients had thymoma. Thymectomy was performed in 50 (28.7%) patients. Pathological evaluation revealed benign results in 37 (21.3%), malign thymoma in 10 (5.7%) patients, and pathology records could not be reached in 3 (1.7%) patients (they declared the result as benign). There was no difference between the age of 50 and older in terms of the presence of thymoma ($p > 0.05$). Thymic hyperplasia ($n = 11$; 48%) was more common in patients under 50 years of age ($p < 0.05$). Thymectomy ($n = 37$; 76%) was performed more frequently in patients under 50 years of age ($p < 0.05$).

The summary of patients' demographic and disease characteristics are shown in Table 1.

Thymus pathologies were found to be more benign ($n = 31$; 84%) in patients under 50 years of age ($p < 0.05$). Most of the patients used CS ($n = 101$; 57.5%) alone or in combination with other immunosuppressants. Short-term (<6 months) CS use was present in 36 (20.7%) and long-term (>6 months) use in 65 (37.4%) patients. Most chronic CS users ($n = 98$; 56.3%) used low dose and remaining ($n = 3$; 1.7%) used high dose. Mild adverse effects were reported in 36 (20.7%), severe in 8 (4.6%) of the patients.

Azathioprine (AZA) was used by 68 (39%) patients. Mycophenolate mofetil (MM) was used by a total of 18 (10.3%) patients; in 17 (25% of AZA users) of these patients, AZA was prescribed as first-line steroid sparing immunosuppressive agent, but they switched from AZA to MM because of adverse effects. Mostly these adverse effects were the persistent elevation of liver enzymes more than twice the normal levels or neutropenia, which normalized after discontinuation of the drug. Only in one (1.5% 0f AZA users) of the patients, a 67 years old female, severe AZA-induced neutropenia developed lasting about 2 weeks, she was hospitalized and followed-up with hematology clinic, and finally her values returned to normal. Two months later, MM was prescribed, she is under MM treatment with good response and no adverse effects for 5 years. In the remainder of the patients who developed AZA-related adverse events, the effects were transient and resolved with drug discontinuation.

There were no remarkable side effects in patients using MM.

Intravenous immunoglobulin (IVIG) was used in 96 (55.2%) patients. Most had a shorter term (<6 months), 22 (12.6%) longer term (≥6 months) IVIG use, and 4 (2.3%) patients who are refractory to single or combination immunosuppressant treatment or with unacceptable side effects, need IVIG as a maintenance treatment with other immunosuppressive agents. Three of these refractory patients are currently under low dose oral CS, MM and IVIG treatment. One of the refractory patients has also rheumatoid arthritis diagnosis, using another immunosuppressive agent, CS and IVIG. Two of these refractory patients were anti-AChR positive and two of them were seronegative, three of them were above 50 years, one was below 50 years. All four of them continue their lives with mild to moderate symptoms.

TABLE 1 Patients' demographic and disease characteristics.

Category	
	Mean (SD; min-max)
Age	54.9 (14.8; 22–84)
Mean duration of disease	7.2 (7.0; 1–37)
Mean follow-up duration	4.8 (2.7; 1–11)
	Number (percentage)
Female	90 (52%)
Male	84 (48%)
≥ 50 years Female	40 (44%)
<50 years Female	50 (56%)
≥ 50 years Male	61 (73%)
<50 years Male	23 (27%)
Ocular MG	43 (25%)
Generalized MG	131 (75%)
Anti-AChR +	91 (52%)
Anti-MuSK +	8 (5%)
Seronegative	39 (22%)
Patients underwent thymectomy	50 (29%)
Patients under the age of 50 who had thymectomy	37 (76%)
Patients with thymoma	17 (10%)
MGFA at the time of application	
I	46 (26%)
II	14 (8%)
IIA	26 (15%)
IIB	41 (23%)
IIIA	12 (7%)
IIIB	22 (13%)
IVA	2 (1%)
IVB	11 (6%)
MGFA-PIS at the last visit	
Complete stable remission	1 (1%)
MM-0	13 (7%)
MM-1	35 (20%)
MM-2	56 (32%)
MM-3	51 (29%)
Change in status, improved	10 (6%)
Change in status, unchanged	8 (5%)

MGFA, Myasthenia Gravis Foundation of America (Clinical classification of MG); MGFA-PIS, MGFA Post-Intervention Status, MM, Minimal Manifestations.

Only in one (1% of IVIG users) patient, a potentially serious side effect, unstable angina pectoris and temporary elevation of troponin was observed due to IVIG.

Patients under 65 years of age had more CS ($n = 46$; 63% vs. $n = 18$; 42%), less AZA ($n = 40$; 31% vs. $n = 30$; 70%) and less IVIG ($n = 63$; 50% vs. $n = 33$; 77%) ($p < 0.05$). AZA use increased with increasing age ($n = 70$; mean = 59.09; SD = 17.709 vs. $n = 102$; mean = 47.12; SD = 16.486) ($p < 0.05$). AZA use ($n = 51$; 52% vs.

$n = 19$; 26%) was more common in patients over 50 years of age ($p < 0.05$).

CS use ($n = 57$; 76% vs. $n = 43$; 44%) was found to be more common under the age of 50 ($p < 0.05$).

Plasmapheresis was used in 11 (6.3%) patients during exacerbations.

There was no difference in terms of hospitalization in the intensive care unit between the groups under 50 years old and over ($p > 0.05$). In terms of myasthenic crisis, there was no difference between the groups under 50 years old and over ($p > 0.05$).

Seven (4%) patients used Rituximab (RTX), four of them were anti-MuSK and three were anti-AChR positive patients. Six of these patients had a favorable response to RTX, one anti-AChR positive patient did not respond well. No significant adverse effect was observed due to RTX.

Remaining four of the anti-MuSK positive patients are clinically stable under quite low dose of CS, and did not need another immunosuppressant agent.

During the follow-up, eight patients (4 females, 4 males, mean age 75.5) died. None of them died because of myasthenic worsening. Two of the patients died due to malignancy and two due to non-COVID infection. Four patients died due to COVID-19 infection; these four patients had serious co-morbidities, and three of them were elderly (>75 years).

One hundred fifty six (89%) patients were in complete stable remission or, mostly, minimal manifestations state according to MGFA-PIS classification (detailed clinical status information is given in Table 1). There was no difference in clinical status between patients younger than 50 years of age and older ($p > 0.05$).

The distribution of treatments is shown in Table 2.

Discussion

According to our findings, MG was more common in women under 65 years of age ($n = 76$; 58%) and in men over 65 years of age ($n = 29$; 64%) ($p < 0.05$). This is compatible with the literature, supporting the bimodal distribution of the disease (2, 10, 11). In the study conducted by Mercelis et al. including a single center in Belgium, women were slightly more than men (53%) (12). In another study (1,060 patients with MG and covering the years 1980–2008), it was found that, the disease started in 66% of men and 42% of women at the age of >50 years (13). Grob et al. reported that MG can occur at any age, but it is more common in women (14). Findings of all these studies show similarities to the gender distribution of our patients. There are some explanations why it is more common in women at an early age. It is thought that sex hormones may particularly affect the production of antibodies (15, 16). Additional findings related to female patients in our study were that, apart from being younger, they had MG for a longer period of time and they needed thymectomy more frequently.

Frequencies of generalized and ocular forms of disease in our patient group showed no significant difference between early and late onset subgroups (>50 and < 50 years of age).

In our study, Anti-AChR antibody positivities were detected in 52.3% of the patients, Anti-MuSK antibody positivity in 4.6%, and seronegativity in 22.4%. Anti-AChR antibody ($n = 58$; 64%) positivity was more common at ≥ 50 years of age, while anti-MuSK positivity was more frequent at <50 (88%) years and in women (88%).

The prevalence of anti-MuSK positive MG varies from country to country. It is low in Northern Europe and rises towards the Mediterranean. In Japan, the prevalence of anti-MuSK antibodies was reported to be 2–3%. Four of the total 8 Anti-MuSK positive patients (100% women <50 years old and 50% of all Anti MuSK positive cases) in our group, showed good clinical course with low dose steroid, in these cases no interventions such as non-steroidal immunosuppressant use or long-term IVIG use were required. Although our patient group is small in number, it is not consistent with the general impression that anti-MuSK positive cases have more severe clinical course (5).

In a study conducted in 13 European countries, the prevalence of seronegative MG cases was found to be 5%–22% (17–22).

The presence of thymoma makes MG a paraneoplastic disease and is present in 10%–15% of MG patients (23). In our patient group, thymic hyperplasia and benign thymus pathologies were found more frequently in the younger patients. Seventeen (9.8%) patients had thymoma. Thymectomy was performed in 28.7% patients.

Pathological evaluation revealed benign results in 21.3%, malign thymoma in 5.7% of all thymectomized patients. Cases with thymoma and especially malignant thymoma are expected to have a more severe clinical course, which is the case in our patient group as well.

Patients with lymphoid follicular hyperplasia or normal thymus have a better prognosis. They are a possible source of antibodies that develop hyperplastic thymus, especially in AChR Ab positive MG patients. This is confirmed by clinical improvement (up to 85%) after thymectomy. In the International thymectomy trial (MGTX), in MG

TABLE 2 Number of patients under different treatments and their clinical and serologic features.

	AZA *n*:51 (29.3%)	MM *n*:18 (10.3%)	RTX *n*:7 (4%)	Only CS *n*: 50 (28.7%)	CS + MM + IVIG *n*:4 (2.3%)	Only symptomatic (pyridostigmine) *n*:44 (25.2%)
Generalized	51	18	7	36	4	3
Ocular	–	–	–	14	–	41
AchR Ab +	43	15	3	22	2	6
MuSK Ab +	–	–	4	4	–	–
Seronegative	8	3	–	14	2	12

AZA, Azathioprine; MM, Mycophenolate mofetil; RTX, Rituximab; CS, Corticosteroids; IVIG, Intravenous immunoglobulin.

patients without thymoma, better clinical outcomes were achieved at three-year follow-up after thymectomy. When the 5-year results after thymectomy were evaluated, 61% of all patients were followed up, and it was understood that the treatment of patients with thymectomy was more successful, they used less CS and required less hospitalization (24). Based on the success of this study, thymectomy was performed more frequently in early-onset generalized MG patients with AChR antibodies. Minimally invasive thymectomy surgery has facilitated this process (25, 26). Thymectomy is not recommended in MuSK Ab positive patients (23).

Consistent with this evidence, thymectomy ($n = 37$; 76%) was performed more frequently in patients under 50 years of age ($p < 0.05$) in our patient group. Thymus pathologies were found to be more benign ($n = 31$; 84%) in patients under 50 years of age ($p < 0.05$).

Most of our patients used steroids (57.5%). Short-term (< 6 months) steroid use was present in 36 (20.7%) and long-term (> 6 months) use in 65 (37.4%) patients. Most steroid users (56.3%) used low dose and remaining (1.7%) used high dose steroids. Mild adverse effects were reported in 20.7%, severe in 4.6% of the patients.

Azathioprine (AZA) was used by 68 (39%) of our patients. It was the most frequently used steroid-sparing immunosuppressive agent in our patient group. In 18 (25%) patients AZA was prescribed as first-line immunosuppressive agent, but it was discontinued because of adverse effects. Mostly these adverse effects were the elevation of liver enzymes more than twice the normal levels or neutropenia, which normalized after discontinuation of the drug. Only in one of the patients (1.5% of AZA users) a severe side effect (neutropenia) was observed, which was reversible. In general, AZA, as a classical immunosuppressant in the treatment of generalized MG, is a highly effective and easily tolerated drug that limits the use of steroids, although the rate of discontinuation due to the development of side effects at the beginning of the treatment can be considered high (25%). Our general observation is that after 3–4 years of AZA use, the disease goes into remission and dose reductions can be made.

Eighteen (10%) of our patients used mycophenolate mofetil (MM) and the need for drug discontinuation was not observed due to side effects. Its side effects are rare. It can be interpreted that it is a well tolerated drug. Our three of the 4 refractory patients who needed maintenance IVIG treatment were using CS and MM. This suggests that MM in MG may be a less effective drug than AZA, although the number of patients is not too large to make a general comment. In the study of Hehir et al., in which the outcomes in 102 patients were documented, it was found to be effective in 50% of patients who received MM, and in the second year, this success was 80% (27). It is recommended for mild and moderate MG, and it is generally used in addition to CS when the effect is low (28).

RTX was used in 4% of patients, four (57%) anti-MuSK and three (43%) anti-AChR positive. Six of them (85%) responded well to RTX, whereas one patient (15%) positive for anti-AChR did not respond to treatment. No significant side effects were observed due to RTX.

Besides its use in myasthenic crisis and exacerbations, four (2.3%) of our patients who are refractory to treatment, needed IVIG as a maintenance treatment with other immunosuppressive agents. Two of these refractory patients were anti-AChR positive and two of them were seronegative. In cases where prednisone combined with another immunosupressant is not sufficient for symptomatic improvement, a single or multiple doses of IVIG are given in cases of bulbar involvement. In chronic use, it is well tolerated and reduces the need for immunosuppressants and acetylcholine esterase inhibitors (29, 30).

Only one (1% of IVIG users) of our patients experienced a serious side effect (elevation of troponin and angina pectoris), but it could be managed.

Two anti-AChR positive and resistant patients who are under adequate immunosuppressant medication but still in need of maintenance IVIG treatment are candidates for second-line treatment (e.g., Eculizumab) (6).

It has been reported that the treatment of late-onset MG patients is more complex and the comorbidity is higher. In their study Cortés-Vicente et al. revealed that late-onset MG patients were mostly male, had more anti-AChR antibodies, had more ocular involvement (31). In a study originating from Turkey, it was stated that the prognosis of late-onset MG was better (32). However, the outputs of our study is contradictory to this finding, since our patients older than 65 years used AZA ($n = 30$, 70% vs. $n = 40$, 31%) and IVIG ($n = 33$, 77% vs. $n = 63$, 50%) more frequently ($p < 0.05$), suggesting that they had more severe course.

As age increases, comorbidity also increases, in this context, mortality was higher and clinical course was more severe in late-onset (>50 years) MG patients (especially in the COVID-19 pandemic), and this affects our treatment choice and success of treatment (especially MG patients after >65 years of age needed more frequent IVIG therapy).

One hundred fifty six (89%) patients were in complete stable remission or, mostly, minimal manifestations state according to MGFA-PIS classification.

Eight patients (four women, four men, mean age 75.5 years) (5%) died during follow-up. None of them died due to myasthenic worsening, two died due to malignancy and two due to non-COVID, four due to COVID-19 infection. A Swedish database study including 4,559 MG patients, concluded that, death rate in patients with MG was not different from the death rate of the Swedish population. The most common cause of death was cancer (19.5%). Deaths in MG patients (11%) were 2.5 times more common due to influenza or pneumonia (33). The presence of thymoma indicates a poor prognosis (34).

During the COVID-19 pandemic, 16 patients (9.2%) had COVID-19 infection. Overall, 75% of these patients did not progress more severely than the general population, and all patients continued their immunosuppressant treatment. Four patients died due to COVID-19 infection, these four patients had serious comorbidities, and three of them were elderly (>75 years). One of the patients who died of COVID-19, 56 years of old male, had a malign thymoma history. MG patients were more adversely affected by the current immune suppression, weakness of respiratory muscles and respiratory failure, pneumonia and pulmonary thromboembolism during the COVID-19 pandemic. There is also an increase in the mortality rate due to ARDS, which occurs with immune dysregulation and respiratory muscle insufficiency (35). When the frequency of COVID-19 was examined in a French MG cohort (CO-MY-COVID registry), COVID-19 was detected in 0.96% ($n = 34$) of 3,558 MG patients. Five patients died. The immunosuppressants and CS they used did not adversely affect the result (36), just like in our patient group.

In a retrospective study conducted in Brazil, the care processes of 15 MG patients with COVID-19 were examined. It was concluded that immunosuppressant treatments had no adverse effects on the clinical course and should be continued in patients with COVID-19 (37). These studies are also consistent with our experiences during the COVID-19 pandemic. In a systematic review, it was reported that among the risk factors that increase the severity of the disease in MG patients with COVID-19, severe MG clinic, old age, long-term steroid use, use of RTX, and comorbidities (35).

The limitations of this study are that it is single-centered and descriptive. However, the scarcity of studies on this subject, the transfer of clinical experience, and the reflection of experiences during the COVID-19 pandemic are among the important outputs of this study.

In conclusion, the age and gender distributions of the patients in our study group are similar to previous studies. MG is more common in women between the ages of 20–40 and in men over the age of 65. The use of corticosteroids was more common under the age of 50, and the use of non-steroidal immunosuppressant agents was more common over the age of 50. AZA is a clinically effective and safe agent that should be used with close follow-up as a classical and first-choice steroid-sparing immunosuppressant agent. MM is seen as an agent with no serious side effects, but whose efficacy may remain low in severe cases. RTX, on the other hand, seems to be effective and safe, especially in anti-MuSK positive cases. Thymectomy is still an important supportive treatment approach in anti-AChR positive and seronegative generalized patients under 50 years of age. IVIG and plasmapheresis are effective treatments during acute exacerbations and bridging periods of treatments. MG is a disease that can be managed very well with close and individualized follow-up. There is a small proportion of resistant patients who do not respond to conventional treatments. In these patients, complement inhibitors can be tried in those who are anti-AChR antibody positive. These agents also have uncertainities such as duration of treatment, long-term side effects, and cost issues. Specific treatments are needed especially for this group of patients.

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: these are patient records of the hospital. Requests to access these datasets should be directed to antalyaeah@saglik.gov.tr.

Ethics statement

The studies involving human participants were reviewed and approved by Antalya Training and Research Hospital, Ethical Committee. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Beloor Suresh A, RMD Asuncion. Myasthenia Gravis. Treasure Island (FL): Stat Pearls Publishing; (2022).

2. Dresser L, Wlodarski R, Rezania K, Soliven B. Myasthenia gravis: epidemiology, pathophysiology and clinical manifestations. *J Clin Med*. (2021) 10:2235. doi: 10.3390/jcm10112235

3. Alanazy MH. Clinical features and outcomes of patients with myasthenia gravis. *Neurosciences (Riyadh)*. (2019) 24:176–84. doi: 10.17712/nsj.2019.3.20190011

4. Deymeer F. Myasthenia gravis: MuSK MG, late-onset MG and ocular MG. *Acta Myol*. (2020) 39:345–52. doi: 10.36185/2532-1900-038

5. Galassi G, Mazzoli M, Ariatti A, Kaleci S, Valzania F, Nichelli PF. Antibody profile may predict outcome in ocular myasthenia gravis. *Acta Neurol Belg*. (2018) 118:435–43. doi: 10.1007/s13760-018-0943-7

6. Alhaidar MK, Abumurad S, Soliven B, Rezania K. Current treatment of myasthenia gravis. *J Clin Med*. (2022) 11:1597. doi: 10.3390/jcm11061597

7. 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

8. Jaretzki A, Barohn RJ, Ernstoff RM, Kaminski HJ, Keesey JC, Penn AS, et al. Myasthenia gravis: recommendations for clinical research standards. *Ann Thorac Surg*. (2000) 70:327–34. doi: 10.1016/S0003-4975(00)01595-2

9. Sanders DB, Wolfe GI, Benatar M, Evoli A, Gilhus NE, Illa I, et al. International consensus guidance for management of myasthenia gravis: executive summary. *Neurology*. (2016) 87:419–25. doi: 10.1212/WNL.0000000000002790

10. Sieb JP. Myasthenia gravis: an update for the clinician. *Clin Exp Immunol*. (2014) 175:408–18. doi: 10.1111/cei.12217

11. Punga AR, Maddison P, Heckmann JM, Guptill JT, Evoli A. Epidemiology, diagnostics, and biomarkers of autoimmune neuromuscular junction disorders. *Lancet Neurol*. (2022) 21:176–88. doi: 10.1016/S1474-4422(21)00297-0

12. Mercelis R, Alonso-Jiménez A, Van Schil P. Current management of myasthenia gravis in Belgium: a single-center experience. *Acta Neurol Belg*. (2023) 123:375–84. doi: 10.1007/s13760-023-02187-0

13. Sanders DB, Raja SM, Guptill JT, Hobson-Webb LD, Juel VC, Massey JM. The Duke myasthenia gravis clinic registry: I. Description and demographics. *Muscle Nerve*. (2021) 63:209–16. doi: 10.1002/mus.27120

14. Grob D, Brunner N, Namba T, Pagala M. Lifetime course of myasthenia gravis. *Muscle Nerve*. (2008) 37:141–9. doi: 10.1002/mus.20950

15. Thomsen JLS, Vinge L, Harbo T, Andersen H. Gender differences in clinical outcomes in myasthenia gravis: a prospective cohort study. *Muscle Nerve*. (2021) 64:538–44. doi: 10.1002/mus.27331

16. Boldingh MI, Maniaol AH, Brunborg C, Weedon-Fekjær H, Verschuuren JJ, Tallaksen CM. Increased risk for clinical onset of myasthenia gravis during the postpartum period. *Neurology*. (2016) 87:2139–45. doi: 10.1212/WNL.0000000000003339

17. Hoch W, McConville J, Helms S, Newsom-Davis J, Melms A, Vincent A. Auto-antibodies to the receptor tyrosine kinase MuSK in patients with myasthenia gravis without acetylcholine receptor antibodies. *Nat Med*. (2001) 7:365–8. doi: 10.1038/85520

18. Skriapa L, Zisimopoulou P, Trakas N, Grapsa E, Tzartos SJ. Expression of extracellular domains of muscle specific kinase (MuSK) and use as immunoadsorbents for the development of an antigen-specific therapy. *J Neuroimmunol*. (2014) 276:150–8. doi: 10.1016/j.jneuroim.2014.09.013

19. Lazaridis K, Tzartos SJ. Autoantibody specificities in myasthenia gravis; implications for improved diagnostics and therapeutics. *Front Immunol*. (2020) 11:212. doi: 10.3389/fimmu.2020.00212

20. Tsonis AI, Zisimopoulou P, Lazaridis K, Tzartos J, Matsigkou E, Zouvelou V, et al. MuSK autoantibodies in myasthenia gravis detected by cell based assay–a multinational study. *J Neuroimmunol*. (2015) 284:10–7. doi: 10.1016/j.jneuroim.2015.04.015

21. Suzuki S, Utsugisawa K, Nagane Y, Satoh T, Kuwana M, Suzuki N. Clinical and immunological differences between early and late-onset myasthenia gravis in Japan. *J Neuroimmunol*. (2011) 230:148–52. doi: 10.1016/j.jneuroim.2010.10.023

22. Leite MI, Jacob S, Viegas S, Cossins J, Clover L, Morgan BP, et al. IgG1 antibodies to acetylcholine receptors in 'seronegative' myasthenia gravis. *Brain*. (2008) 131:1940–52. doi: 10.1093/brain/awn092

23. Romi F. Thymoma in myasthenia gravis: from diagnosis to treatment. *Autoimmune Dis*. (2011) 2011:474512. doi: 10.4061/2011/474512

24. Nikolic A, Djukic P, Basta I, Lj H, Stojanovic VR, Stevic Z, et al. The predictive value of the presence of different antibodies and thymus pathology to the clinical outcome in patients with generalized myasthenia gravis. *Clin Neurol Neurosurg*. (2013) 115:432–7. doi: 10.1016/j.clineuro.2012.06.013

25. Farrugia ME, Goodfellow JA. A practical approach to managing patients with myasthenia gravis-opinions and a review of the literature. *Front Neurol*. (2020) 11:604. doi: 10.3389/fneur.2020.00604

26. Verschuuren JJ, Palace J, Murai H, Tannemaat MR, Kaminski HJ, Bril V. Advances and ongoing research in the treatment of autoimmune neuromuscular junction disorders. *Lancet Neurol*. (2022) 21:189–202. doi: 10.1016/S1474-4422(21)00463-4

27. Hehir MK, Burns TM, Alpers J, Conaway MR, Sawa M, Sanders DB. Mycophenolate mofetil in AChR-antibody-positive myasthenia gravis: outcomes in 102 patients. *Muscle Nerve*. (2010) 41:593–8. doi: 10.1002/mus.21640

28. Heatwole C, Ciafaloni E. Mycophenolate mofetil for myasthenia gravis: a clear and present controversy. *Neuropsychiatr Dis Treat*. (2008) 4:1203–9. doi: 10.2147/ndt.s3309

29. Gilhus NE, Verschuuren JJ. Myasthenia gravis: subgroup classification and therapeutic strategies. *Lancet Neurol*. (2015) 14:1023–36. doi: 10.1016/S1474-4422(15)00145-3

30. Guo Y, Tian X, Wang X, Xiao Z. Adverse effects of immunoglobulin therapy. *Front Immunol*. (2018) 9:1299. doi: 10.3389/fimmu.2018.01299

31. Cortés-Vicente E, Álvarez-Velasco R, Segovia S, Paradas C, Casasnovas C, Guerrero-Sola A, et al. Clinical and therapeutic features of myasthenia gravis in adults based on age at onset. *Neurology*. (2020) 94:e1171–80. doi: 10.1212/WNL.0000000000008903

32. Tireli H, Yuksel G, Tutkavul K. Late-onset myasthenia gravis: is it a different clinical entity? *Neurol Sci Neurophysiol*. (2021) 38:127–34. doi: 10.4103/nsn.nsn_201_20

33. Westerberg E, Punga AR. Mortality rates and causes of death in Swedish myasthenia gravis patients. *Neuromuscul Disord*. (2020) 30:815–24. doi: 10.1016/j.nmd.2020.08.355

34. Christensen PB, Jensen TS, Tsiropoulos I, Sørensen T, Kjaer M, Højer-Pedersen E, et al. Mortality and survival in myasthenia gravis: a Danish population based study. *J Neurol Neurosurg Psychiatry*. (1998) 64:78–83. doi: 10.1136/jnnp.64.1.78

35. Tugasworo D, Kurnianto A, Retnaningsih AY, Ardhini R, Budiman J. The relationship between myasthenia gravis and COVID-19: a systematic review. *Egypt J Neurol Psychiatr Neurosurg*. (2022) 58:83. doi: 10.1186/s41983-022-00516-3

36. Solé G, Mathis S, Friedman D, Salort-Campana E, Tard C, Bouhour F, et al. Impact of coronavirus disease 2019 in a French cohort of myasthenia gravis. *Neurology*. (2021) 96:e2109–20. doi: 10.1212/WNL.0000000000011669

37. Camelo-Filho AE, Silva AMS, Estephan EP, Zambon AA, Mendonça RH, Souza PVS, et al. Myasthenia gravis and covid-19: clinical characteristics and outcomes. *Front. Neurol.* (2020) 11:1053. doi: 10.3389/fneur.2020.01053

frontiers | Frontiers in Neurology

TYPE Review
PUBLISHED 04 August 2023
DOI 10.3389/fneur.2023.1229112

OPEN ACCESS

EDITED BY
Francesco Saccà,
University of Naples Federico II, Italy

REVIEWED BY
Marc De Baets,
Maastricht University, Netherlands
Cherie L. Butts,
Biogen Idec, United States

*CORRESPONDENCE
Vera Bril
✉ vera.bril@utoronto.ca

RECEIVED 25 May 2023
ACCEPTED 19 July 2023
PUBLISHED 04 August 2023

CITATION
Bhandari V and Bril V (2023) FcRN receptor antagonists in the management of myasthenia gravis.
Front. Neurol. 14:1229112.
doi: 10.3389/fneur.2023.1229112

FcRN receptor antagonists in the management of myasthenia gravis

Vinaya Bhandari and Vera Bril*

Ellen and Martin Prosserman Centre for Neuromuscular Diseases, Toronto General Hospital, University Health Network, University of Toronto, Toronto, ON, Canada

Myasthenia gravis (MG) is an autoimmune disorder characterized by autoantibodies specifically directed against proteins located within the postsynaptic membrane of the neuromuscular junction. These pathogenic autoantibodies can be reduced by therapies such as plasma exchange, IVIG infusions and other immunosuppressive agents. However, there are significant side effects associated with most of these therapies. Since there is a better understanding of the molecular structure and the biological properties of the neonatal Fc receptors (FcRn), it possesses an attractive profile in treating myasthenia gravis. FcRn receptors prevent the catabolism of IgG by impeding their lysosomal degradation and facilitating their extracellular release at physiological pH, consequently extending the IgG half-life. Thus, the catabolism of IgG can be enhanced by blocking the FcRn, leading to outcomes similar to those achieved through plasma exchange with no significant safety concerns. The available studies suggest that FcRn holds promise as a versatile therapeutic intervention, capable of delivering beneficial outcomes in patients with distinct characteristics and varying degrees of MG severity. Efgartigimod is already approved for the treatment of generalized MG, rozanolixizumab is under review by health authorities, and phase 3 trials of nipocalimab and batoclimab are underway. Here, we will review the available data on FcRn therapeutic agents in the management of MG.

KEYWORDS

neonatal Fc receptor (FcRn), myasthenia gravis (MG), immunoglobulins (IgG), clinical trials, Fc receptor inhibitors

Introduction

MG represents an autoimmune disorder characterized by autoantibodies specifically directed against proteins located within the postsynaptic membrane of the neuromuscular junction (1, 2). This results in the development of focal or generalized muscle weakness in the skeletal muscles. The clinical spectrum encompasses a variety of manifestations, ranging from isolated ocular involvement to profound weakness affecting the limbs, bulbar region, and respiratory muscles. The weakness is fatigable and fluctuating in nature, improving with rest. The prevalence of MG, a relatively uncommon disease, ranges from 5.3 to 35 cases per 100,000 individuals, while the incidence ranges from 0.3 to 2.8 new cases per 100,000 individuals (3–5). Over the past few decades, the prevalence of MG has shown a steady increase, attributed to factors such as improved diagnostic capabilities, advancements in therapeutic options, and increased life expectancy of MG patients.

Pathogenesis of myasthenia gravis

In normal neuromuscular transmission, the presynaptic membrane releases acetylcholine (ACh) which then binds to the acetylcholine receptor (AChR) situated on the postsynaptic membrane. This interaction produces an end plate potential (EPP), the magnitude of which is dictated by the quantity of ACh released at the presynaptic membrane and its interaction with the receptor. Under normal circumstances, the EPP rises above the depolarization threshold to produce an action potential and muscle contraction. In MG, impaired neuromuscular transmission and reduced safety factor (EPP) amplitude reduces muscle contraction (6, 7). About 80% of patients with MG in some series have anti-acetylcholine receptor antibodies (anti-ACHR ab) (8, 9). The prevalence of the IgG1 and IgG3 subclasses of antibodies is most observed in patients diagnosed with myasthenia gravis (10). These autoantibodies bind to AChR at the terminal expansions of the junctional folds that cause activation of the complement system forming membrane attack complexes (MAC), causing accelerated internalization, degradation of ACHR and destruction of the ACHR receptors. In addition, there is cross-linking of the autoantibodies, causing a conformational change in the ACHR receptor, which also interferes with neuromuscular transmission (11). Another mechanism affecting the neuromuscular junction in MG is the functional blockade of ACHR receptors by antibodies and the disruption of junctional folds. This causes the postsynaptic membrane to be distorted and simplified (12–15). These processes lead to neuromuscular failure, muscle weakness and characteristic worsening with an extended period of activity. There is a decremental response of the motor unit potential to repetitive nerve stimulation (RNS) as there is a reduction in safety factor. Up to 50% of patients with anti-ACHR negative generalized MG (ACHR- gMG) may have circulating antibodies against muscle-restricted receptor tyrosine kinase (MuSK), constituting about 5% of the total generalized MG cases. These are mainly IgG4 antibodies and do not affect the complement system. These antibodies mainly affect the clustering of the ACHR, thereby causing a functional blockage at the neuromuscular junction (16, 17). Roughly 2% of individuals diagnosed with double seronegative generalized myasthenia gravis (gMG) display low-density lipoprotein receptor-related protein 4 antibodies (anti-LRP antibodies) (18). The IgG1 subclass accounts for most anti-LRP (lipoprotein receptor-related protein) antibodies and causes damage to the postsynaptic function by complement-mediated damage and possibly interfering with agrin-induced MUSK activation (19, 20). A proportion of patients with MG have a presence of low-affinity antibodies, which cannot be detected by routine radioimmunoassays (RIAs) and can be detected by specific assays such as cell-based assays (21).

MG does not follow a classic Mendelian inheritance pattern, indicating that it is not a hereditary disease. However, there is an increased likelihood of family members of MG patients developing the disease compared to the general population (22). Studies have shown a concordance rate of 35% in monozygotic twins and 5% in dizygotic twins, suggesting a genetic contribution to MG pathogenesis. Nonetheless, environmental factors also play a crucial role (23). Various HLA types, such as DR2, DR3, B8, and DR1, are associated with an increased predisposition to MG.

The breakdown of mechanisms responsible for maintaining immune tolerance to self-antigens is the underlying cause of autoimmunity. During development, most auto-reactive T cells are eradicated in the thymus, establishing central tolerance. Regulatory mechanisms involved in maintaining immune tolerance encompass the generation of regulatory T cells (Treg cells), which exert control over self-reactive T cells that escape thymic elimination. Essential roles in the clonal deletion and Treg cell selection are performed by transcription factors, notably the autoimmune regulator gene (AIRE) (24). The thymus is pivotal in MG pathogenesis, as 85% of patients exhibit thymic gland abnormalities, with 70% showing thymic hyperplasia and 10% having a thymoma. The regulation of autoreactive T cells is impaired due to thymic hyperplasia or thymoma, possibly due to altered expression of AIRE and inefficient Treg generation in patients with MG (25). There is a breakdown of immune tolerance to self-antigens that leads to autoimmunity.

The pathophysiology of MG is complex and multifactorial, attributed to the interplay between genetic and environmental factors leading to complex immune-mediated dysfunction at the neuromuscular junction.

There is upregulation of CD4+ T cells due to the breakdown of immune tolerance, leading to the release of various proinflammatory cytokines (IL-2, IL-4 and IL-6), leading to B cell stimulation, eventually leading to antibody production (5). As a consequence of these mechanisms, mature T and B lymphocytes in the thymus gland become activated. The activation of T cells results in the secretion of proinflammatory cytokines like IFN-gamma and IL-17, leading to an imbalance between regulatory T cells (Treg), which are deficient, and hyperactivated Th17 cells, further amplifying antibody production in MG (26–28).

Conventional treatment in myasthenia gravis

The treatment of MG ranges includes various non-immunosuppressive, immunosuppressive therapeutic agents and newer biological agents which are target specific (29). The acetylcholinesterase agents such as pyridostigmine provide symptomatic treatment of myasthenia gravis; however, seldom as sole therapy. In older literature, about 30–50% of MG patients were reportedly on pyridostigmine alone (30). Corticosteroids likely act by inhibiting T lymphocytes and monocyte–macrophage activation and are used in many MG patients (31). Azathioprine, mycophenolate mofetil, tacrolimus, methotrexate, and cyclophosphamide are some of the immunosuppressive agents used in the treatment of MG include, and these act by varied mechanisms that suppress the immune system. The main role of immunosuppressive agents is to act as steroid-sparing agents and to prevent the long-term side effects caused by corticosteroids, which include diabetes mellitus, weight gain, cataracts, hypertension, osteoporosis, and gastric ulcer (32). However, the immune suppressive agents have their own set of side effects and increase the propensity to cause infection and cancers such as squamous cell carcinomas.

IVIG and PLEX have proven to be efficacious in acute exacerbations of MG and MG crises (33, 34). IVIG is more widely available and has fewer side effects than PLEX. Several studies have shown no difference in the overall efficacy of IVIG over PLEX (35, 36). In cases of chronic refractory disease or when patients do not respond to standard immunosuppressant therapy, intravenous immunoglobulin (IVIG) and

subcutaneous immunoglobulin (SCIG) Infusions have shown beneficial effects (37). However, the management of IVIG has its own challenges. Various side-effects associated with IVIG administration include aseptic meningitis, headaches, increased propensity to thrombosis, and renal impairment (38).

PLEX is a therapeutic approach employed in the treatment of myasthenia gravis since 1976 (39). This includes the elimination of pathogenic and normal immunoglobulins and high molecular weight components like albumin and proinflammatory factors that contribute to the autoimmune process. The effects of PLEX last for 2–4 weeks (40). PLEX requires specialized equipment, central venous access, and nurse supervision. As coagulation factors are removed during PLEX treatment, it is performed on alternate days to allow natural recovery. However, tissue IgG is redistributed between the PLEX sessions, and the serum IgG rises again. Thus, there is a need for agents that mimic the role of PLEX or IVIG but have a sustained effect with fewer side effects.

Fc gamma receptors (FcγR) and neonatal Fc receptors (FcRn) as therapeutic agents

Antibodies constitute the most important part of adaptive immunity. Immunoglobulin G (IgG) is the predominant class of antibodies, accounting for approximately 75–80% of the total immunoglobulin pool (41). These antibodies can be found in both circulation and extracellular fluids. Immunoglobulins exhibit a structural arrangement composed of two heavy chains and two light chains, giving rise to a molecular configuration encompassing two fragment antigen binding domains (Fab) and a glycosylated crystallizable fragment (Fc). The hinge region connects the two Fab fragments and the Fc fragment, enabling conformational flexibility to the Fab fragment. Because the Fab fragments are identical, they can bind to specific target antigens (42–44). The effector function of the Fc region is facilitated through its interaction with various receptor molecules such as Fc gamma receptors (FcγR) and the first subcomponent of the C1 complex (C1q), facilitating crucial functions such as induction of mediator secretion, antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), endocytosis of opsonized particles, complement-dependent cytotoxicity (CDC) (45, 46).

FcγR is a diverse family of proteins encompassing classical membrane-bound surface receptors, atypical intracellular receptors, and cytoplasmic glycoproteins. Cells of hemopoietic origin widely express receptors and can be either activating receptors (FcγRI, FcγRIIA, FcγRIIC, FcγRIIIA, and FcγRIIIB) or inhibitory receptors (FcγRIIB) and differ in their affinity to various IgG subclasses (47) FcRn belongs to the FcγRs is structurally unique and differs from the classic members of the receptor family in various aspects. It was initially discovered that the transfer of maternal antibodies to neonates was possible due to proteins called neonatal Fc receptors (FcRn) (48). FcRn is a beta-2 macroglobulin-associated protein exhibiting structural similarity to the major histocompatibility class I (MHC-I) family. FcRn is monomorphic, quasi-ubiquitously expressed, and expressed in various body tissues, including epithelia, endothelia, hemopoietic cells, intestinal cells, kidney, liver, and liver placenta (49–53).

Amongst all the serum proteins, albumin and IgG have the longest half-life period of approximately 3 weeks compared to other serum proteins, which have a half-life period of approximately 5–7 days (54). FcRn uniquely binds both IgG and albumin, despite the structural and functional dissimilarity between these two molecules. Intracellularly, FcRn binds to IgG and albumin at non-overlapping sites within endosomes at pH 5–6.5. It then prevents the catabolism of IgG and albumin by preventing their lysosomal degradation and releasing them outside the cell at physiologic pH, thus prolonging the half-life of the albumin and IgG (55–58).

The presence of pathogenic autoantibodies characterizes autoimmune disorders such as MG. Current therapies such as plasma exchange, IVIG infusions and other immunosuppressive agents aim to reduce the pathogenic antibodies. However, most of these therapies exhibit a broader mechanism of action and have significant side effects. Since there is a better understanding of the molecular structure and its biological properties, the FcRn possesses an attractive profile in treating myasthenia gravis and other autoimmune disorders whereby the autoantibodies can be reduced by blocking the FcRn. By blocking the FcRn, the catabolism of IgG will be enhanced, and similar results will be achieved with plasma exchange (Figure 1). This mechanism will be useful in various autoimmune disorders; degradation of IgG molecules can be achieved by blocking the FcRn receptors is a rational therapeutic approach (59, 60).

FcRn therapeutic agents in the management of MG

Table 1 gives an overview of the various FcRn blocking agents developed in the management of MG. The remainder of this article focuses on the evidence for these agents in the treatment of MG.

Efgartigimod

Efgartigimod is a modified Fc fragment derived from human IgG1. It has been specifically engineered to enhance its binding affinity to FcRn receptors under both acidic and physiological pH conditions while maintaining its pH-dependent properties. Flow cytometry and microscopic analysis suggested an augmented affinity and/or avidity of efgartigomod toward the FcRn receptors due to the demonstration of an elevated concentration of efgartigomod specifically within FcRn-positive compartments within cells, concomitant with enhanced lysosomal accumulation.

Phase 1 study

Ulrichts et al. conducted a double-blind, placebo-controlled phase 1 study on 62 healthy volunteers (61). There was an enhanced clearing of IgG1 levels following multiple ascending doses (MAD)of intravenous (IV) efgartigomod compared to administration of IV single ascending dose (SAD), with a 50% reduction in the IgG levels following SAD and a 75% reduction in IgG levels following MAD of efgartigimod. Following the last infusions of efgartigimod, IgG levels returned to their baseline levels after approximately 8 weeks. There were no notable alterations in the IgA, IgE, IgM, and IgD levels, indicating no significant impact on these immunoglobulin subclasses. Additionally, efgartigimod did not interfere with albumin homeostasis.

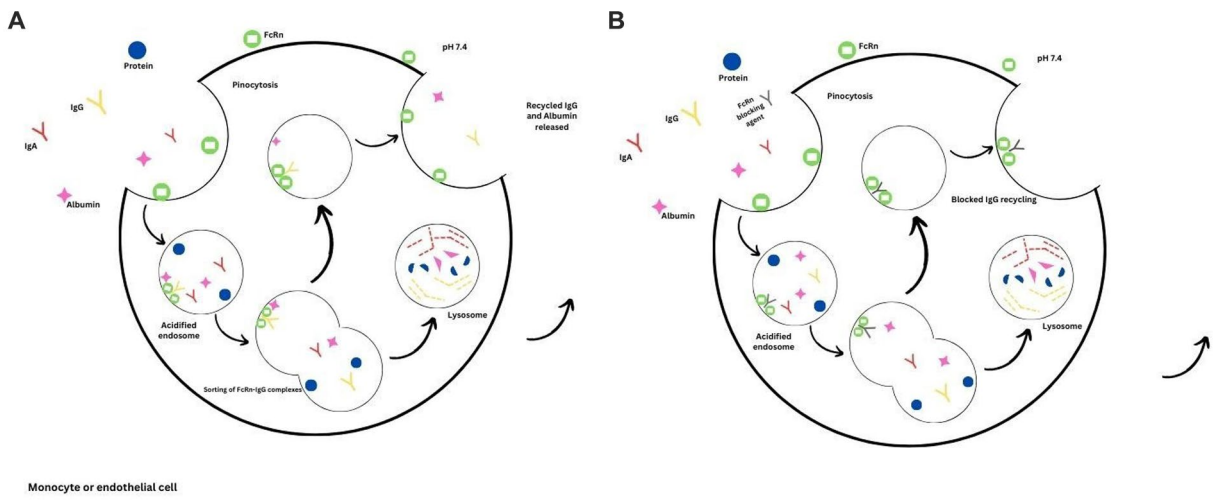


FIGURE 1
FcRn mediated recycling. **(A)** The binding of IgG and albumin to the FcRn receptors prolongs their half-lives due to the prevention of lysosomal degradation. **(B)** Binding of the FcRn therapeutic agent, thus preventing the recycling of IgG due to increased lysosomal degradation.

TABLE 1 Overview of the FcRn receptors.

Agent	Company	Molecule	Current status
Efgartigimod	ARGENX	Humanized IgG1 Fc Fragment	Approved for treatment of AChR+ gMG
Rozanolixizumab	UCB	Humanized IgG4 Monoclonal	Phase 3 completed
Nipocalimab	Johnson and Johnson	Humanized aglycosylated monoclonal ab	Phase 3 study ongoing
Batoclimab	Immunovant	Humanized IgG1 monoclonal ab	Phase 3 study ongoing
ABY-039	Affibody	Bilvalent antibody	Phase 1 study was prematurely terminated

The drug had no safety concerns, with most adverse effects being mild and self-limiting and most side effects being seen at higher doses. Mild headache was the most common side effect encountered, which subsided with minimal intervention. In the phase 1 study, no significant production of anti-drug antibodies was observed.

Phase 2 study

In the phase 2 exploratory study, Howard et al. conducted a multicenter, randomized, double-blind, placebo-controlled trial of anti-ACR positive generalized myasthenia gravis (ACHR+ gMG) subjects. This study represents the first investigation providing a clinical profile of Fc receptor antagonism in generalized myasthenia gravis (gMG) (62).

The study included participants who had impaired daily activities of living, as indicated by an MG-ADL score of 5 or higher at screening and baseline, with more than 50% of the score attributed to nonocular items and was diagnosed with MGFA class II-IVa disease. Individuals who had a recent malignancy or thymectomy within 3 months of screening were excluded. A total of 24 patients were randomly assigned to receive 10 mg/kg IV efgartigimod or a matched placebo in a 1:1 ratio for a total of 4 doses over 3 weeks, in addition to their standard-of-care therapy. The study aimed to assess the impact of efgartigimod on patient safety and the effectiveness of the treatment in managing ACR+ gMG. The secondary endpoints included an assessment of efficacy based on the improvement in the outcome measures at week 11. In addition, pharmacokinetics (PK), pharmacodynamics (PD), and immunogenicity were evaluated. The scales deployed to assess the efficacy, including the quantitative myasthenia gravis score (QMG), myasthenia gravis activity of daily living (MG-ADL), myasthenia gravis composite disease scores (MGC) and the revised 15-item MG-quality of life (MG-QoL15r) (Table 2).

Efgartigimod was reported to be safe and well-tolerated by all the patients who received the drug and had no serious adverse effects (SAE) or severe treatment-emergent adverse effects (TEAE) reported with no significant difference in the side effect profile between the efgartigimod group compared to the placebo group. No incompatibility was seen between efgartigimod and the standard of care therapy used in MG. Reduced monocyte count and headaches were the most frequent side effects noted in the study, with most reported side effects being mild in severity. Other side effects included rhinorrhea, pruritis, injection site reaction and herpes zoster infection. Of note, the patient diagnosed with herpes zoster was already on prednisone and mycophenolate mofetil, and the authors were uncertain if efgartigimod was the causative factor leading to herpes zoster.

Patients who received efgartigimod demonstrated clinically meaningful and sustained improvement, consistently observed across all clinical scales, including MG-ADL, QMG, MGC, and MG-QoL15r (Table 3). Maximum clinical improvement was observed 1 week following the final infusion dose and persisted even after stopping the medication. Maximum mean changes in efgartigimod vs. placebo for QMG score (−5.7 vs. −2.1), MG-ADL (−4.4 *VS* -2.9), MGC (−9.4 vs.

TABLE 2 Methodology of the phase 2 and phase 3 results.

Therapeutic name	Phase	Treatment arms	Inclusion	Sample size	Route and dose of administration	Primary end-points	Secondary endpoints
Efgartigimod	2	Double blinded RCT (1:1).	AChR+gMG MG-ADL ≥ 5 MGFA II-IV	24	10 mg/kg efgartigomod or placebo IV wkly 4 doses over 3 wks	Safety and efficacy	Change from baseline to 11 weeks: QMG MG-ADL MGC MGQoL15r
Efgartigimod	3	Double blinded RCT (1:1)	gMG MG-ADL ≥ 5 MGFA II-IV	167	10 mg/kg efgartigomod or placebo IV wkly 4 doses and then repeat dosing if needed	MG-ADL responders.	QMG responders Early MG-ADL responders
Rozanolixizumab	2	Double blinded 2 Period RCT (1:1).	gMG QMG ≥11	43.	Period 1: 7 mg/kg rozanolixizumab. or placebo Qwkly for 3wks. Period 2: 7 mg/kg or 4 mg/kg Rozanolixizumab Qwkly for 3wks	Change from baseline to day 29: QMG.	Change from baseline to day 29: MG-ADL MGC Safety
Rozanolixizumab	3	Double blinded RCT (1:1:1)	ACHR+ MG Musk+MG MGFA II-IVa MG-ADL ≥ 3	200	7 mg/kg Qwkly or 10 mg/kg Qwkly of rozanolixizumab for 6 wks.	Change from baseline to day 43: MG-ADL	Change from baseline to day 43: MGC QMG
Nipocalimab	2	Double blinded 5 arm RCT (1:1:1: 1:1)	gMG	68	5 mg/kg q4wkly 30 mg/kg q4wkly 60 mg/kg q2wkly 60 mg/kg q2wkly, or placebo q2wkly for 8 weeks	Change from baseline to day 57 of MG-ADL Effect on total IgG and anti-ACHR ab level	Change from baseline to day 57: QMG, MG-QoL15r
Nipocalimab	3	Double blinded RCT (1:1)	AChR+ gMG MGFA II-Iva MG-ADL ≥ 6		30 mg/kg at first infusion, 15 mg/kg thereafter Q2wks for 24 wks	Change from baseline to wks 22,23,24 of MG-ADL	
Batoclimab	2	Double blinded 3arm RCT (1:1:1) with OLE	AChR+ gMG MGFA II-Iva QMG score ≥ 12	15	Batoclimab 680 mg, Batoclimab 340 mg, Placebo Qwkly SC for 6wks	Safety and efficacy	Change from baseline to day 43: QMG MG-ADL MG-QoL15r
Batoclimab	3	Quadruple blinded 3 arm 2 period RCT (1:1:1) with OLE	gMG MGFA II-Iva QMG score ≥ 11 MG-ADL ≥ 5		Period 1: Batoclimab 680 mg, Batoclimab 340 mg, Placebo Qwkly SC for 6wks. Period 2: Batoclimab 340 mg QWkly, Batoclimab 340 mg Q2Wkly, Placebo Qwkly SC for 6wks	Change from baseline to 12 wks of MG-ADL In ACHR+MG	Change from baseline to 12 wks of QMG in ACHR+MG Change from baseline to 24 wks of MG-ADL, QMG in ACHR+MG

−4.4) and MG-QoL15r (−6 vs. −2.1). 75% of the patients treated with efgartigimod obtained a clinically significant, statistically significant improvement in MG-ADL for a period of 6 weeks. In contrast, only 25% of patients in the placebo group showed similar improvement. There was evidence of rapid reduction of total IgG and IgG subtypes in all patients who received efgartigimod. A reduction of up to 40% was seen after the first administration, and a maximum reduction of up to 70% was seen around 1 week after the last infusion. Reduction in anti-ACHR ab levels mimicked the reduction in total IgG levels. The clinical benefit correlated with the initial drop in IgG levels, but the clinical effects persisted for 8 weeks even when the IgG levels returned almost to baseline.

Phase 3 study

The ADAPT trial was a phase 3 randomized multicentre, double-blinded, placebo-controlled trial of efgartigimod in patients with gMG with or without anti ACHR antibodies with the disease classified as class II-IVa as per MGFA classification with MG-ADL of at least 5 with more than 50% of the score attributed to nonocular symptoms (63). The study excluded patients with thymectomy within 3 months of screening. Patients were randomized in a 1:1 ratio to receive efgartigimod or placebo. The patient received the same dose of efgartigimod of 10 mg/kg IV or matching placebo as four weekly infusions per cycle. After each cycle, there were at least 5 weeks of follow-up, and retreatment was possible if the patient was a clinical

TABLE 3 Summary of the phase 2 and phase 3 results.

Therapeutic drug	Phase	Primary end point results (Mean change from baseline)	Secondary end point results (Mean change from baseline)	Adverse effects
Efgartigimod	2	Safe and well tolerated	QMG −5.7 MG-ADL −4.4 MG-QoL15r −6	No serious side effects Most common side effects: headaches, rhinitis, pruritis
Efgartigimod	3	MG-ADL responders (68%)	QMG responders (63%) Early MG-ADL responders (57%)	5% patients had serious side effects. Most common side effects: headaches, nasopharyngitis
Rozanolixizomab	2	QMG −1.8	MG-ADL −1.8 MGC −3.1	No serious side effects Most common side effects: headaches
Rozanolixizomab	3	7 mg/kg MG-ADL −3.70 10 mg/kg MG-ADL −3.40	7 mg/kg MGC -5.93, QMG −5.40 10 mg/kg QMG −6.67, QMG −7.55	No serious side effects Most common side effects: headaches diarrhea, nausea, pyrexia
Nipocalimab	2	30 mg/kg q4wkly MG-ADL −3.7 60mh/kgQ2wkly		No serious side effects Most common side effects: diarrhea, headache, nasopharyngitis
Batoclimab	2	Safe and well tolerated 680 mg SC Total IgG −776.1. 340 mg SC Total IgG −59.3	MG-ADL −3.8 QMG −3.9 MGC −8.0	No serious side effects

responder with an MG-ADL score of ≥5 and no longer had a meaningful clinical response. Clinically meaningful improvement was defined as a reduction in ≥2 points in MG-ADL compared to the baseline sustained for ≥4 weeks, the first improvement occurring in the 4th week of the 1st cycle. The retreatment was possible no sooner than 8 weeks. In the study period of 26 weeks, a maximum of 3 cycles were possible. The study primarily assessed the proportion of ACHR-positive patients who were MG-ADL responders in the first treatment cycle.

Secondary outcome measures encompassed evaluating the proportion of individuals exhibiting a favourable response in terms of the QMG score (clinically meaningful improvement was defined as ≥3 point reduction, with the first improvement occurring the 4th week of the 1st cycle), percentage of MG-ADL responders in the overall population after cycle 1, the proportion of time patients showed a meaningful response in ACR+ gMG patients up to 126 days, time from last infusion of cycle 1 to no longer have a clinically meaningful response in ACHR positive patients and proportion of early MG-ADL responders (first MG-ADL response of reduction in ≥2 points from baseline occurring within the first 2 weeks of the cycle 1) in ACHR+ gMG patients (Table 2).

167 patients were enrolled in the trial, with 129 ACHR+ gMG. 68% of patients in the efgartigomod group achieved all primary outcomes compared to the 30% in the placebo group. Secondary endpoints were met in the efgartigimod group with improvement in outcome measures which was statistically significant (Table 3). There was a greater percentage of QMG responders (63%) compared to placebo (14%), MG-ADL responders (all patients) in cycle 1 (68%) compared to placebo (37%), early MG-ADL responders (57%) compared to placebo (25%) and there was a clinically meaningful improvement in MG-ADL score for the efgartigimod group 48.7% of the time compared to placebo 26.6%. The drug was safely tolerated, with no deaths associated with the administration of the efgartigomod. Most TEAEs were mild to moderate; however, 5% of patients reported serious adverse effects. The most common adverse effect reported following efgartigimod administration was headaches, followed by nasopharyngitis. The IV formulation of the drug was approved by FDA, EMA and in Japan for use in patients with ACHR+ gMG (Table 3). A different route of administration, such as subcutaneous therapy, may be effective for MG, lessen the side effects and allow treatment to be continued.

ADAPT+ study

Of the 167 patients enrolled in the ADAPT trial, 151 (91%) entered the ADAPT + trial, an extended open-label trial assessing the safety and efficacy of efgartigimod. 106 ACHR+ gMG and 33 patients with anti-acetylcholine receptor antibody-negative generalized myasthenia gravis (ACHR- gMG) were included, of which 66 were previously in the placebo group (64). A dose of 10 mg/kg of efgartigimod was given IV, following a treatment schedule consisting of once-weekly infusions for a period of 4 weeks. Subsequent treatment cycles were determined based on clinical evaluation. Throughout the study, patients received an average of 5.1 treatment cycles comprising 20.5 infusions. The median duration of participation in the study was 371 days, resulting in a cumulative observation time of 138 patient-years. Efficacy was assessed during each cycle utilizing MG-ADL and QMG scales. The mean change in the baseline for MG-ADL was −5.1, and for QMG was −4.7, suggesting the efficacy of long-term treatment with efgartigimod consistent across multiple cycles with no safety concerns identified, with most of the AE being mild to moderate.

Rozanolixizumab

Rozanolixizumab is a human anti- FcRn IgG4 antibody having a high affinity to the FcRn receptors (65). The drug was first studied in animals and was reported to be well tolerated, with no mortality or serious side effects following its IV or subcutaneous (SC) administration. There was a 75–90% reduction in IgG levels, with the maximum effect seen on day 10. No susceptibility to increased

infections or raised acute phase reactants was noted in patients who received the drug. There was no change in serum concentration of IgA and IgM levels; however, a small decrease in albumin levels was observed, most likely due to steric hindrance due to bound antibodies. There was a treatment-related effect on the relative or absolute number of lymphocyte counts on immunophenotyping (65, 66).

Phase 1 study

This study investigated the dose escalation of IV or SC rozanolixizumab in healthy individuals (66). A total of 49 patients were subjected to randomization, where they were assigned to receive either rozanolixizumab or a placebo. The doses administered were 1 mg/kg, 4 mg/kg, and 7 mg/kg. The drug was administered as a single infusion over 1 h. The 7 mg/kg SC route administration had a better safety profile and tolerability than the IV group. After IV drug administration, there was a dose-dependent increase in side effects of headache, vomiting, nausea, and pyrexia. 4 severe TEAEs were reported following higher doses of the IV formulation. There were no severe side effects reported in patients who received SC drugs. A dose-dependent decrease in IgG levels was similar in both IV and SC groups. There was a 48% reduction in IgG levels at the highest dose of IV formulation and a 43% reduction in IgG levels following SC formulation. The reduction in serum IgG levels was maximum at day 7–10, persisted for weeks and gradually returned to baseline.

Phase 2 study

This multicenter, double-masked, placebo-controlled trial was conducted in two periods. The subjects were randomized to receive either three once-weekly SC infusions of rozanolixizumab at a dosage of 7 mg/kg or a placebo in the initial period (67). Following a two-week drug-free interval, patients were re-randomized in the second period to receive rozanolixizumab at either 7 mg/kg or 4 mg/kg, and an observation period between days 44 and 99 followed the second treatment period.

The study primarily evaluated the change in QMG on day 29. Secondary objectives included assessing the change in MG-ADL and MGC at day 29, responder rates (defined as a reduction of at least 3 points) in the MG scores and monitoring the safety profile of rozanolixizumab. Patients at least 18 years of age diagnosed with generalized MG with positive anti-ACHR antibody titre or anti-Musk antibody titre with a QMG score of at least 11 or more with eligibility for IVIG or PLEX indicating moderate to severe disease were included in the study. Patients with a thymectomy within 6 months of screening were excluded from the study. Of the 43 patients randomized, 21 patients received rozanolixizumab, and 22 received a placebo (Table 2).

At day 29, the mean change in QMG score from baseline was −1.8 for rozanolixizumab compared to −1.2 for placebo. For MG-ADL, the mean change was −1.8 for rozanolixizumab and − 0.4 for placebo. For the MGC score, the mean change was −3.1 for rozanolixizumab and − 1.2 for placebo. Although the rozanolixizumab group showed an overall improvement in outcome measures compared to the placebo group from baseline to day 29, the difference did not reach statistical significance. On day 29, the responder rates were higher in patients receiving rozanolixizumab 7 mg compared to placebo for QMG (38% vs. 23%), MG-ADL (48% vs. 14%), and MGC (48% vs. 27%). Interestingly, even greater responder rates were observed on day 22 for QMG scores (52%) and MGC scores (55%) compared to day 29. The reduction in QMG score reached its lowest point on day 21 and returned to baseline on day 29, just before the start of period 2. The short subcutaneous treatment duration could be one of the reasons that the primary endpoint was not achieved on day 29. The continuation of rozanolixizumab treatment at a 7 mg/kg dose demonstrated further improvements in outcome measures during period 2. Notably, the nadir of QMG and MG-ADL scores was observed on day 21 after reinitiating rozanolixizumab at the 7 mg/kg dosage. However, by the end of the 99-day observation period, all the measured outcomes returned to their baseline values. These findings suggest that the mode of action of the drug was reversible (Table 3).

There was a moderate reduction in the assessed outcome measures in subjects who received 4 mg/kg of rozanolixizumab with a sustained reduction in QMG score until day 78. Of note, both groups demonstrated dose-dependent improvements in the measured outcome measures when compared to the placebo group.

In period 1, a rapid decline in IgG levels (52%) was seen compared to placebo (4%) at day 29. Nadir in IgG level in the rozanolixizumab group occurred on day 22 (61%). In period 2 of the study, there was a dose-dependent decrease in IgG levels observed among the groups that received Rozanolixizumab at doses of 7 mg/kg and 4 mg/kg, in comparison to the placebo group.

The most commonly encountered adverse effects were mild to moderate headaches (57% rozanolixizumab 7 mg/kg group vs. 14% in the placebo group), which responded well to standard therapy. There were no serious infections or opportunistic infections noted.

Phase 3 study

MycarinG study was a large multicentre trial compared SC administration of 7 mg/kg, 10 mg/kg of rozanolixizumab or placebo for 6 weeks in patients with ACHR/MuSK positive MG (68). The study incorporated subjects with MGFA class II-IVa, with QMG scores exceeding 11 and MG-ADL scores of at least 3. The primary endpoint of the study aimed to evaluate the change in MG-ADL scores from baseline to day 43. The secondary endpoints assessed the changes in MGC, QMG, and Myasthenia gravis patient-related outcomes (MG-PRO) from baseline to day 43 (Table 2). 200 patients were enrolled in the study. There were statistically significant and clinically meaningful improvements in the various outcome measures used in both 7 mg/kg and 10 mg/kg doses (69). For the dose of 7 mg/kg vs. placebo mean change from baseline to day 43 for MG-ADL (−3.37 vs. −0.78), MGC (−5.93 vs. −2.03), and QMG (−5.40 vs. −1.92). For subjects with 10 mg/kg group vs. placebo change in baseline to day 43 for MG-ADL (−3.40 *vs*-0.78), MGC (−7.55 vs. −2.03) and QMG (−6.67 vs. −1.92). 72% of patients in the 7 mg/kg group were MG-ADL responders, and 69% of patients in the 10 mg/kg group were MG-ADL responders. The study was not statistically powered to compare the two doses of rozanolixizumab. Both doses were well tolerated, and no severe side effects were seen, with the most frequent TEAE being headaches followed by diarrhea and pyrexia (Table 3).

Rozanolixizumab is currently awaiting approval for treatment in MG

Nipocalimab

Nipocalimab is a glycosylated fully human monoclonal antibody of the IgG1 class that exhibits high affinity for FcRn exhibiting pico

affinity to FcRn at both endosomal and extracellular pH levels. This molecule shows selective binding to, saturation of, and blocking of the IgG binding site on the endogenous FcRn. As a result, it inhibits the FcRn-mediated recycling of IgG, reducing pathogenic IgG levels with no impact on IgG production. One of the pharmacokinetic properties of the drug is the minimal transfer of the drug across the human placental lobule so that it does not reach the fetal circulation (70).

Phase 1 study

The Phase 1 study was designed as a two-part ascending dose study (71). Part 1 studied SAD up to 60 mg/kg, and Part 2 studied MAD of 15 or 30 mg/kg weekly for 4 weeks. 50 healthy volunteers were recruited for the study. The single dose of nipocalimab resulted in dose-dependent serum IgG levels reduction similar across all IgG classes. The single dose of nipocalimab at 30–60 mg/kg doses maintained serum IgG levels at or below 50% of the baseline for 18 and 27 days, respectively. Multiple doses of 15 to 30 mg/kg achieved a mean reduction in IgG levels by approximately 85% of the baseline and maintained levels below 75% or more for up to 24 days. There was no effect on other immunoglobulins such as IgM, IgA, IgE or other inflammatory cytokines. At a higher dose, there was a mild reduction in albumin noted. The drug was well tolerated in all cohorts, with no deaths, infusion site reactions, systemic allergic reactions or severe TEAEs noted. Due to this pharmacokinetic property, the minimal transfer of the drug across the human placenta may help treat patients in the reproductive age group where teratogenicity and fetal health are of major concern. There is a further exploration of this agent in pregnant women at risk of autoimmune hemolytic disease of newborns (72).

Phase 2 study

Vivacity-MG is a phase 2 multicenter study that employed a randomized, double-blinded placebo trial design to assess the efficacy of nipocalimab in moderate-to-severe gMG (73). A total of 68 patients were enrolled and randomly assigned in a 1:1:1:1:1 ratio to receive various intravenous doses of nipocalimab (5 mg/kg every 4 weeks, 30 mg/kg every 4 weeks, a single dose of 60 mg/kg, 60 mg/kg every 2 weeks) or placebo every 2 weeks during the 8-week treatment period. The primary outcome measure of the study was the change in the MG-ADL score from baseline to day 57. The secondary endpoints included assessing the changes in the QMG score and MG-QoL15r at day 57 (Table 2).

The maximum and most consistent reductions were observed in subjects in the nipocalimab 30 mg/kg q4w and 60 mg/kg q2w treatment groups compared to placebo for MG-ADL (−3.7 vs. −1.3) even though statistical significance was not reached. Following single-dose administration of 60 mg/kg, the mean change from baseline to day 29 vs. 57 for MG-ADL was (−3.9 vs. −1.5), indicating the effect of a single dose of nipocalimab may not last for more than 1 month. In contrast to the placebo group, it was observed that 52% of patients who received nipocalimab across all four dosing regimens experienced a substantial and long-lasting reduction in MG-ADL (a change of ≥2 points for at least four consecutive weeks). Only 15% of participants in the placebo group exhibited a similar response. Clinically meaningful changes were observed in a rapid timeframe, with noticeable improvements occurring within 2 weeks. A dose-dependent significant reduction of the total serum IgG and anti-ACR antibody titres significantly correlated with the MG-ADL improvement. The maximum reduction in serum total IgG levels of 80% was seen following the highest dose of 60 mg/kg q2wkly administration. There was a reduction in total IgG 1 week following the first infusion, which returned to baseline 8 weeks after the last infusion.

The drug had a favourable safety outcome with no significant adverse effects. There was an equal incidence of infections and headaches in the patients who received nipocalimab and placebo. No adverse side effects led to treatment discontinuation in any of the groups. Similar results for albumin were observed in healthy participants with dose-related, self-limiting reductions and was maximum with a dose of 60 mg/kg q2wkly. The most common TEAE reported were diarrhea, headaches and nasopharyngitis (Table 3).

Phase 3 study

An ongoing phase 3 multicenter study is currently being conducted to assess the efficacy, the safety profile of nipocalimab in adult patients with seropositive gMG (74). Inclusion criteria include patients diagnosed with gMG classified as MGFA class II-IVa and have an MG-ADL of 6 or higher. Individuals who underwent thymectomy within 12 months of screening are excluded from the study. Participants in the study were randomly assigned in a 1:1 ratio to receive either nipocalimab or a placebo. The administration of nipocalimab consisted of an initial infusion of 30 mg/kg, followed by subsequent infusions of 15 mg/kg, given once every 2 weeks for a total duration of 24 weeks. The placebo group received corresponding intravenous infusions at the same intervals. The study will primarily ascertain the efficacy of Nipocalimab compared to placebo, using MG-ADL over weeks 22, 23, and 24 (Table 2).

Batoclimab

Batoclimab is a fully human IgG1 monoclonal antibody which can be used for SC or IV administration.

Phase 1 study

A phase 1 study (RVT-1401-1,001) investigated the effects of batoclimab on serum total IgG and albumin levels (75). This trial included IV and subcutaneous SC administration of batoclimab at various doses and durations. Batoclimab effectively reduced total IgG concentrations in a dose-dependent and reversible manner. The maximum reductions ranged from 13 to 67% for single IV doses and 14 to 48% for single SC doses. The SC weekly dose groups showed low inter-subject variability in total IgG reduction. The 680 mg dose exhibited a more consistent effect than the 340 mg dose. The weekly SC dose of 680 mg led to a total IgG reduction of 78%, consistent with other anti-FcRn agents in development. The time course of total IgG reduction was independent of the route of administration (IV or SC) and dose. However, the time to return to baseline was dose-dependent, ranging from 29 to ≥85 days for single doses and 21 to 24 days for weekly SC doses. Serum total IgG concentrations returned to normal levels for most subjects after discontinuing dosing. Batoclimab had a lesser impact on albumin levels compared to total IgG. Albumin reductions were 22 and 34% for the weekly SC dose groups. However, albumin levels returned to normal within a few weeks after the last dose.

A similar phase I randomized controlled trial was conducted in the Chinese population, where healthy subjects were given a single

subcutaneous dose of batoclimab or placebo in doses of 340 mg, 510 mg, 680 mg, with an equal allocation ratio of 1:1:1:1 (76). The participants were followed up for 85 days. Twenty-four subjects were included in the study. There was evidence of a rapid dose-dependent reduction in total IgG levels, reaching a nadir at day 11 with the steady recovery of the IgG levels from day 11 to day 85. No serious side effects were reported, and the most reported illness in subjects who received the drug was an influenza-like rash.

Phase 2 study

A phase IIa clinical trial employing randomization, double-blinding, and placebo control, investigated the safety and effectiveness of subcutaneous administration of batoclimab (77). Subjects with ACHR + gMG with (MGFA) Class II-IVa and QMG score ≥ 12 baselines were included in the study. Subjects with a thymectomy in the past 12 months were excluded. Patients included in the study were assigned to one of three groups receiving a weekly dose of batoclimab (680 mg), a weekly dose of batoclimab (340 mg), or a placebo for a duration of 6 weeks. Following this initial phase, an open-label extension was initiated, during which patients received batoclimab (340 mg) on days 50, 64, and 78. Assessment of safety and efficacy was the primary endpoint of the study, measured by percentage changes in total IgG levels. Secondary endpoints included QMG, MG-ADL, and MGC scores, with a change in scores on day 43 from baseline (Table 2).

Improvement in all the outcome measures, including MG-ADL scores, was reported on day 43. There was a dose-dependent rapid and sustained decline in the IgG levels with a −76.1% change in IgG levels with the dose of 680 mg QWkly treatment arm, −59.3% change in 340 mg SC treatment group compared to a 1.5% change in the placebo group. The mean change in QMG at day 43 from baseline for batoclimab vs. placebo was (−3.8 vs. +0.6), MG-ADL (−1.8 *VS* -0.4) and MGC (−8.0 vs. +1.4). In the group receiving batoclimab, the MG-ADL responder rates, which indicate the percentage of patients with a > 2-point improvement, were 60%. In contrast, the placebo group had a responder rate of 20%. The maximum benefit from the treatment was reported 4 weeks after the initial intervention, and this benefit was sustained for a period of 3 weeks. Both doses of the drug were found to be safe and well-tolerated. The reduction in the pathogenic Ig levels correlated with the clinical benefit (Table 3). Adverse effects were mild to moderate, with no serious adverse effects or death (3).

In another phase 2 trial conducted in China, the tolerability and effectiveness of subcutaneously administered batoclimab were evaluated in patients with moderate to severe gMG. Eligible patients were randomly assigned to receive either batoclimab (680 mg), batoclimab (340 mg), or a placebo weekly for 6 weeks. Following this, there was an open-label extension phase where all patients received batoclimab (340 mg) weekly for another 6 weeks. The primary aim of the study was to determine the change in MG-ADL score, measured from baseline to day 43, as the main objective. On day 43, significant improvements were observed in all outcome measures, including MG-ADL scores. Top-line results indicated that both administered doses of batoclimab exhibited prompt and substantial reductions in disease severity, as evaluated by the MG-ADL, in a manner that was clinically and statistically significant when compared to the placebo. Furthermore, the drug demonstrated a favourable safety profile, with most reported adverse events being of mild intensity and generally well tolerated by the patients (78).

Phase 3 study

Study IMVT-1401-3,101 is an ongoing Phase 3 pivotal multicenter, randomized, quadruple-blind, two-period placebo-controlled study done to assess the effectiveness and how safe batoclimab is as induction and maintenance therapy in adult participants with gMG (79). Patients with mild to severe disease with an MG-ADL of ≥5 at baseline were enrolled in the trial. Other inclusion criteria included MGFA Class II-IVa and QMG score of ≥11 with a ≥ 50% score attributed to nonocular symptoms will be included in the study. Patients with a thymectomy in the past 6 months will be excluded from the study. In Period 1, subjects will be randomized 1:1:1 to receive batoclimab 680 mg SC once a week or 340 mg SC once weekly or a placebo as induction therapy. Primary endpoints of ≥2-point improvement in MG-ADL from baseline to week 12 will be assessed. Re-randomized (1:1:1) subjects will receive batoclimab 340 mg SC once weekly or 340 mg SC Q2weeks or receive placebo treatment according to their treatment assignment in Period 1 and the change from Period 1 baseline in MG-ADL score (< 2-point improvement or ≥ 2-point improvement from Period 1 baseline) at Weeks 10 and 12. Only those participants who demonstrate a ≥ 2-point improvement in MG-ADL score from Period 1 baseline during at least one of the 2 final visits of either Period 1 or Period 2 will proceed to enter the long-term extension, which will extend for 52 weeks. The primary endpoint of the study involved evaluating the change in MG-ADL score from baseline to week 12. The secondary endpoints included assessing the change in QMG scores from baseline to week 12 and MG-ADL scores from baseline to week 24. The overall duration of the study is expected to be approximately 80 weeks (Table 2).

Phase 1 study

ABY-039, a bivalent antibody-mimetic, has completed a phase 1 trial involving healthy volunteers. It specifically targets the neonatal Fc receptor (80). It has a potent effect on lowering plasma IgG titers in healthy volunteers in Phase 1. ABY-039 program was terminated in June 2020 due to tolerability observations that would limit the target product profile of high subcutaneous doses once monthly maintenance injections.

Discussion and conclusion

The FcRn blocking agents have been recently developed and have a targeted approach for MG treatment. Given the rapid reduction in pathogenic IgG autoantibodies, many authorities consider this treatment to be medical plasma exchange. The drugs included in this class have been found to be safe and efficacious in the various phase 1, phase 2 and phase 3 trials conducted thus far. FcRn receptor inhibitors demonstrate efficacy across diverse subgroups and a wide range of MG severity, supported by robust clinical evidence. Efgartigimod has been approved in the management of gMG, rozanolixizumab is under review by health authorities, and there are phase 3 trials ongoing for nipocalimab and batoclimab. The optimal dosage and duration of treatment in managing MG still need to be better understood, and more research is needed. Long-term safety, cost of the medications, and optimal usage are some of the factors influencing the use of these newer agents and requiring their judicious

use in an appropriate clinical scenario. Overall, the FcRn blocking agents have an exciting and promising role in the management of MG.

Author contributions

ViB: writing, review, and editing. VeB: writing, review, editing and supervision. All authors contributed to the article and approved the submitted version.

Conflict of interest

VeB has been a consultant for: Grifols, CSL, UCB, Argenx, Takeda, Alnylam Octapharma, Pfizer, Powell Mansfield Inc., Akcea, Ionis Immunovant, Sanofi, Momenta (J&J), Roche, Janssen, AZ-Alexion, NovoNordisk, Japan tobacco. VeB had research support from: AZ-Alexion, Grifols, CSL, UCB, Argenx, Takeda, Octapharma, Akcea, Momenta (J&J), Immunovant, Ionis.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Lindstrom JM, Seybold ME, Lennon VA, Whittingham S, Duane DD. Antibody to acetylcholine receptor in myasthenia gravis: prevalence, clinical correlates, and diagnostic value. *Neurology*. (1976) 26:1054–4. doi: 10.1212/WNL.26.11.1054

2. Hoch W, McConville J, Helms S, Newsom-Davis J, Melms A, Vincent A. Auto-antibodies to the receptor tyrosine kinase MuSK in patients with myasthenia gravis without acetylcholine receptor antibodies. *Nat Med*. (2001) 7:365–8. doi: 10.1038/85520

3. Breiner A, Widdifield J, Katzberg HD, Barnett C, Bril V, Tu K. Epidemiology of myasthenia gravis in Ontario, Canada. *Neuromuscul Disord*. (2016) 26:41–6. doi: 10.1016/j.nmd.2015.10.009

4. Carr AS, Cardwell CR, McCarron PO, McConville J. A systematic review of population based epidemiological studies in myasthenia gravis. *BMC Neurol*. (2010) 10:46. doi: 10.1186/1471-2377-10-46

5. Menon D, Bril V. Pharmacotherapy of generalized myasthenia gravis with special emphasis on newer Biologicals. *Drugs*. (2022) 82:865–87. doi: 10.1007/s40265-022-01726-y

6. Katz B, Miledi R. Further observations on the distribution of acetylcholine-reactive sites in skeletal muscle. *J Physiol*. (1964) 170:379–88. doi: 10.1113/jphysiol.1964.sp007338

7. Wood SJ, Slater CR. Safety factor at the neuromuscular junction. *Prog Neurobiol*. (2001) 64:393–429. doi: 10.1016/S0301-0082(00)00055-1

8. Wang S, Breskovska I, Gandhy S, Punga AR, Guptill JT, Kaminski HJ. Advances in autoimmune myasthenia gravis management. *Expert Rev Neurother*. (2018) 18:573–88. doi: 10.1080/14737175.2018.1491310

9. Leite MI, Jacob S, Viegas S, Cossins J, Clover L, Morgan BP, et al. IgG1 antibodies to acetylcholine receptors in 'seronegative' myasthenia gravis†. *Brain*. (2008) 131:1940–52. doi: 10.1093/brain/awn092

10. Morgan BP, Chamberlain-Banoub J, Neal JW, Song W, Mizuno M, Harris CL. The membrane attack pathway of complement drives pathology in passively induced experimental autoimmune myasthenia gravis in mice. *Clin Exp Immunol*. (2006) 146:294–302. doi: 10.1111/j.1365-2249.2006.03205.x

11. Drachman DB, Angus CW, Adams RN, Michelson JD, Hoffman GJ. Myasthenic antibodies cross-link acetylcholine receptors to accelerate degradation. *N Engl J Med*. (1978) 298:1116–22. doi: 10.1056/NEJM197805182982004

12. Cole RN, Ghazanfari N, Ngo ST, Gervásio OL, Reddel SW, Phillips WD. Patient autoantibodies deplete postsynaptic muscle-specific kinase leading to disassembly of the ACh receptor scaffold and myasthenia gravis in mice: actions of MuSK autoantibodies in experimental myasthenia gravis. *J Physiol*. (2010) 588:3217–29. doi: 10.1113/jphysiol.2010.190298

13. Drachman DB, Adams RN, Josifek LF, Self SG. Functional activities of autoantibodies to acetylcholine receptors and the clinical severity of myasthenia gravis. *N Engl J Med*. (1982) 307:769–75. doi: 10.1056/NEJM198209233071301

14. Engel AG, Arahata K. The membrane attack complex of complement at the endplate in myasthenia gravis. *Ann N Y Acad Sci*. (1987) 505:326–32. doi: 10.1111/j.1749-6632.1987.tb51301.x

15. Skeie GO, Apostolski S, Evoli A, Gilhus NE, Illa I, Harms L, et al. Guidelines for treatment of autoimmune neuromuscular transmission disorders: autoimmune neuromuscular disorders. *Eur J Neurol*. (2010) 17:893–902. doi: 10.1111/j.1468-1331.2010.03019.x

16. McConville J, Farrugia ME, Beeson D, Kishore U, Metcalfe R, Newsom-Davis J, et al. Detection and characterization of MuSK antibodies in seronegative myasthenia gravis. *Ann Neurol*. (2004) 55:580–4. doi: 10.1002/ana.20061

17. Niks EH, Van Leeuwen Y, Leite MI, Dekker FW, Wintzen AR, Wirtz PW, et al. Clinical fluctuations in MuSK myasthenia gravis are related to antigen-specific IgG4 instead of IgG1. *J Neuroimmunol*. (2008) 195:151–6. doi: 10.1016/j.jneuroim.2008.01.013

18. Zisimopoulou P, Evangelakou P, Tzartos J, Lazaridis K, Zouvelou V, Mantegazza R, et al. A comprehensive analysis of the epidemiology and clinical characteristics of anti-LRP4 in myasthenia gravis. *J Autoimmun*. (2014) 52:139–45. doi: 10.1016/j.jaut.2013.12.004

19. Shen C, Lu Y, Zhang B, Figueiredo D, Bean J, Jung J, et al. Antibodies against low-density lipoprotein receptor–related protein 4 induce myasthenia gravis. *J Clin Invest*. (2013) 123:5190–202. doi: 10.1172/JCI66039

20. Rivner MH, Quarles BM, Pan J, Yu Z, Howard JF, Corse A, et al. Clinical features of LRP4 /agrin-antibody–positive myasthenia gravis: a multicenter study. *Muscle Nerve*. (2020) 62:333–43. doi: 10.1002/mus.26985

21. Motomura M, Narita MT. Autoantibodies in myasthenia gravis. *Shikei kenkyū no shinpo*. (2013) 65:433–9. doi: 10.11477/mf.1416101472

22. Pirskanen R. Genetic aspects in myasthenia gravis: a family study of 264 Finnish patients. *Acta Neurol Scand*. (2009) 56:365–88. doi: 10.1111/j.1600-0404.1977.tb01445.x

23. Ramanujam R, Pirskanen R, Ramanujam S, Hammarström L. Utilizing twins concordance rates to infer the predisposition to myasthenia gravis. *Twin Res Hum Genet*. (2011) 14:129–36. doi: 10.1375/twin.14.2.129

24. Nomura T, Sakaguchi S. Foxp3 and Aire in thymus-generated Treg cells: a link in self-tolerance. *Nat Immunol*. (2007) 8:333–4. doi: 10.1038/ni0407-333

25. Truffault F, De Montpreville V, Eymard B, Sharshar T, Le Panse R, Berrih-Aknin S. Thymic germinal centers and corticosteroids in myasthenia gravis: an Immunopathological study in 1035 cases and a critical review. *Clin Rev Allergy Immunol*. (2017) 52:108–24. doi: 10.1007/s12016-016-8558-3

26. Sánchez-Tejerina D, Sotoca J, Llaurado A, López-Diego V, Juntas-Morales R, Salvado M. New targeted agents in myasthenia gravis and future therapeutic strategies. *J Clin Med*. (2022) 11:6394. doi: 10.3390/jcm11216394

27. Dalakas MC. Immunotherapy in myasthenia gravis in the era of biologics. *Nat Rev Neurol*. (2019) 15:113–24. doi: 10.1038/s41582-018-0110-z

28. Thiruppathi M, Rowin J, Li Jiang Q, Sheng JR, Prabhakar BS, Meriggioli MN. Functional defect in regulatory T cells in myasthenia gravis. *Ann N Y Acad Sci*. (2012) 1274:68–76. doi: 10.1111/j.1749-6632.2012.06840.x

29. Menon D, Barnett C, Bril V. Novel treatments in myasthenia gravis. *Front Neurol*. (2020) 11:538. doi: 10.3389/fneur.2020.00538

30. Beekman R, Kuks JBM, Oosterhuis HJGH. Myasthenia gravis: diagnosis and follow-up of 100 consecutive patients. *J Neurol*. (1997) 244:112–8. doi: 10.1007/s004150050059

31. Mantegazza R, Beghi E, Pareyson D, Antozzi C, Peluchetti D, Sghirlanzoni A, et al. A multicentre follow-up study of 1152 patients with myasthenia gravis in Italy. *J Neurol*. (1990) 237:339–44. doi: 10.1007/BF00315656

32. Sanders DB, Wolfe GI, Benatar M, Evoli A, Gilhus NE, Illa I, et al. International consensus guidance for management of myasthenia gravis: executive summary. *Neurology*. (2016) 87:419–25. doi: 10.1212/WNL.0000000000002790

33. Ebadi H, Barth D, Bril V. Safety of plasma exchange therapy in patients with myasthenia gravis: PLEX in MG. *Muscle Nerve*. (2013) 47:510–4. doi: 10.1002/mus.23626

34. Barth D, Nabavi Nouri M, Ng E, Nwe P, Bril V. Comparison of IVIg and PLEX in patients with myasthenia gravis. *Neurology*. (2011) 76:2017–23. doi: 10.1212/WNL.0b013e31821e5505

35. Bril V, Barnett-Tapia C, Barth D, Katzberg HD. IVIG and PLEX in the treatment of myasthenia gravis: IVIG and PLEX in MG. *Ann N Y Acad Sci*. (2012) 1275:1–6. doi: 10.1111/j.1749-6632.2012.06767.x

36. Jensen P, Bril V. A comparison of the effectiveness of intravenous immunoglobulin and plasma exchange as preoperative therapy of myasthenia gravis. *J Clin Neuromuscul Dis*. (2008) 9:352–5. doi: 10.1097/CND.0b013e3181660807

37. Alcantara M, Sarpong E, Barnett C, Katzberg H, Bril V. Chronic immunoglobulin maintenance therapy in myasthenia gravis. *Eur J Neurol*. (2021) 28:639–46. doi: 10.1111/ene.14547

38. Guo Y, Tian X, Wang X, Xiao Z. Adverse effects of immunoglobulin therapy. *Front Immunol*. (2018) 9:1299. doi: 10.3389/fimmu.2018.01299

39. Pinching A. Remission of myasthenia gravis following plasma-exchange. *Lancet*. (1976) 308:1373–6. doi: 10.1016/S0140-6736(76)91917-6

40. Guptill JT, Juel VC, Massey JM, Anderson AC, Chopra M, Yi JS, et al. Effect of therapeutic plasma exchange on immunoglobulins in myasthenia gravis. *Autoimmunity*. (2016) 49:472–9. doi: 10.1080/08916934.2016.1214823

41. Raghavan M, Bjorkman PJ. Fc receptors and their interaction with immunoglobulins. *Annu Rev Cell Dev Biol*. (1996) 12:181–220. doi: 10.1146/annurev.cellbio.12.1.181

42. Amzel LM, Poljak RJ. Three-dimensional structure of immunoglobulins. *Annu Rev Biochem*. (1979) 48:961–97. doi: 10.1146/annurev.bi.48.070179.004525

43. Davies DR, Metzger H. Structural basis of antibody function. *Annu Rev Immunol*. (1983) 1:87–115. doi: 10.1146/annurev.iy.01.040183.000511

44. Wilson IA, Stanfield RL. Antibody-antigen interactions: new structures and new conformational changes. *Curr Opin Struct Biol*. (1994) 4:857–67. doi: 10.1016/0959-440X(94)90267-4

45. Rouard H, Tamasdan S, Moncuit J, Moutel S, Michon J, Fridman WH, et al. Fc receptors as targets for immunotherapy. *Int Rev Immunol*. (1997) 16:147–85. doi: 10.3109/08830189709045707

46. Taylor RP, Lindorfer MA. Fcγ-receptor–mediated trogocytosis impacts mAb-based therapies: historical precedence and recent developments. *Blood*. (2015) 125:762–6. doi: 10.1182/blood-2014-10-569244

47. Nimmerjahn F, Ravetch JV. Fcγ receptors as regulators of immune responses. *Nat Rev Immunol*. (2008) 8:34–47. doi: 10.1038/nri2206

48. Raghavan M, Bjorkman PJ. Fc receptors and their interactions with immunoglobulins. *Annu Rev Cell Dev Biol*. (1996) 12:181–220. doi: 10.1146/annurev.cellbio.12.1.181

49. Chiu ML, Goulet DR, Teplyakov A, Gilliland GL. Antibody structure and function: the basis for engineering therapeutics. *Antibodies*. (2019) 8:55. doi: 10.3390/antib8040055

50. Hornby PJ, Cooper PR, Kliwinski C, Ragwan E, Mabus JR, Harman B, et al. Human and non-human primate intestinal FcRn expression and immunoglobulin G transcytosis. *Pharm Res*. (2014) 31:908–22. doi: 10.1007/s11095-013-1212-3

51. Lozano NA, Lozano A, Marini V, Saranz RJ, Blumberg RS, Baker K, et al. Expression of FcRn receptor in placental tissue and its relationship with IgG levels in term and preterm newborns. *Am J Reprod Immunol*. (2018) 80:e12972. doi: 10.1111/aji.12972

52. Haymann JP, Levraud JP, Bouet S, Kappes V, Hagège J, Nguyen G, et al. Characterization and localization of the neonatal fc receptor in adult human kidney. *J Am Soc Nephrol*. (2000) 11:632–9. doi: 10.1681/ASN.V114632

53. Blumberg RS, Koss T, Story CM, Barisani D, Polischuk J, Lipin A, et al. A major histocompatibility complex class I-related fc receptor for IgG on rat hepatocytes. *J Clin Invest*. (1995) 95:2397–402. doi: 10.1172/JCI117934

54. Baldwin WM, Valujskikh A, Fairchild RL. The neonatal fc receptor: key to homeostasic control of IgG and IgG-related biopharmaceuticals. *Am J Transplant*. (2019) 19:1881–7. doi: 10.1111/ajt.15366

55. Chaudhury C, Brooks CL, Carter DC, Robinson JM, Anderson CL. Albumin binding to FcRn: distinct from the FcRn−IgG interaction. *Biochemistry*. (2006) 45:4983–90. doi: 10.1021/bi052628y

56. Ghetie V, Hubbard JG, Kim JK, Tsen MF, Lee Y, Ward ES. Abnormally short serum half-lives of IgG in β2-microglobulin-deficient mice. *Eur J Immunol*. (1996) 26:690–6. doi: 10.1002/eji.1830260327

57. Junghans RP, Anderson CL. The protection receptor for IgG catabolism is the beta2-microglobulin-containing neonatal intestinal transport receptor. *Proc Natl Acad Sci*. (1996) 93:5512–6. doi: 10.1073/pnas.93.11.5512

58. Roopenian DC, Akilesh S. FcRn: the neonatal fc receptor comes of age. *Nat Rev Immunol*. (2007) 7:715–25. doi: 10.1038/nri2155

59. Liu L, Garcia AM, Santoro H, Zhang Y, McDonnell K, Dumont J, et al. Amelioration of experimental autoimmune myasthenia gravis in rats by neonatal FcR blockade. *J Immunol*. (2007) 178:5390–8. doi: 10.4049/jimmunol.178.8.5390

60. Sesarman A, Sitaru AG, Olaru F, Zillikens D, Sitaru C. Neonatal fc receptor deficiency protects from tissue injury in experimental epidermolysis bullosa acquisita. *J Mol Med*. (2008) 86:951–9. doi: 10.1007/s00109-008-0366-7

61. Ulrichts P, Guglietta A, Dreier T, van Bragt T, Hanssens V, Hofman E, et al. Neonatal fc receptor antagonist efgartigimod safely and sustainably reduces IgGs in humans. *J Clin Invest*. (2018) 128:4372–86. doi: 10.1172/JCI97911

62. Howard JF, Bril V, Burns TM, Mantegazza R, Bilinska M, Szczudlik A, et al. Randomized phase 2 study of FcRn antagonist efgartigimod in generalized myasthenia gravis. *Neurology*. (2019) 92:e2661–73. doi: 10.1212/WNL.0000000000007600

63. Howard JF, Bril V, Vu T, Karam C, Peric S, Margania T, et al. Safety, efficacy, and tolerability of efgartigimod in patients with generalised myasthenia gravis (ADAPT): a multicentre, randomised, placebo-controlled, phase 3 trial. *Lancet Neurol*. (2021) 20:526–36. doi: 10.1016/S1474-4422(21)00159-9

64. Howard J, Bril V, Vu T, Karam C, Peric S, De Bleecker J, et al. Long-term safety and efficacy of Efgartigimod in patients with generalized myasthenia gravis: interim results of the ADAPT+ study. *Neurology*. (2022) 99:S37–8. doi: 10.1212/01.wnl.0000903308.81107.e2

65. Smith B, Kiessling A, Lledo-Garcia R, Dixon KL, Christodoulou L, Catley MC, et al. Generation and characterization of a high affinity anti-human FcRn antibody, rozanolixizumab, and the effects of different molecular formats on the reduction of plasma IgG concentration. *MAbs*. (2018) 10:1111–1130. doi: 10.1080/19420862.2018.1505464

66. Kiessling P, Lledo-Garcia R, Watanabe S, Langdon G, Tran D, Bari M, et al. The FcRn inhibitor rozanolixizumab reduces human serum IgG concentration: a randomized phase 1 study. *Sci Transl Med*. (2017) 9:eaan1208. doi: 10.1126/scitranslmed.aan1208

67. Bril V, Benatar M, Andersen H, Vissing J, Brock M, Greve B, et al. Efficacy and safety of rozanolixizumab in moderate-to-severe generalised myasthenia gravis: a phase 2 RCT. *Neurology*. (2020). doi: 10.1212/WNL.0000000000011108

68. Bril V, Drużdż A, Grosskreutz J, Habib AA, Mantegazza R, Sacconi S, et al. Safety and efficacy of rozanolixizumab in patients with generalised myasthenia gravis (MycarinG): a randomised, double-blind, placebo-controlled, adaptive phase 3 study. *Lancet Neurol*. (2023) 22:383–94.

69. UCB announces positive Phase 3 results for rozanolixizumab in generalized myasthenia gravis | UCB [Internet]. Available at: https:// www. ucb. com/ stori es-media/ Press- Relea ses/ artic le/ UCB- annou nces- posit ive- Phase-3- resul ts- for-rozan olixi zumabin- generalized- myast henia- gravis

70. Roy S, Nanovskaya T, Patrikeeva S, Cochran E, Parge V, Guess J, et al. M281, an anti-FcRn antibody, inhibits IgG transfer in a human ex vivo placental perfusion model. *Am J Obstet Gynecol*. (2019) 220:498.e1–9. doi: 10.1016/j.ajog.2019.02.058

71. Ling LE, Hillson JL, Tiessen RG, Bosje T, Iersel MP, Nix DJ, et al. M281, an anti-FcRn antibody: pharmacodynamics, pharmacokinetics, and safety across the full range of IgG reduction in a first-in-human study. *Clin Pharmacol Ther*. (2019) 105:1031–9. doi: 10.1002/cpt.1276

72. Castleman JS, Moise KJ, Kilby MD. Medical therapy to attenuate fetal anaemia in severe maternal red cell alloimmunisation. *Br J Haematol*. (2021) 192:425–32. doi: 10.1111/bjh.17041

73. Vivacity-MG. A phase 2, multicenter, randomized, double-blind, placebo-controlled study to evaluate the safety, tolerability, efficacy, pharmacokinetics, pharmacodynamics, and immunogenicity of Nipocalimab administered to adults with generalized myasthenia gravis (2157)Jeffrey Guptill, Carlo Antozzi, Vera Bril, Josep Gamez, Sven G. Meuth, Jose Luis Muñoz Blanco, RichardJ. Nowak, Dianna Quan, Teresa Sevilla, Andrzej Szczudlik, Brooke Hegart, Marie-Helene Jouvin, Jim Jin. *Santiago Arroyo Neurol*. (2021) 96:2157.

74. Janssen Research & Development, LLC. *Phase 3, multicenter, randomized, double-blind, placebo-controlled study to evaluate the efficacy, safety, pharmacokinetics, and pharmacodynamics of Nipocalimab administered to adults with generalized myasthenia gravis*. clinicaltrials.gov; (2021). Report No.: NCT04951622.

75. Collins J, Jones L, Snyder M, Sicard E, Griffin P, Webster L, et al. *RVT-1401, a novel anti-FcRn monoclonal antibody, is well tolerated in healthy subjects and reduces plasma IgG following subcutaneous or intravenous administration (P 5.2–079)*. (2019); Available at: https://www.semanticscholar.org/paper/RVT-1401%2C-A-Novel-Anti-FcRn-Monoc lonal- Antib ody% 2C-Is-Colli ns- Jones/33910 16595 f96d3 dc5e8 6bddd c670e 36578 eb1a9

76. Yap DYH, Hai J, Lee PCH, Zhou X, Lee M, Zhang Y, et al. Safety, tolerability, pharmacokinetics, and pharmacodynamics of HBM9161, a novel FcRn inhibitor, in a phase I study for healthy Chinese volunteers. *Clin Transl Sci*. (2021) 14:1769–79. doi: 10.1111/cts.13019

77. Immunovant Sciences GmbH. *A phase 2a, multicenter, randomized, double-blind, placebo-controlled study with an open-label extension of RVT-1401 in myasthenia gravis patients*. clinicaltrials.gov; (2021). Report No.: NCT03863080. Available at: https://clinicaltrials.gov/ct2/show/NCT03863086

78. Harbour Bio Med (Guangzhou) Co. Ltd. *A multicenter, randomized, double-blind, placebo-controlled study to evaluate the efficacy, safety and Pharmacodynamic and pharmacokinetic of HBM9161 (HL161) subcutaneous injection in patients with generalized myasthenia gravis*. clinicaltrials. gov; (2021). Report No.: NCT04346888. Available at: https://clinicaltrials.gov/ct2/show/NCT04346888

79. https://clinicaltrials.gov/ct2/show/NCT05403541

80. https://www.affibody.se/affibody-announces-termination-of-aby-039-fcrn-program

frontiers | Frontiers in Neurology

TYPE Review
PUBLISHED 02 October 2023
DOI 10.3389/fneur.2023.1275533

OPEN ACCESS

EDITED BY
Ernestina Santos,
University Hospital Center of Porto, Portugal

REVIEWED BY
Yuwei Da,
Capital Medical University, China
Paolo Emilio Alboini,
Home for Relief of Suffering (IRCCS), Italy

*CORRESPONDENCE
Elena Cortés-Vicente
✉ ecortes@santpau.cat

RECEIVED 10 August 2023
ACCEPTED 11 September 2023
PUBLISHED 02 October 2023

CITATION
Vesperinas-Castro A and Cortés-Vicente E (2023) Rituximab treatment in myasthenia gravis.
Front. Neurol. 14:1275533.
doi: 10.3389/fneur.2023.1275533

Rituximab treatment in myasthenia gravis

Ana Vesperinas-Castro[1,2,3,4] and Elena Cortés-Vicente[1,2,3,4]*

[1]Neuromuscular Diseases Unit, Department of Neurology, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, [2]Department of Medicine, Universitat Autònoma de Barcelona, Barcelona, Spain, [3]Biomedical Research Institute Sant Pau (IIB Sant Pau), Barcelona, Spain, [4]Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Instituto de Salud Carlos III, Madrid, Spain

Myasthenia gravis (MG) is a chronic autoimmune disease mediated by antibodies against post-synaptic proteins of the neuromuscular junction. Up to 10%–30% of patients are refractory to conventional treatments. For these patients, rituximab has been used off-label in the recent decades. Rituximab is a monoclonal antibody against the CD20 protein that leads to B cell depletion and to the synthesis of new antibody-secreting plasma cells. Although rituximab was created to treat B-cell lymphoma, its use has widely increased to treat autoimmune diseases. In MG, the benefit of rituximab treatment in MuSK-positive patients seems clear, but a high variability in the results of observational studies and even clinical trials has been reported for AChR-positive patients. Moreover, few evidence has been reported in seronegative MG and juvenile MG and some questions about regimen of administration or monitoring strategies, remains open. In this review, we intend to revise the available literature on this topic and resume the current evidence of effectiveness of Rituximab in MG, with special attention to results on every MG subtype, as well as the administration protocols, monitoring strategies and safety profile of the drug.

KEYWORDS

myasthenia (myasthenia gravis—MG), Rituximab, refractory patients, efficacy and safety, B-cell depletion

1. Introduction

Myasthenia Gravis (MG) is a chronic autoimmune disease mediated by antibodies against the acetylcholine receptor (AChR), Muscle-Specific Kinase (MuSK) or other proteins in the neuromuscular junction such as Low-Density Lipoprotein Receptor Related Protein-4 (LRP4). There is a 10%–15% of patients without detectable antibodies in serum, named seronegative MG (1).

The main symptom is weakness, which characteristically get worse with sustained exercise and can affect extraocular, bulbar, limb, and axial muscles. Fifteen percent of patients have only ocular symptoms whereas most of them have a generalized presentation. Respiratory muscles can occur times, with the subsequent need for ventilatory support. This life-threatening situation is called myasthenic crisis and implies a mortality risk of 5%–12% (1–3).

MG therapeutic approach usually includes a combination of symptomatic treatment with acetylcholinesterase inhibitors which increase acetylcholine levels at the neuromuscular junction, thymectomy in selected patients and long-term immunosuppressive medications, with a wide range of options which goes from conventional agents to new immunomodulatory therapies (4, 5). Corticosteroids remain the first-line treatment but long-term use is limited by the burden of adverse events. Immunosuppressants, such as

Azathioprine, Mycophenolate Mofetil, Cyclosporine or Tacrolimus are essential for reducing prednisone to the lowest possible dose and prevent relapses.

Biologic therapies have emerged in the last decades with a highly selective target and better security profile than classic immunosuppressants. Rituximab leads to depletion of B cells and its use remains off-label. Two recently developed treatment strategies are complement blockade (such as Eculizumab, Ravulizumab, and Zilucoplan) and neonatal Fc receptor (FcRn) antagonism (Efgartigimod and Rozanolixizumab) (5, 6).

Thanks to all these therapeutic advances, MG prognosis has markedly improved in the last decades. A multicenter study in Norwegian population did not find any increased mortality in patients with MG compared with controls (7).

Despite this, most patients do not reach complete clinical remission, and they do persist symptomatic or they need lifelong immunosuppressing treatments to control the disease. Moreover, there are a 10%–30% of patients with refractory MG, which do not respond to therapies (2, 8).

Rituximab (RTX) has been postulated as a therapeutic option in refractory MG. However, there are some controversies in literature and unresolved questions, such as its efficacy in every serological group, regimen of administration or monitoring to decide retreatment. Recently, several studies including two clinical trials have been published, providing new evidence to this matter.

In this paper, we aim to review the current evidence of the use of Rituximab in MG, with special attention to the serological subtypes.

2. Mechanism of action

Rituximab is a human/murine chimeric monoclonal antibody against the CD20 protein, administered via intravenous infusion (9).

It is a molecule composed of the CD20-recognizing regions of murine origin, fused to the constant region of the heavy chain of human IgG1 and human kappa light chain.

CD20 is a glycosylated transmembrane phosphoprotein present on the surface of developing B lymphocyte cells, while progenitor cells and mature plasma cells do not express this marker. Although its cellular function is not fully understood, it is believed to participate in cellular development and activation processes through the regulation of transmembrane calcium flux (10).

Its limited expression in intermediate stages of B cell maturation, but not in progenitor or mature cells or other normal cell lines, makes CD20 an effective and safe potential therapeutic target without permanent side effects.

The binding of the monoclonal antibody to the CD20 receptor induces cell death through four different mechanisms, three of which are dependent on the patient's immune system: antibody-dependent cellular cytotoxicity through the activation of NK cells, complement-dependent cytotoxicity through cascade activation and, finally, membrane attack complex-dependent and antibody-dependent phagocytosis through macrophage activation. The last mechanism is independent of the immune system and is based on the activation of intracellular mechanisms such as the caspase pathway and lysosomal activation, leading to cell apoptosis (9, 10).

This leads to depletion of circulating B lineage cells and, therefore, the synthesis of new antibody-secreting plasma cells.

Despite the main function of B lymphocytes being the production of antibodies, in recent years, other functions of these cells have been recognized. On one hand, B cells play a role as antigen-presenting cells through the major histocompatibility complex type II to CD4+ Th lymphocytes, participating in their activation alongside dendritic cells. Another important function is cytokine secretion. Regulatory B cells are a subset of B cells that contribute to inflammation control by secreting IL-10, promoting the differentiation of CD4 T lymphocytes into regulatory T cells. Various studies have demonstrated the benefit of B cell depletion therapies in autoimmune diseases mediated by self-reactive T cells (11–13).

3. Drug history and indications

Rituximab is the first monoclonal antibody implemented in oncology and remains the most widely used to this day. It was created by Ronald Levy with the goal of targeting malignant B cells, and in 1982, the first case of a successfully treated cancer patient with this antibody was published. In 1994, the first phase I clinical trial of rituximab was conducted in patients with Non-Hodgkin's lymphoma (10). This led to FDA approval in the United States in 1997 for the treatment of Non-Hodgkin's lymphoma. Since then, it has been approved for other indications such as rheumatoid arthritis (2006), Wegener's granulomatosis, microscopic polyangiitis (2011), chronic lymphocytic leukemia (2017), and pemphigus vulgaris (2018) (14).

However, Rituximab is used off-label for numerous indications. In a retrospective study conducted in the United States that reviewed Rituximab administration indications, an increase in off-label indications was observed from 1.2% in 2009 to 55.6% in 2017 (15).

In this same study, the main off-label indication was neurological diseases, including multiple sclerosis, other inflammatory CNS diseases, neuropathies such as CIDP, Stiff-Person syndrome, refractory MG, among others (15, 16).

4. Use of rituximab in myasthenia gravis

The first reported case of Rituximab use in MG was in 1999, in a 27-year-old patient who developed refractory myasthenia gravis after a hematopoietic stem cell transplant in the context of acute non-lymphocytic leukemia. The patient experienced improvement in myasthenia gravis, supporting the efficacy of Rituximab in other autoimmune disorders mediated by autoantibodies (17). Since then, the use of rituximab in MG has been widely extended to patients with refractory disease (2, 18) and various articles have reported its efficacy in up to 50%–84%, depending on the report (5, 19–21).

Several series of cases were reported at the beginning of the 2000s. In 2008, Isabel Illa et al. published an observational study including their experience with 6 patients with refractory MG (22). In the next years, several retrospective and prospective studies came out, including a wider number of patients (20, 23–25). In 2014 was published the first systematic review (19) and other meta-analysis have appeared later (26, 27). Recently, in 2022 two clinical trials have been published to increase evidence about this subject.

Table 1 resumes the main characteristics of previous studies.

TABLE 1 Resume of characteristics of previous studies.

References	Sample size (AChR/ Musk/SNMG)	Follow-up after RTX, mean, months	RTX regimen	Primary outcome	Result
Clinical trials					
Nowa et al. (28)	52 (52/0/0)	13	2 cycles of 375 mg/m^2 × 4 weeks every 6 months.	Steroid-sparing effect.	Futility (60% with RTX vs. 56% with placebo).
Piehl et al. (29)	47 (45/0/2)	12	Single infusion of 500 mg.	Minimal disease manifestations (QMG < 4 or prednisolone < 10 mg/day).	Positive (71% with RTX vs. 29% in placebo arm).
Meta-analysis					
Feng et al. (30)	196 (138/43/15)	Variable among studies.	Low dose (375 mg/m^2 twice a month) vs. high dose (any other regimen).	Change in QMG.	Reduction of QMG: 4.16. No difference between Rituximab and Eculizumab.
Zhao et al. (27)	417 (242/155/20)	Variable among studies.	Most patients received 375 mg/m^2 × 4 weeks or 1 g × 2 weeks. Variable regimens in the rest of patients.	Proportion of patients achieving MMs and change in QMG.	64% achieving MMS or better. Mean reduction of QMG 1.55.
Tandan et al. (26)	169 (99/57/7)	Variable among studies.	Most patients received 375 mg/m^2 × 4 weeks or 500 mg × 2 weeks. Variable regimens in the rest of patients.	Proportion of patients achieving MMs.	70% between MuSK-positive patients but 30% between AChR.
Iorio et al. (19)	168 (91/70/7)	Variable among studies.	Most patients received 375 mg/m^2 × 4 weeks or 500 mg × 2 weeks or 1 g × 2 weeks. Variable regimens in the rest of patients	Change in MGFA-PIS.	Response in 83.9%.
Observational studies					
Nelke et al. (3)	56	24	1 g × 2 weeks.	Compare the change in QMG after treatment with RTX and eculizumab	Greater benefit with eculizumab.
Li et al. (31)	19 (19/0/0)	51.3 (30.3–72.5)	Based on CD19 count at baseline and repopulation.	Change in QMG score.	Positive (median QMG decreased from 18 to 11).
Fatehi et al. (32)	34 (17/9/8)	12	1 g × 2 weeks and reinfusions every 6 months.	Change in average score on MGC, MGQoL-15, MGFA and MG-ADL. Change in prednisolone and pyridostigmine doses.	Improvement in MG-QoL and MGC. Reductions in the average daily dose of both drugs.
Zhou et al. (33)	12 (0/12/0)	6	600 mg single infusion.	Change in QMG, MGC, MMT, MG-ADL y MG QOL-15.	Decrease in all scales.
Martínez-Monte et al. (34)	20 (16/2/2)	31,7 (15,2)	NA	Clinical response (complete/partial/absence).	75% of response (60% complete).
Doughty et al. (35)	40 (28/9/3)	12	375 mg/m^2 × 4 weeks or 1 g × 2 weeks.	Proportion of patients reaching a "Improved" or better in MGFA-PIS.	76.9% achieved primary endpoint.
Litchman et al. (36)	33 (17/16/0)	62.1 (31.8)	375 mg/m^2 × 4 weeks.	Change in median MFGA and proportion of patients achieving MMs or better at 12 months and last visit.	MGFA change from II to 0 and MMs or better was attained in 64.7% (AChR+) and 75% (MuSK+).
Choi et al. (37)	17 (9/6/2)	24.5 (11.3)	375 mg/m^2 × 2 weeks.	Achieving MMs or better in MGFA-PIS with prednisolone dose ⩽5 mg/day.	65% achieved primary endpoint.

(Continued)

10.3389/fneur.2023.1275533

TABLE 1 (Continued)

References	Sample size (AChR/ Musk/SNMG)	Follow-up after RTX, mean, months	RTX regimen	Primary outcome	Result
Lu et al. (38)	12 (12/0/0)	18	600 mg every 6 months.	Change in QMG.	Decreased from 18.25 ± 4.03 to 8.42 ± 3.99.
Topakian et al. (39)	56 (39/14/3)	20 (10–53.5)	Most patients received 375 mg/m^2 × 2 weeks or 500 mg × 2 weeks. Variable regimens in the rest of patients	MGFA-PIS.	MMs or better 67.9% at last follow-up.
Roda et al. (40)	27 (10/13/4)	NA	375 mg/m^2 × 4 weeks or 1 g × 2 weeks.	(1) Efficacy of stopping conventional immunosuppressants. (2) Reduction in steroids daily dose. (3) Improvement in MGFA-PIS.	(1) Discontinuation in all the cohort. (2) Decreased from an average of 19.9 to 10.2 mg/day. (3) MMs or better in 55.5%.
Beecher et al. (25)	22 (10/9/3)	28.8 (19.0)	375 mg/m^2 × 4 weeks + two reinfusions of 750 mg within 2 months.	Change in the MMT.	Mean reduction in MMT score from 10.3 to 3.3.
Landon-Cardinal et al. (41)	11 (11/0/0)	18	1 g × 2 weeks.	Improvement of at least 20-points on the MMS at 12 months.	Only one patient (9%) achieved primary endpoint.
Cortes-Vicente et al. (42)	25 (0/25/0)	60 (39.6)	375 mg/m^2 × 4 weeks or 375 mg/m^2 × 4 weeks + two reinfusions monthly or 1 g × 2 weeks.	Proportion of patients achieving MMs or better in MGFA-PIS.	Primary endpoint achieved in 100%.
Afanasiev et al. (21)	28 (21/3/4)	27.2 (16.6)	375 mg/m^2 × 4 weeks or 1 g × 2 weeks.	Change in MGFA-PIS.	50% achieved Improved Status.
Hehir et al. (24)	24 (0/24/0)	45 (6~116)	375 mg/m^2 × 4 weeks.	MGSTI score.	58% achieved MGSTI level 2 or better.
Robeson et al. (43)	16 (16/0/0)	56.1 (20.1)	375 mg/m^2 × 4 weeks.	MGFA-PIS.	63% achieved CSR; 19% Pharmacological Remission; 19% MMs.
Blum et al. (44)	14 (11/3/0)	14.4 (11.3)	500 mg × 2 weeks.	Change in MGFA-PIS.	78.5% achieved Improved or better.
Maddison et al. (45)	10 (7/3/0)	12 ~ 48	375 mg/m^2 × 4 weeks.	MGFA-PIS.	25% of CSR and 33% of MMs or Improved.
Díaz-Manera et al. (46)	17 (11/6/0)	31	375 mg/m^2 × 4 weeks.	MGFA-PIS.	100% of MuSK+ achieved MMs. 90.9% of AChR+ achieved Improved.
Collongues et al. (23)	13 (8/3/2)	26 (13)	375 mg/m^2 × 4 weeks or 1 g × 2 weeks.	ARR and MGFA scores.	Decrease ARR from 2.1 to 0.3 and lower MGFA scores in both refractory and non-refractory patients.
Nowak et al. (20)	14 (6/8/0)	12	375 mg/m^2 × 4 weeks.	Change in corticosteroids dose.	Prednisone dose decreased a mean of 93.8% after cycle 3 of RTX.

SNMG, Seronegative myasthenia gravis; RTX, Rituximab; QMG, Quantitative myasthenia gravis; MMs, Minimal manifestations status; MGFA-PIS, Myasthenia Gravis Foundation of America—Postinterventional Status; MGC, Myasthenia gravis composite; MGQoL-15, Myasthenia gravis quality of life 15; MGFA, Myasthenia Gravis Foundation of America; MG-ADL, Myasthenia gravis activities of daily living; MMT, Manual muscle testing; MMS, Myasthenic muscle score; MGSTI, Myasthenia gravis status and treatment intensity; ARR, Annualized relapse rate; CSR, Complete stable remission.

10.3389/fneur.2023.1275533

However, all these studies have often reported contradictory results and differences among different subgroups of autoantibodies. Other questions such as the adequate dosage, monitoring strategies, re-infusion regimen or long-term security profile remain also unclear.

4.1. Rituximab treatment in patients with anti-AChR antibodies

4.1.1. Evidence of use

During the decade of 2010, several observational studies were published reporting good responses of patients with AChR-positive MG patients and reductions in the corticosteroid doses after treatment with Rituximab (21–23). Although those studies pointed toward a treatment benefit, the evaluated outcomes and population baseline characteristics were different between studies and results exhibited high variability in the degree of improvement.

In a single-center study of patients with refractory MG treated with Rituximab published in 2017, the impact on patients' quality of life and the difference in annual healthcare costs per patient compared to the year before rituximab initiation were analyzed, showing a favorable cost-effectiveness balance (47).

In the same year, a meta-analysis of the evidence on the use of Rituximab in MG was published, including a total of 169 patients. Among patients with positive anti-AChR antibodies, it was observed that 30% of patients achieved a status of minimal manifestations or better on the Myasthenia Gravis Foundation of America—Post Interventional Status score (MGFA-PIS) and a 46% reduction in the Quantitative Myasthenia Gravis score (QMG) (26).

Two new meta-analyses have been published in recent years, including patients with refractory MG and positive anti-AChR antibodies treated with rituximab. Both showed a proportion of patients reaching a state of minimal manifestations or better on the MGFA-PIS scale after Rituximab treatment of 51% (27) and 54% (48).

However, in 2018, the first randomized, double-blind, placebo-controlled clinical trial in MG patients with anti-AChR antibodies treated with Rituximab (BEAT-MG) was conducted and published in 2022. A non-inferiority design was used, with the primary efficacy objectives being the reduction in daily corticosteroid dose and the score on the MGC scale. This objective was achieved by 60% of the patients, a rate of benefit which is consistent with the results of previous studies. However, there was a high percentage of patients in the placebo group who also achieved this objective (56%), so the study did not show statistically significant differences between placebo and Rituximab in these patients. Moreover, no differences were found in quantitative scales such as QMG or MGC scores (49).

On the other hand, at the end of 2022, the results of the RINOMAX trial were published, a randomized, double-blind, placebo-controlled study in which 47 patients (45 of whom were positive for anti-AChR antibodies, and only two were MuSK-positive) were randomized to receive Rituximab vs. placebo. The primary objective was the proportion of patients with minimal manifestations, defined as QMG < 4 with a prednisolone dose of less than 10 mg/day. This was achieved by 71% of patients with rituximab compared to 29% of patients with placebo. A lower need for hospitalizations and rescue treatments with immunoglobulin or plasmapheresis in the rituximab group, as well as a lower corticosteroid dose at the end of the study in the rituximab group were also found. However, the study did not meet the secondary objectives of reducing QMG and MG-ADL scores, although patients who received rescue treatments during the study were excluded from the analysis, which had a greater impact on the placebo group, affecting the power to detect differences between arms.

Another limitation of this trial was an imbalance in important characteristics of the baseline populations in the two arms. Patients in placebo group were younger, had higher titers of antibodies and were taking lower doses of oral corticosteroids. More patients were classified as MGFA III in the placebo group whereas in Rituximab most of them were classified as II. Although predictive factors of response to treatment remain unknown, those are important characteristics which might had impacted on results.

In a recent meta-analysis published in 2023, including clinical trials of new therapies for myasthenia gravis, rituximab was the only one of the three analyzed strategies (anti-CD20, anti-FcRn, and anti-complement) that did not show statistically significant differences compared to placebo in reducing scores on the MG-ADL, MGC, QMG, and patients' quality of life. However, as the authors themselves noted, none of these scales were the primary outcome of the trials and the number of recruited patients was significantly lower than for the rest of therapies, giving place to large CI (6).

This discordance among the results of different observational studies, clinical trials, and meta-analyses can be attributed to significant heterogeneity in the methods and conduct of the studies, with considerable variability in the analyzed variables as objectives, which complicates comparisons. This encourages the need to conduct new clinical trials with a uniform methodology, where primary objectives were common, and baseline characteristics of the study population were carefully recorded.

4.1.2. Recommendation of use

In the latest international consensus guidelines for the management of MG updated in 2021, the efficacy of rituximab in patients with anti-AChR antibodies is still considered uncertain, although its use is considered an acceptable alternative in refractory cases (50).

4.2. Rituximab treatment in patients with anti-MuSK antibodies

4.2.1. Evidence of use

Patients with anti-MuSK antibodies constitute approximately 5% of all MG cases (2). These patients have been associated with a bulbar phenotype of the disease and with increased severity and refractoriness to other immunosuppressive treatments and intravenous immunoglobulin. This has been highlighted in studies showing a higher proportion of patients in the MuSK positive group requiring second-line immunosuppressive therapies such as Rituximab (22).

To the best of our knowledge, in 2006 was reported the first case of MuSK-positive MG patient successfully treated with Rituximab (51). Isabel Illa et al. published a series of 6 patients treated with Rituximab, highlighting a differential response between patients with anti-AChR antibodies and MuSK-positive, with greater and more sustained responses in this last group (22). In the light of these findings, the same group conducted a retrospective study comparing the results of Rituximab treatment between different serologic groups

in 17 MG patients, confirming the better and lasted longer response in the MuSK-positive group (46).

In 2014, the first meta-analysis on the use of Rituximab in patients with MG was published. However, in this article, although the authors point to better outcomes among MuSK-positive patients, differences were minimal (88.8% improvement in MGFA-PIS vs. 85.6% in anti-AChR) and did not reach statistical significance (19). Subsequent studies have supported this evidence, finding greater differences between the two groups, suggesting that MuSK-positive patients benefit more from rituximab therapy, showing a faster response, tolerate a greater reduction in oral immunosuppressants and corticosteroids, and take longer to relapse (25, 27, 36, 52).

Also, in a multicenter retrospective study of all patients treated with Rituximab in the healthcare system of Austria, MuSK positivity was identified as the only independent variable associated with response to Rituximab (39). This finding was subsequently confirmed in another similar meta-analysis (26).

Despite the lack of clinical trials specifically focusing on the use of rituximab in MuSK positive patients (only 2 patients were included in RINOMAX), evidence from observational studies supports the same line of findings. In a prospective multicenter, double-blind study comparing MuSK-positive MG patients treated with rituximab vs. other conventional immunosuppressants, better responses and lower corticosteroid doses were demonstrated in the intravenous treatment arm (24).

4.2.2. Recommendation of use

Currently, international consensus guidelines for the management of MG consider Rituximab as an early-line treatment in MuSK-positive patients who have failed first-line immunotherapy (50).

4.3. Rituximab treatment in seronegative patients

4.3.1. Evidence of use

The evidence for the use of Rituximab in seronegative Myasthenia gravis (MG) patients is scarce and limited to the inclusion of a small number of patients in observational studies.

In 2015 and subsequently in 2017, two meta-analyses were published that included 4 and 7 seronegative patients, respectively. While the first meta-analysis showed an 85.6% improvement in MGFA-PIS scale, the second meta-analysis reported improvement in only one patient (19, 26). In 2018, the results of a prospective study on the use of rituximab in refractory MG were published, which included three seronegative patients. The study did not demonstrate improvement in the primary outcome (reduction in Manual Muscle Testing score), although one of the three patients was able to discontinue corticosteroid therapy (25). In 2019, another retrospective study included 4 seronegative patients, of whom 3 showed Improvement status on MGFA-PIS after treatment with Rituximab. In all cases, corticosteroid-sparing therapy could be discontinued, and the prednisone dose was reduced in two patients (40). However, in the same year, another retrospective study based on the Austrian healthcare population did not show clinical remission in any of the three seronegative patients included in the study and treated with Rituximab (39). Finally, the study with the largest number of seronegative patients included is a meta-analysis published in 2021, which included 20 seronegative patients treated with Rituximab. 40% of these patients achieved a status of minimal manifestations or better, compared to 51% in anti-AChR patients and 79% in MuSK-positive patients. The reduction in the mean daily corticosteroid dose was also lower in this subgroup, although withdrawal of other immunosuppressive agents was observed in up to 91% of patients (27).

4.3.2. Recommendation of use

The data to date is limited and contradictory in the literature. Although it is not possible to establish an evidence-based recommendation for the use of rituximab in these patients, there are reported cases of improvement in seronegative patients with refractory MG, which supports the use of rituximab in selected cases.

4.4. Rituximab treatment in juvenile myasthenia gravis

Juvenile Myasthenia Gravis (JMG) is defined as the onset of the disease in patients younger than 18 years old. It is a rare condition with an incidence of around 1.5 patients per million inhabitants per year and represents 3%–15% of all MG cases in Europe, while in Asia, it is estimated to reach up to 50% (53, 54). Pure ocular forms are more common in female patients with prepubertal onset, especially in the Asian population. The rate of generalization in JMG patients varies depending on the studies, ranging from approximately 25 to 35%, which is much lower than in adult patients. Generalized forms of JMG typically debut in the post-pubertal period (53–55). Another difference compared to adult patients is the prevalence of antibodies, with a high rate of seronegative patients (36%–50%) (54) and a higher rate of seroconversion during the course of the disease compared to adults (55).

Regarding the treatment of JMG, corticosteroids are the first-line immunosuppressive therapy. However, high rates of corticosteroid dependence have been reported among pediatric patients (54). In refractory cases or when symptoms reappear and corticosteroid dose reduction is not possible, second-line immunomodulatory therapies are indicated.

4.4.1. Evidence of use

The experience with Rituximab in JMG is limited to case reports, generally showing favorable outcomes in patients with seropositivity for anti-AChR and anti-MuSK antibodies, as well as in seronegative patients (56–61).

Two subsequent larger studies documented the experience with five patients each (seven patients with anti-AChR antibodies and three MuSK-positive patients), all of whom showed improvement (54, 62). Despite the improvement, two children with anti-MuSK antibodies required high doses of corticosteroids to maintain their condition (54).

A recent multicenter retrospective study conducted in several French hospitals included 27 pediatric patients treated with Rituximab (63). The patients treated with Rituximab showed better outcomes compared to other conventional therapies, allowing for a reduction in

corticosteroid doses and withdrawal of immunosuppressants. No adverse events were reported during the study.

4.4.2. Recommendation of use

Currently, the evidence is limited, but the results indicate that Rituximab is a well-tolerated and effective option in the treatment of JMG.

5. The physiopathology behind the evidence

After antigen recognition by B-Cell Receptors (BCR), activated B cells give rise to short-lived plasmablasts and plasma cells, which are effectors of the initial humoral response. The sustained exposure to the antigen eventually leads to the development of long-lived plasma cells. On the other hand, antigen-activated B cells can undergo affinity maturation of the BCR to give rise to memory B cells, which, along with long-lived plasma cells, provide long-term immunity (64).

As we said before, Rituximab causes depletion of circulating B cells and a 70% reduction of this lineage in narrow bone, including plasmablasts, thus suppressing the initial humoral response. Rituximab also produces depletion of memory B cells, leading to repopulation of the periphery with naive B cells and generating an altered balance between different cell populations and their activity. Some evidence has emerged showing how administration of Rituximab also leads to an increased percentage of the T regulator lymphocytes (11, 65).

This abortive effect over the first humoral immunity has several implications:

On one side, this is probably the reason behind the differential response to Rituximab between MuSK and AChR-positive MG patients. Anti-MuSK antibodies are predominantly of the IgG4 subtype which are thought to be mainly produced by short-lived B cells and plasmablasts, a pool of cells that requires constant replenishment from B cell precursors and which are depleted after Rituximab administration, justifying the better and sustained response in these patients (52).

This evidence is supported by the quick reduction in titers of MuSK antibody after the treatment (26, 46). Elevations in anti-MuSK antibody titers have also been described in patients who experience relapses. In a study analyzing samples from patients who had relapsed following initial improvement after rituximab, the presence of specific memory B cells against MuSK and an increase in peripheral blood plasmablasts were demonstrated, as well as an increase in anti-MuSK antibody titers compared to patients who remained in complete remission or controls (66).

The other implication of depleting the effectors of the early humoral response is the possible impact of disease duration in the response to Rituximab. It is speculated that early administration of anti-CD20 therapies in autoimmune diseases may abort the process of forming autoantibody-secreting plasma cells, maintaining sustained therapeutic response (64, 65, 67).

This could explain the differences between the two clinical trials in MG, since in RINOMAX, the included patients had 12 months or less of disease duration, while in BEAT-MG, there was no limit on disease duration (mean disease duration was 5.5 years). This is consistent with previous studies in which disease duration is the only factor correlated with a faster response to rituximab (19, 67).

6. Regimen of administration

There is no clear consensus regarding the appropriate administration regimen of Rituximab for patients with MG. The most commonly used guidelines are those indicated for patients with B-cell lymphoma, which consist of either weekly doses of 375 mg/m^2 for 4 consecutive weeks or two doses of 1 g, with a 2-week interval between them (27).

In recent years, several authors have started to suggest the use of lower doses of Rituximab in patients with autoimmune diseases, as the lymphocyte burden is lower compared to patients with hematological neoplasms. These lower dose regimens have already proved efficacy in other autoimmune diseases (68). In MG, a meta-analysis by Li and colleagues reported that up to 34% of patients receive these reduced-dose regimens, which can vary greatly: two doses of 500 mg separated by 2 weeks, a single induction dose of 600 mg, two weekly doses of 375 mg/m^2, etc. (69). However, results are contradictory. A meta-analysis comparing MG patients with anti-AChR antibodies who had received the standard dose vs. a reduced dose found no differences in clinical response, reduction in corticosteroid dosage, or withdrawal of conventional immunosuppressants (69). These findings are in line with previous studies, which also did not observe differences in the need for reinfusion (19, 26, 39). In contrary, another meta-analysis including 196 refractory MG patients showed a higher rate of Minimal Manifestations MGFA-PIS between the high-dose group of Rituximab (84% vs. 39%) (30). In another retrospective multicenter study of patients with anti-MuSK MG, a higher risk of relapse and shorter time to relapse were observed in patients who had received two weekly doses of 1 gram compared to patients who had received 6 doses of 375 mg/m2, with no differences in safety between both regimens (42).

Since the evidence reported to date are controversial, the question about the adequate dosage of Rituximab for MG patients remains still unclear.

7. Monitorization after treatment

Regarding maintenance therapy, there is no established protocol for the need for reinfusion. Several monitoring strategies have been proposed based on analytic markers. Since anti-AChR and anti-MuSK antibodies have a pathogenic role in the disease (22), monitoring titers of antibodies has been postulated as a possible option.

As for the AChR antibodies, neither of the two clinical trials conducted so far, proved a significant reduction in antibody titers after Rituximab treatment (49). Other observational studies did report a reduction in titers, although none of them demonstrated a correlation with clinical improvement, so monitoring these antibodies is not indicated as a predictor of the patient's clinical course (26, 48).

In contrast to anti-AChR antibodies, anti-MuSK antibody titers do closely correlate with disease severity (47), with reductions of up to 90% in titers being described in patients who respond to rituximab administration (26, 46). Elevations in anti-MuSK antibody titers have also been described in patients who experience relapses compared to those who remained in complete remission (66).

As Rituximab treatment induces B cell depletion, B cell counts in peripheral blood has also been used to monitor in some studies and seems to have a better correlation with the clinical response than the antibodies titer (69). In several autoimmune diseases, including MG,

monitoring CD20 cells have proved its value as a predictor of clinical relapses (70, 71). However, Choi and colleagues reported B-cell recovery appeared to be in parallel with clinical relapse on the group level, although it was not a good predictor at the individual-level, with B-cell repopulation observed only at 57% of clinical relapses (37).

In recent years, the levels of CD27+ cells, corresponding to memory B cells, have been described as possible monitoring marker, with a stronger correlation than antibody titers or total B lymphocytes. In a study by Lebrun et al., no patients with low levels of CD27+ cells experienced relapses, while an increase in the levels of these cells correlated with the appearance of symptoms in all cases (72). Using this marker to guide re-treatment decreased the number of annual cycles without a higher number of relapses. However, other authors have noted that, despite of a good sensitivity as a risk of relapse marker, only a 21% of patients with levels of CD27+ cells above threshold manifested a clinical worsening, which could lead to an overtreatment (71). The applicability of this marker in clinical practice is not yet well defined.

In literature, in most studies and reported cases, the decision to administer a new cycle is based on the reappearance of symptoms or the count of CD20+ cells in peripheral blood, while a minority of cases administered Rituximab on a periodic basis (39, 42, 69).

8. Safety profile

As previously mentioned, experience with Rituximab dates back to the last two decades, so there is abundant evidence regarding the drug's safety.

In 2015, safety results of over 3,000 patients with rheumatoid arthritis treated with Rituximab were published, following an 11-year follow-up. The results showed that Rituximab does not pose a higher risk of serious adverse effects, including severe infections, cardiovascular events, or neoplasms (73).

In MG, the rate of adverse events reaches 15%–20% depending on the studies. In most cases, these are mild events that occur within the first 6 months of treatment and are related to infusion reactions, which can present as flushing, flu-like symptoms, fever, etc. (26, 27).

Another concern regarding the use of Rituximab is infections. In MG patients, a risk of serious infections of 0.05/100 patient-years has been reported, including respiratory and gastrointestinal infections, erysipelas, or herpes zoster reactivation. No increased risk of infections has been demonstrated with Rituximab compared to placebo (4).

Rituximab has been associated with the induction of hypogammaglobulinemia, which is associated with a higher risk of serious infections (73, 74). In a retrospective multicenter study of Rituximab-treated MG patients with anti-AChR and anti-MuSK antibodies, it was observed that 37% of patients developed hypogammaglobulinemia, of which 70% was mild. However, no association was demonstrated between hypogammaglobulinemia and the development of serious infections (74).

An infection that deserves special attention due to its poor prognosis is progressive multifocal leukoencephalopathy (PML), an opportunistic infection of the central nervous system caused by the John Cunningham (JC) virus. PML infection has been associated with the use of Rituximab with a frequency of 1 in 20,000 treated patients (16). To date, three confirmed cases of PML have been reported in MG patients associated with the use of Rituximab, although all of them had previously received other conventional immunosuppressive therapies (21, 74, 75).

On the other hand, resistance to Rituximab mediated by inhibitory human anti-chimeric antibodies has been described in 1% of patients with hematological malignancies. As far as we know, only one case of resistance to Rituximab have been reported in MG patients to date. A 28 years old female patient who was tested for these antibodies due to absence of response after the third infusion of Rituximab (26, 74).

In summary, Rituximab is considered a safe alternative in MG patients, with a complication rate similar to other immunosuppressants (27).

9. Discussion

Since the first use of Rituximab in a patient with MG in 1999, a large number of studies have shown benefits of this therapy over the past 20 years. However, results between studies have often been inconsistent, and it has been suggested that the treatment's efficacy depends on the patient's serotype.

Currently, the benefit of Rituximab therapy in MuSK-positive patients seems clear. Although no clinical trials have been conducted in this patient subgroup, accumulated evidence from observational studies and meta-analyses over the past few decades has been consistent and has shown a positive effect not only in the clinical improvement of these patients but also as a steroid-sparing and other immunosuppressive medications, making Rituximab a therapeutic option even ahead of conventional immunosuppressants (27, 36, 46, 50).

The situation is different, however, for AChR-positive seropositive patients. The high variability in the results of observational studies is evident in systematic reviews, which have shown benefits ranging from 30% to 54% to negative results (6, 26, 27). In addition, two recently published randomized clinical trials have been conducted in these patients. The first trial, BEAT-MG, concluded with a treatment futility outcome. The second trial, the RINOMAX trial, has generated controversies because, despite achieving the primary endpoint, the baseline populations of both arms were significantly different in terms of age, corticosteroid dose, and disease severity. Furthermore, this trial was negative in the secondary outcomes (49).

Therefore, with the available evidence to date, it is not possible to ascertain a clinical benefit of Rituximab in AChR-positive patients, and in our opinion, the use of Rituximab in these patients should be restricted to refractory cases and after individualized therapeutic decision-making.

Another issue yet to be clarified is the appropriate administration regimen in these patients. Although most studies have been conducted following classical treatment guidelines (375 mg/m^2 × 4 weekly doses or 1 g × 2 fortnightly doses), in recent years, an increasing number of authors have suggested that lower doses of Rituximab achieve the same clinical effect with a better safety profile and cost-effectiveness (26, 39, 69).

This uncertainty in the administration regimen also applies to the re-infusion schedule, and there is an increasing need for a marker that guides monitoring and retreatment before clinical relapse occurs. Unlike anti-AChR antibody titers, it is clear that anti-MuSK antibody

titers have a good correlation with patient symptomatology and can predict relapses in the majority of cases (26, 46). Other markers that have been proposed as promising in recent years are the count of CD27-positive memory B cells (72). However, in neither case are there guidelines that apply this correlation in clinical practice.

Finally, Rituximab appears to be a safe long-term therapy for patients with MG. In general, most adverse effects are related to infusion reactions, and there has been no demonstrated increase in the risk of serious infections or neoplasms in these patients (27).

Despite the extensive experience accumulated over 20 years of using this treatment, many questions remain unresolved. In our opinion, new clinical trials are needed to clarify the question of efficacy in anti-AChR-positive patients, conducted with a stratification process that ensures similarity between both arms of the trial, as well as new guidelines that standardize the use of Rituximab regimen among different studies and centers and allow the implementation of new markers in the monitoring of these patients.

Author contributions

AV-C: Writing – original draft, Writing – review & editing, Conceptualization, Methodology. EC-V: Writing – original draft, Writing – review & editing, Conceptualization, Funding acquisition, Methodology, Supervision.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was funded by the Instituto de Salud Carlos III through the project PI22/01786 (cofunded by the European Union ERDF), PI E. Gallardo and EC-V. EC-V was supported by a Juan Rodés grant (JR19/00037) from the Fondo de Investigación en Salud, Instituto de Salud Carlos III and co-funded by European Union (ERDF/ESF, "A way to make Europe"/"Investing in your future"), Ministry of Health (Spain).

Acknowledgments

AV-C and EC-V are members of the European Reference Network for Neuromuscular Diseases; work on a CSUR (centro, servicio, unidad de referencia) on rare neuromuscular diseases and are members of XUECs (Xarxes d'unitats d'expertesa clínica en malalties minoritàries).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Gilhus NE, Verschuuren JJ. Myasthenia gravis: subgroup classification and therapeutic strategies. *Lancet Neurol*. (2015) 14:1023–36. doi: 10.1016/S1474-4422(15)00145-3

2. Gilhus NE, Tzartos S, Evoli A, Palace J, Burns TM, Verschuuren JJGM. Myasthenia gravis. *Nat Rev Dis Primers*. (2019) 5:30. doi: 10.1038/s41572-019-0079-y

3. Nelke C, Stascheit F, Eckert C, Pawlitzki M, Schroeter CB, Huntemann N, et al. Independent risk factors for myasthenic crisis and disease exacerbation in a retrospective cohort of myasthenia gravis patients. *J Neuroinflammation*. (2022) 19:89. doi: 10.1186/s12974-022-02448-4

4. Menon D, Bril V. Pharmacotherapy of generalized myasthenia gravis with special emphasis on newer biologicals. *Drugs*. (2022) 82:865–87. doi: 10.1007/s40265-022-01726-y

5. Dalakas MC. Immunotherapy in myasthenia gravis in the era of biologics. *Nat Clin Pract Neurol*. (2019) 15:113–24. doi: 10.1038/s41582-018-0110-z

6. Saccà F, Pane C, Espinosa PE, Sormani MP, Signori A. Efficacy of innovative therapies in myasthenia gravis: a systematic review, meta-analysis and network meta-analysis. *Eur J Neurol*. (2023). 1–14. doi: 10.1111/ene.15872

7. Owe JF, Daltveit AK, Gilhus NE. Causes of death among patients with myasthenia gravis in Norway between 1951 and 2001. *J Neurol Neurosurg Psychiatry*. (2006) 77:203–7. doi: 10.1136/jnnp.2005.072355

8. Silvestri NJ, Wolfe GI. Treatment-refractory myasthenia gravis. *J Clin Neuromuscul Dis*. (2014) 15:167–78. doi: 10.1097/CND.0000000000000034

9. Pescovitz MD. Rituximab, an anti-CD20 monoclonal antibody: history and mechanism of action. *Am J Transplant*. (2006) 6:859–66. doi: 10.1111/j.1600-6143.2006.01288.x

10. Pierpont TM, Limper CB, Richards KL. Past, present and future of rituximab-the Wordl's first oncology monoclonal antibody therapy. *Front Oncol*. (2018) 8:163. doi: 10.3389/fonc.2018.00163

11. Jing S, Lu J, Song J, Luo S, Zhou L, Quan C, et al. Effect of low-dose rituximab treatment on T-and B-cell lymphocyte imbalance in refractory myasthenia gravis. *J Neuroimmunol*. (2019) 332:216–23. doi: 10.1016/j.jneuroim.2019.05.004

12. Prieto Martín A, Barbarroja Escudero J, Barcenilla Rodríguez H, Díaz Martín D. Funciones de los linfocitos B. lymphocyte functions. *Medicine*. (2013) 11:1752–9. doi: 10.1016/S0304-5412(13)70552-3

13. Bouaziz J-D, Yanaba K, Venturi GM, Wang Y, Tisch RM, Poe JC, et al. Therapeutic B cell depletion impairs adaptive and autoreactive CD4+ T cell activation in mice. *Proc Natl Acad Sci U S A*. (2007) 104:20878–83. doi: 10.1073/pnas.0709205105

14. Hanif N, Rituximab AF. *[Updated 2022 Sep 26]. In: StatPearls [Internet]*. Treasure Island (FL): StatPearls Publishing. (2023) Available at: https://www.ncbi.nlm.nih.gov/books/NBK564374/.

15. Delate T, Hansen ML, Gutierrez AC, le KN. Indications for rituximab use in an integrated health care delivery system. *J Manag Care Spec Pharm*. (2020) 26:832–8. doi: 10.18553/jmcp.2020.26.7.832

16. Randall KL. Rituximab in autoimmune diseases. *Aust Prescr*. (2016) 39:131–4. doi: 10.18773/austprescr.2016.053

17. Zaja F, Russo D, Fuga G, Perella G, Baccarani M. Rituximab for myasthenia gravis developing after bone marrow transplant. *Ann Neurol*. (2000) 55:1062–3. doi: 10.1212/WNL.55.7.1062-a

18. Cortés-Vicente E, Álvarez-Velasco R, Pla-Junca F, Rojas-Garcia R, Paradas C, Sevilla T, et al. Drug-refractory myasthenia gravis: clinical characteristics, treatments, and outcome. *Neurology*. (2022) 9:122–31. doi: 10.1002/acn3.51492

19. Iorio R, Damato V, Alboini PE, Evoli A. Efficacy and safety of rituximab for myasthenia gravis: a systematic review and meta-analysis. *J Neurol*. (2015) 262:1115–9. doi: 10.1007/s00415-014-7532-3

20. Nowak RJ, DiCapua DB, Zebardast N, Goldstein JM. Response of patients with refractory myasthenia gravis to rituximab: a retrospective study. *Ther Adv Neurol Disord*. (2011) 4:259–66. doi: 10.1177/1756285611411503

21. Afanasiev V, Demeret S, Bolgert F, Eymard B, Laforêt P, Benveniste O. Resistant myasthenia gravis and rituximab: a monocentric retrospective study in 28 patients. *Neuromuscul Disord*. (2017) 27:251–8. doi: 10.1016/j.nmd.2016.12.004

22. Illa I, Diaz-Manera J, Rojas-Garcia R, Pradas J, Rey A, Blesa R, et al. Sustained response to rituximab in anti-AChR and anti-MuSK positive myasthenia gravis patients. *J Neuroimmunol*. (2008) 201-202:90–4. doi: 10.1016/j.jneuroim.2008.04.039

23. Collongues N, Casez O, Lacour A, Tranchant C, Vermersch P, de Seze J, et al. Rituximab in refractory and non-refractory myasthenia: a retrospective multicenter study. *Muscle Nerve*. (2012) 46:687–91. doi: 10.1002/mus.23412

24. Hehir MK, Hobson-Webb LD, Benatar M, Barnett C, Silvestri NJ, Howard JF Jr, et al. Rituximab as treatment for anti-MuSK myasthenia gravis. *Neurology*. (2017) 89:1069–77. doi: 10.1212/WNL.0000000000004341

25. Beecher G, Anderson D, Siddiqi ZA. Rituximab in refractory myasthenia gravis: extended prospective study results. *Muscle Nerve*. (2018) 58:452–5. doi: 10.1002/mus.26156

26. Tandan R, Hehir MK II, Waheed W, Howard DB. Rituximab treatment of myasthenia gravis: a systematic review. *Muscle Nerve*. (2017) 56:185–96. doi: 10.1002/mus.25597

27. Zhao C. Effectiveness and safety of rituximab for refractory myasthenia gravis: a systematic review and single-arm Meta-analysis. *Front Neurol*. (2021) 12:736190. doi: 10.3389/fneur.2021.736190

28. Nowa RJ, Coffey CS, Goldstein JM, Dimachki MM, Benatar M, Kissel JT, et al. NeuroNEXT NN103 BeatMG Study Team. Phase 2 Trial of Rituximab in Acetylcholine Receptor Antibody-Positive Generalized Myasthenia Gravis: The BeatMG Study. *Neurology*. (2021) 98:e376–89.

29. Piehl F, Eriksson-Dufva A, Budzianowska A, Feresiadou A, Hansson W, Hietala MA, et al. Efficacy and Safety of Rituximab for New-Onset Generalized Myasthenia Gravis: The RINOMAX Randomized Clinical Trial. *JAMA Neurol*. (2022) 79:1105–1112.

30. Feng X, Song Z, Wu M, Liu Y, Luo S, Zhao C, et al. Efficacy and safety of immunotherapies in refractory myasthenia gravis: a systematic review and Meta-analysis. *Front Neurol*. (2021) 12:700. doi: 10.3389/fneur.2021.725700

31. Li H, Huang Z, Jia D, Xue H, Pan J, Zhang M, et al. Low-dose rituximab treatment for new-onset generalized myasthenia gravis. *J Neuroimmunol*. (2021) 354:577528.

32. Fatehi F, Moradi K, Okhovat AA, Shojatalab G, Boostani R, Sarraf P, et al. Zytux in Refractory Myasthenia Gravis: A Multicenter, Open-Labeled, Clinical Trial Study of Effectiveness and Safety of a Rituximab Biosimilar. *Front Neurol*. (2021) 12:682622.

33. Zhou Y, Chen J, Li Z, Tan S, Yan C, Luo S, et al. Clinical Features of Myasthenia Gravis With Antibodies to MuSK Based on Age at Onset: A Multicenter Retrospective Study in China. *Front Neurol*. (2022) 13:879261.

34. Martínez-Monte E, Gascón-Giménez F, Domínguez-Morán JA, Láinez-Andres JM. Rituximab for the treatment of generalised myasthenia gravis: experience in clinical practice. *Rev Neurol*. (2021) 73:416–20.

35. Doughty CT, Suh J, David WS, Amato AA, Guidon AC. Retrospective analysis of safety and outcomes of rituximab for myasthenia gravis in patients ≥65 years old. *Muscle Nerve*. (2021) 64:651–6.

36. Litchman T, Roy B, Kumar A, Sharma A, Njike V, Nowak RJ. Differential response to rituximab in anti-AChR and anti-MuSK positive myasthenia gravis patients: a single-center retrospective study. *J Neurol Sci*. (2020) 411:116690. doi: 10.1016/j.jns.2020.116690

37. Choi K, Hong Y-H, Ahn S-H, Baek S-H, Kim J-S, Shin J-Y, et al. Repeated low-dose rituximab treatment based on the assessment of circulating B cells in patients with refractory myasthenia gravis. *Ther Adv Neurol Disord*. (2019) 12:175628641987118. doi: 10.1177/1756286419871187

38. Lu J, Zhong H, Jing S, Wang L, Xi J, Lu J, et al. Low-dose rituximab every 6 months for the treatment of acetylcholine receptor-positive refractory generalized myasthenia gravis. *Muscle Nerve*. (2020) 61:311–5.

39. Topakian R, Zimprich F, Iglseder S, Embacher N, Guger M, Stieglbauer K, et al. High efficacy of rituximab for myasthenia gravis: a comprehensive nationwide study in Austria. *J Neurol*. (2019) 266:699–706. doi: 10.1007/s00415-019-09191-6

40. Roda RH, Doherty L, Corse AM. Stopping Oral steroid-sparing agents at initiation of rituximab in myasthenia gravis. *Neuromuscul Disord*. (2019) 29:554–61. doi: 10.1016/j.nmd.2019.06.002

41. Landon-Cardinal O, Friedman D, Guiguet M, Laforêt P, Heming N, Salort-Campana E, et al. Efficacy of Rituximab in Refractory Generalized anti-AChR Myasthenia Gravis. *J Neuromuscul Dis*. (2018) 5:241–9.

42. Cortés-Vicente E, Rojas-Garcia R, Díaz-Manera J, Querol L, Casasnovas C, Guerrero-Sola A, et al. The impact of rituximab infusion protocol on the long-term outcome in anti-MuSK myasthenia gravis. *Ann Clin Transl Neurol*. (2018) 5:710–6. doi: 10.1002/acn3.564

43. Robeson KR, Kumar A, Keung B, DiCapua DB, Grodinsky E, Patwa HS, et al. Durability of the Rituximab Response in Acetylcholine Receptor Autoantibody-Positive Myasthenia Gravis. *JAMA Neurol*. (2017) 74:60–6.

44. 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) 22:1164–9.

45. Maddison P, Ambrose PA, Sadalage G, Vincent A. A Prospective Study of the Incidence of Myasthenia Gravis in the East Midlands of England. *Neuroepidemiology*. (2019) 53:93–9.

46. Diaz-Manera J, Martinez-Hernandez E, Querol L, Klooster R, Rojas-Garcia R, Suarez-Calvet X, et al. Long-lasting treatment effect of rituximab in MuSK myasthenia. *Neurology*. (2012) 78:189–93. doi: 10.1212/WNL.0b013e3182407982

47. Peres J, Martins R, Alves JD, Valverde A. Rituximab in generalized myasthenia gravis: clinical, quality of life and cost–utility analysis. *Porto Biomed J*. (2017) 2:81–5. doi: 10.1016/j.pbj.2017.02.002

48. Di Stefano V. Rituximab in AChR subtype of myasthenia gravis: systematic review. *J Neurol Neurosurg Psychiatry*. (2020) 91:392–5. doi: 10.1136/jnnp-2019-322606

49. Nowak RJ. Phase 2 trial of rituximab in acetylcholine receptor antibody-positive generalized myasthenia gravis. *Neurology*. (2021) 98:e376–89. doi: 10.1212/WNL.0000000000013121

50. Narayanaswami P, Sanders DB, Wolfe G, Benatar M, Cea G, Evoli A, et al. International consensus guidance for Management of Myasthenia Gravis. *Neurology*. (2021) 96:114–22. doi: 10.1212/WNL.0000000000011124

51. Hain B, Jordan K, Deschauer M, Zierz S. Successful treatment of MuSK antibody-positive myasthenia gravis with rituximab. *Muscle Nerve*. (2006) 33:575–80. doi: 10.1002/mus.20479

52. Marino M, Basile U, Spagni G, Napodano C, Iorio R, Gulli F, et al. Long-lasting rituximab-induced reduction of specific—but not Total—IgG4 in MuSK-positive myasthenia gravis. *Front Immunol*. (2020) 11:613. doi: 10.3389/fimmu.2020.00613

53. O'Connell K, Ramdas S, Palace J. Management of Juvenile Myasthenia Gravis. *Front Neurol*. (2020) 11:743. doi: 10.3389/fneur.2020.00743

54. Barraud C, Desguerre I, Barnerias C, Gitiaux C, Boulay C, Chabrol B. Clinical features and evolution of juvenile myasthenia gravis in a French cohort. *Muscle Nerve*. (2018) 57:603–9. doi: 10.1002/mus.25965

55. Anlar B, Şenbil N, Köse G, Değerliyurt A. Serological follow-up in juvenile myasthenia: clinical and acetylcholine receptor antibody status of patients followed for at least 2 years. *Neuromuscul Disord*. (2005) 15:355–7. doi: 10.1016/j.nmd.2005.01.010

56. Weger S, Appendino JP, Clark IH. Longstanding and refractory anti-muscle specific tyrosine kinase antibody-associated myasthenia gravis (anti-MuSK-MG) in a child successfully treated with rituximab. *J Binocul Vis Ocul Motil*. (2019) 69:26–9. doi: 10.1080/2576117X.2019.1578164

57. Koul R, al-Futaisi A, Abdelrahim R, Mani R, Abdwani R, al-Asmi A. Rituximab treatment in myasthenia gravis: report of two paediatric cases. *Sultan Qaboos Univ Med J*. (2018) 18:223–e227. doi: 10.18295/squmj.2018.18.02.018

58. Wylam ME, Anderson PM, Kuntz NL, Rodriguez V. Successful treatment of refractory myasthenia gravis using rituximab: a pediatric case report. *J Pediatr*. (2003) 143:674–7. doi: 10.1067/S0022-3476(03)00300-7

59. Skjei KL, Lennon VA, Kuntz NL. Muscle specific kinase autoimmune myasthenia gravis in children: a case series. *Neuromuscul Disord*. (2013) 23:874–82. doi: 10.1016/j.nmd.2013.07.010

60. Govindarajan R, Iyadurai SJ, Connolly A, Zaidman C. Selective response to rituximab in a young child with MuSK-associated myasthenia gravis. *Neuromuscul Disord*. (2015) 25:651–2. doi: 10.1016/j.nmd.2015.03.014

61. Koul R, al Futaisi A, Abdwani R. Rituximab in severe seronegative juvenile myasthenia gravis: review of the literature. *Pediatr Neurol*. (2012) 47:209–12. doi: 10.1016/j.pediatrneurol.2012.05.017

62. Zingariello CD, Elder ME, Kang PB. Rituximab as adjunct maintenance therapy for refractory juvenile myasthenia gravis. *Pediatr Neurol*. (2020) 111:40–3. doi: 10.1016/j.pediatrneurol.2020.07.002

63. Molimard A, Gitiaux C, Barnerias C, Audic F, Isapof A, Walther-Louvier U, et al. Rituximab therapy in the treatment of juvenile myasthenia gravis. *Neurology*. (2022) 98:e2368–76. doi: 10.1212/WNL.0000000000200288

64. Nutt SL, Hodgkin PD, Tarlinton DM, Corcoran LM. The generation of antibody-secreting plasma cells. *Nat Rev Immunol*. (2015) 15:160–71. doi: 10.1038/nri3795

65. Lee DSW, Rojas OL, Gommerman JL. B cell depletion therapies in autoimmune disease: advances and mechanistic insights. *Drug Discov*. (2021) 20:179–99. doi: 10.1038/s41573-020-00092-2

66. Stathopoulos P, Kumar A, Nowak RJ, O'Connor KC. Autoantibody-producing plasmablasts after B cell depletion identified in muscle-specific kinase myasthenia gravis. *Insight J Clin Invest*. (2017) 2:94263. doi: 10.1172/jci.insight.94263

67. Brauner S, Eriksson-Dufva A, Hietala MA, Frisell T, Press R, Piehl F. Comparison between rituximab treatment for new-onset generalized myasthenia gravis and refractory generalized myasthenia gravis. *JAMA Neurol*. (2020) 77:974. doi: 10.1001/jamaneurol.2020.0851

68. Nepal G, Shing YK, Yadav JK, Rehrig JH, Ojha R, Huang DY, et al. Efficacy and safety of rituximab in autoimmune encephalitis: a meta-analysis. *Acta Neurol Scand*. (2020) 142:449–59. doi: 10.1111/ane.13291

69. Li T, Zhang G-Q, Li Y, Dong S-A, Wang N, Yi M, et al. Efficacy and safety of different dosages of rituximab for refractory generalized AChR myasthenia gravis: a meta-analysis. *J Clin Neurosci*. (2021) 85:6–12. doi: 10.1016/j.jocn.2020.11.043

70. Trouvin A-P, Jacquot S, Grigioni S, Curis E, Dedreux I, Roucheux A, et al. Usefulness of monitoring of B cell depletion in rituximab-treated rheumatoid arthritis patients in order to predict clinical relapse: a prospective observational study. *Clin Exp Immunol*. (2015) 180:11–8. doi: 10.1111/cei.12481

71. Ruetsch-Chelli C, Bresch S, Seitz-Polski B, Rosenthal A, Desnuelle C, Cohen M, et al. Memory B cells predict relapse in rituximab-treated myasthenia gravis. *Neurotherapeutics*. (2021) 18:938–48. doi: 10.1007/s13311-021-01006-9

72. Lebrun C, Bourg V, Bresch S, Cohen M, Rosenthal-Allieri MA, Desnuelle C, et al. Therapeutic target of memory B cells depletion helps to tailor administration frequency of rituximab in myasthenia gravis. *J Neuroimmunol*. (2016) 298:79–81. doi: 10.1016/j.jneuroim.2016.07.009

73. van Vollenhoven RF, Fleischmann RM, Furst DE, Lacey S, Lehane PB. Longterm safety of rituximab: final report of the rheumatoid arthritis global clinical trial program over 11 years. *J Rheumatol*. (2015) 42:1761–6. doi: 10.3899/jrheum.150051

74. Caballero-Ávila M, Álvarez-Velasco R, Moga E, Rojas-Garcia R, Turon-Sans J, Querol L, et al. Rituximab in myasthenia gravis: efficacy, associated infections and risk of induced hypogammaglobulinemia. *Neuromuscul Disord*. (2022) 32:664–71. doi: 10.1016/j.nmd.2022.06.006

75. Kanth KM, Solorzano GE, Goldman MD. PML in a patient with myasthenia gravis treated with multiple immunosuppressing agents. *Neurol Clin Pract Neurol Clin Pract*. (2016) 6:e17–9. doi: 10.1212/CPJ.0000000000000202

frontiers | Frontiers in Neurology

TYPE Review
PUBLISHED 05 October 2023
DOI 10.3389/fneur.2023.1277596

OPEN ACCESS

EDITED BY
Elena Cortés-Vicente,
Hospital Santa Creu i Sant Pau, Spain

REVIEWED BY
Nils Erik Gilhus,
University of Bergen, Norway
Ruksana Huda,
University of Texas Medical Branch at Galveston, United States

*CORRESPONDENCE
Saiju Jacob
✉ saiju.jacob@uhb.nhs.uk

RECEIVED 14 August 2023
ACCEPTED 18 September 2023
PUBLISHED 05 October 2023

CITATION
San PP and Jacob S (2023) Role of complement in myasthenia gravis. *Front. Neurol.* 14:1277596. doi: 10.3389/fneur.2023.1277596

Role of complement in myasthenia gravis

Pyae Phyo San[1] and Saiju Jacob[1,2]*

[1]Institute of Immunology and Immunotherapy, University of Birmingham, Birmingham, United Kingdom, [2]Department of Neurology, Queen Elizabeth Hospital Birmingham, University Hospitals Birmingham NHS Foundation Trust, Birmingham, United Kingdom

Myasthenia gravis is a prototypic neuroimmune disorder with autoantibodies targeting the acetylcholine receptor complex at the neuromuscular junction. Patients present with mainly ocular muscle weakness and tend to have a generalized muscle weakness later in the clinical course. The weakness can be severe and fatal when bulbar muscles are heavily involved. Acetylcholine receptor antibodies are present in the majority of patients and are of IgG1 and IgG3 subtypes which can activate the complement system. The complement involvement plays a major role in the neuromuscular junction damage and the supporting evidence in the literature is described in this article. Complement therapies were initially studied and approved for paroxysmal nocturnal hemoglobinuria and in the past decade, those have also been studied in myasthenia gravis. The currently available randomized control trial and real-world data on the efficacy and safety of the approved and investigational complement therapies are summarized in this review.

KEYWORDS

myasthenia gravis, AChR antibody, complement, C5, eculizumab, ravulizumab, zilucoplan, meningococcal

1. Introduction

Myasthenia gravis (MG) is a neuroimmunological disorder where the autoantibodies target the nicotinic acetylcholine receptor (AChR) complex at the postsynaptic membrane of the neuromuscular junction (NMJ) of various skeletal muscles.

The incidence varies from 1.7–21.3 per million person-years for all myasthenia types and 4.3 to 18 per million person-years for AChR MG and an estimated United Kingdom (UK) prevalence of 15 per 100,000 population (1, 2).

Clinical presentation arises from the fatigability of various skeletal muscles. At the onset, it is limited to extraocular muscles in about 85% of patients, giving rise to symptoms such as diplopia, blurred vision, and ptosis. The muscles involved will become generalized in about 80% of such patients, mainly within 2 years from onset (3). Neck, limb, bulbar, and respiratory muscles can be involved with various presentations such as head drop, dysarthria, dysphagia, dyspnoea, and limb weakness. About 40% of patients have severe muscle weakness involving the bulbar and respiratory muscles. One in five patients with severe muscle weakness require ventilator support with endotracheal intubation. With the lack of such ventilation assistance in the past, respiratory failure and pneumonia used to be the causes of almost 100% mortality in the earlier centuries. Despite the advances in ventilator support, mortality remains around 5% to 10% (3).

MG is a prototypic T-cell dependent B-cell mediated autoimmune disorder and anti-AChR antibody is elevated in 90% of patients with generalized MG and 50% with localized ocular MG (3). Muscle specific kinase (MuSK) antibody is found to be positive in about 70% of AChR

antibody-negative patients (4). In about 8% of double seronegative patients, low-density lipoprotein receptor-related protein 4 (LRP4) antibody is positive (5–7).

Among the different antibodies identified in myasthenia gravis, AChR antibody is of IgG1 and IgG3 subtype and can activate the complement system. In this article, we will only review AChR-MG with the focus on the role of the complement system in the pathogenesis and its therapeutic potential.

2. Role of complement in AChR-MG

2.1. Proposed pathogenic mechanisms of AChR antibody

AChR is of the larger ligand-gated ion channel gene superfamily and the best-known nicotinic AChR of the family. It is a transmembrane glycoprotein structure and composed of five homologous subunits α2βγδ as fetal AChR, and in the adult type, the γ subunit is replaced by the ε subunit.

The AChR is a very potent immunogen (8). The ability to induce experimental autoimmune MG in several animal models either actively by heterologous or homologous AChR or its parts or passively by polyclonal or monoclonal AChR antibodies has been shown in several studies (9, 10).

Over half of the autoantibodies were observed to bind to the α subunit of AChR, especially to the major immunogenic region (MIR) formed by overlapping epitopes in the Extracellular domain of the α subunit (α 67–76). Autoantibodies can bind all AChR subunits, including the γ subunit in fetal AChR. However, α subunit binding antibodies were found to be more pathogenic (8, 11, 12).

Three pathogenic mechanisms of AChR antibodies have been proposed in the literature and are schematically presented in Figure 1.

The first proposed mechanism is the direct AChR blockade, where anti-AChR binds to and directly inhibits AChR function. Various groups have explored this hypothesis using human, mice and rat cells, transfected cell lines and intact neuromuscular junctions however such studies failed to show a unanimous neurotransmission failure (13). At the physiological NMJ, AChRs are densely packed at around 9,000 receptors per square micrometre (14) and perhaps the results may have been consistent if this had been taken into consideration. Cetin et al. (13) exploited this and demonstrated that by mimicking a physiological NMJ by clustering AChRs using rapsyn in CN21 cell line, AChR antibody sera from patients were able to rapidly and more potently block AChR currents than in the cell line with unclustered AChRs.

However, the magnitude of AChR antibody bound to AChR at the post-synaptic membrane was observed to be directly proportionate to the AChR index (residual AChR) and the mini-end plate potential in the muscle biopsies from myasthenic patients. This suggested that direct antibody blockade may not be the most important mechanism and the receptor depletion mechanisms may play a larger role for neurotransmission failure (15).

The AChR population at the post synaptic membrane is rather dynamic due to internalization and either recycling or degradation and replacement with new receptors (16). Engel at al demonstrated that this process was accelerated in EAMG rats compared with healthy control ones however this was also compensated by increased synthesis and release of AChR in mild or subclinical EAMG rats (15). Heinemann et al. (17) demonstrated similar findings in rat diaphragm using anti-AChR rat sera in rat diaphragm and Dranchman et al. (18) by patient-derived immunoglobulins in rat muscle cell cultures. This antigenic modulation by anti-AChR antibody seems to be mediated by its receptor cross-linking ability (19, 20).

In most severely affected EAMG mice, junctional folds were however destroyed and shedding of labelled AChR into the synaptic space was seen and Engel et al. inferred that this cannot by explained purely by an increased AChR internalization mechanism (15).

In the muscle biopsies of myasthenic patients, similar ultrastructural changes were also observed such as widening of primary synaptic clefts with significant debris of junctional folds in the synaptic clefts, simplification of junctional folds (by shallowing and widening or reduction in the number of secondary synaptic clefts), remodeling of the endplate and moving away of the nerve terminal from the destroyed endplate to an adjacent region where a new endplate region was formed (illustrated in Figure 1) (15, 21, 22). Such NMJ loss suggested complement involvement in the pathogenesis of myasthenia gravis.

2.2. Complement in myasthenia gravis

Complement system is part of innate immune response and central functions include inducing acute inflammation, killing microbes by opsonization for phagocytosis and osmotic/colloidal lysis and removing apoptotic host cells. It can help solubilize or remove antigen-antibody complexes from circulation. It is also involved in adaptive immune response by helping regulate T and B cell activation (23–25).

It is an integrated system of nearly 50 proteins present abundantly in blood but not in normal extravascular tissues. Complement is activated on cell surfaces of mainly microbes and damaged host cells and autoimmunity is suppressed by complement regulators present at the intact cell surfaces (see below for a further review in relation to MG pathogenesis). It operates in a cascade via a series of proteolytic cleavages after activation. IgM and IgG are major immunoglobulins that can activate the complement cascade via the classical pathway. IgG can diffuse into normal extravascular tissues. In contrast IgM can only enter those with increased vascular permeability induced by tissue inflammation.

There are three pathways to activate the complement cascade however only the classical pathway is the most relevant in this review, and it will be described.

2.2.1. The classical pathway

Multivalent C1q can either be activated by direct binding to microbes or by antigen antibody complexes and subsequent enzymatically active C1r and C1s are generated. C1s cleaves C4 to C4a and C4b. C1r, C1s and C4b in combination cleaves C2 to C2a and C2b. C4b2a complex (C3 convertase) cleaves C3 to C3a and C3b. C3C4b2a3b forms C5 convertase and initiates terminal complement pathway by cleaving C5 into C5a and C5b. C3a, C4a and 5a are anaphylatoxins, which are proinflammatory and responsible for increased vascular permeability, smooth muscle contraction and leucocyte recruitment. C5b subsequently exposes a binding site for C6 and C5bC6 reversibly binds to the cell surface

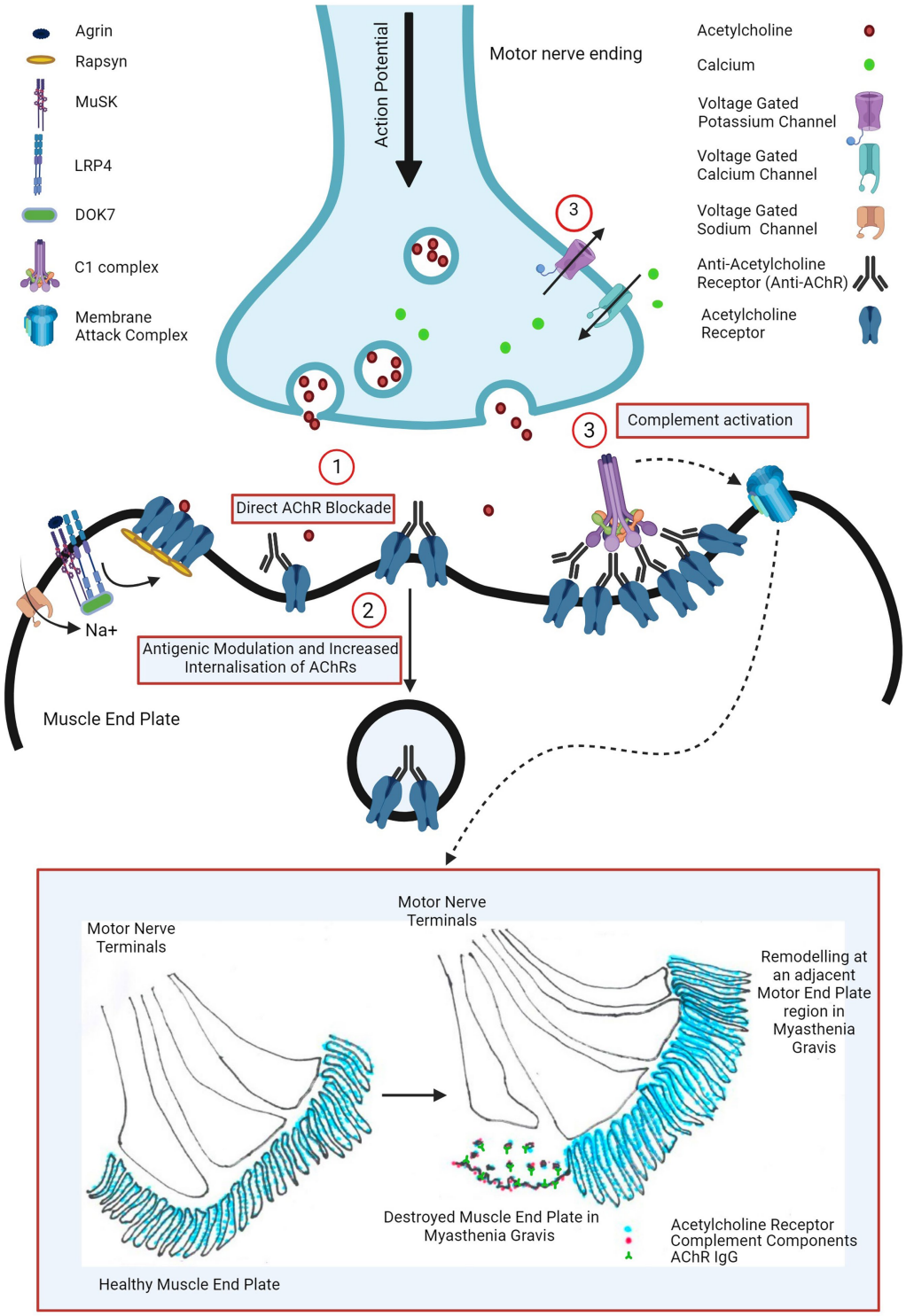


FIGURE 1
Pathogenic mechanisms of AChR antibody in myasthenia gravis: (1) direct AChR blockade, (2) antigenic modulation and increased AChR internalization, and (3) complement activation leading to complement mediated NMJ destruction (widening of primary synaptic cleft (space between motor nerve terminal and muscle end plate), destruction of junctional folds with simplification of secondary clefts (fewer and wider clefts), increased AChR, AChR IgG and complement bound junctional fold debris in the primary synaptic cleft) and remodeling of nearby motor end plate region (motor nerve terminal moving away from the damaged muscle end plate area with formation of a new end plate in a nearby region). Image created with the help of ©BioRender.com.

and forms the foundation for membrane attack complex (MAC). C7 binds to C5bC6 to form C5bC6C7, which subsequently induces transmembrane insertion of C8α and C8β, forming unstable pores. C9 binds to C8α and attracts polymerization of multiple C9

molecules to stabilize the pores with a maximum diameter of 10 nm. This C5bC6C7C8C9 forms MAC, which lyses the cell via several mechanisms (25). The classical pathway is illustrated in Figure 2 alongside the approved and investigational therapeutic targets.

2.2.2. Telltale of NMJ destruction by the complement system

The possibility of the complement system involvement in the pathogenesis of myasthenia was first considered in 1960s when sera from myasthenic patients were able to cause cytolytic destruction of frog sartorius muscle fibers correlating with serum complement levels outside a normal range in most patients (26).

Studies have demonstrated that antibodies binding AChR leads to complement deposition at the NMJ (27, 28). In the muscle biopsies of patients with myasthenia, IgG and C3 deposition were localized at the identical sites such as the post-synaptic membrane, synaptic cleft debris and on disintegrating junctional folds suggesting that the complement pathway had been activated by anti-AChR and it had been completed to C3 phase (15). C9 is one of the major components in assembling the final and stable membrane attack complex, which is responsible for destruction of neuromuscular junction in case of myasthenia gravis. As definitive evidence of destruction of NMJ by the complement system, Engel et al. (15) demonstrated that deposition of C9 was seen at the MG end plate regions however the most intense depositions were observed in association with the most abnormal looking and destroyed neuromuscular junctions. Such findings were also reflected in EAMG models (29).

In myasthenic patients with high AChR antibody concentrations, the evidence of consumption of complement was also observed and C3 levels were inversely correlated with clinical severity in AChR-MG patients (30, 31). The electrophysiological tests often correlate with *in vitro* serum complement-fixing ability of clustered AChR ab (32).

The essential role that the complement plays in the pathogenesis of AChR-MG was also supported by prevention of murine EAMG either by depleting the complement with cobra venom factor or by knocking out C3, C4, C5 or C6 (33–36). In all of these studies, despite visualization of antibodies attached to AChR at NMJ, neither complement deposition nor NMJ destruction were observed.

2.2.3. How anti-AChR IgG could trigger complement cascade

In the classical pathway, IgM, a natural pentamer in blood, when bound to an antigen, can strongly active the complement cascade. However, it is not relevant to AChR-MG as the most specific antibody is IgG. Unlike the pentameric IgM, IgGs in oligomers, can bind C1q with a sufficient avidity to activate the complement system (37, 38). Among IgG subclasses, IgG 1 and 3 are known to be the strongest complement activators.

Six IgG monomers can form a hexamer via Fc:Fc interactions and are able to bind and activate C1q and subsequently the downstream complete cascade (39–41). For lateral recruitment model on a sparsely antigenic surface, IgG must be monovalent to be able to form hexamers as bivalent binding seems to suppress oligomerization by lateral collision, however with vertical recruitment from the solution, IgGs can be bivalent (41). For high density antigenic expression like

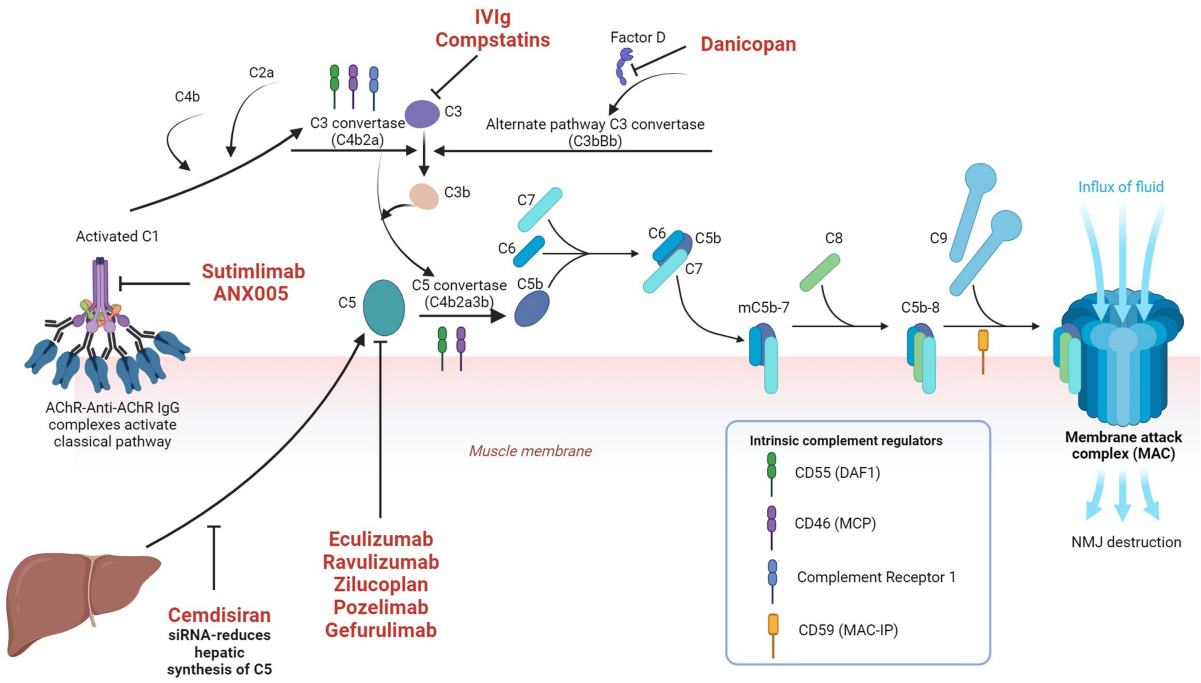


FIGURE 2
Classical complement pathway in myasthenia gravis and the approved and investigational therapeutic targets in the complement system Image created with the help of ©BioRender.com.

nicotinic NMJ AChRs, the former model is not important (41). This has been illustrated in a few studies but not in myasthenia. Such Fc:Fc interactions can be modified by Fc mutations or Fc domain deglycosylation (39, 40).

AChR antibodies are of bivalent IgG1 and 3 subtype and can cross-link the receptors with antibodies against the major immunogenic region being the most potent (11). It can be deducted that densely populated AChRs with an estimated distance of a mere 10 nm between them favour a very close clustering of Anti-AChR monomers, formation of hexamers via the vertical pathway mentioned above and thereby a multivalent binding of C1q multivalent head. However, the direct visualizable evidence of how the complement cascade is triggered by AChR IgGs in AChR-MG is yet to be investigated.

2.2.4. Complement regulators

Complement regulators are present at host cell surfaces to prevent autologous destruction by the complement system (42). Decay accelerating factor 1 (DAF1) or CD55 inhibits C3 and C5 convertases and accelerates their decay (43). Membrane cofactor protein (CD46) is a cofactor of cleavage of C3b and C4b, which form C3/4 convertases (44). Complement receptor 1 accelerates the decay of C3/5 convertases and degradation of C3b/4b (45). Membrane attack complex inhibitory protein (MAC-IP) or CD59 inhibits the formation of membrane attack complex (46, 47).

Mice that are deficient in DAF1 alone or both DAF1 and CD59 were observed to be much susceptible to EAMG with significant receptor loss, muscle weakness and NMJ damage, with the double knockout mice showing a significantly worse EAMG even leading to crisis (48, 49).

Complement regulator activities (mRNA and protein expression) were observed to be lower at extraocular muscles (EOM) than diaphragm at baseline or after EAMG induction in mice. This supports increased predilection of EOM involvement in AChR-MG patients (50).

2.2.5. Complement system as therapeutic targets

2.2.5.1. High dose intravenous immunoglobulin

Among the established immunomodulatory therapies for MG, intravenous immunoglobulin (IVIg) appears to inhibit the complement system by neutralization of C3a and C5a and at high concentrations, by inhibiting the uptake of C3b and C5b onto the cell surface and subsequent complement mediated tissue damage (51, 52).

2.2.5.2. FDA approved anti-C5 therapies

2.2.5.2.1. Eculizumab

Eculizumab is a recombinant humanized monoclonal antibody against C5. It binds to C5 and prevents its breakdown to C5a and C5b, thereby reducing inflammatory cells recruitment and membrane attack complex formation. United States Food and Drug Administration (U.S. FDA) first approved its use in paroxysmal nocturnal hemoglobinuria (PNH) in 2007 and atypical haemolytic uremic syndrome (aHUS) in 2011, where uncontrolled complement activation is largely responsible for the pathogeneses.

It was then studied in 2 neurological conditions such as myasthenia gravis and aquaporin-4 (Aqp-4) antibody positive neuromyelitis optica spectrum disorder (NMOSD), where complement involvement is seen but less well-delineated. Phase 3 randomized controlled trial (RCT) data for Aqp-4-positive NMOSD (PREVENT, prevention of relapses in neuromyelitis optica; NCT01892345) showed a remarkable 94% relative risk reduction of relapses (53) however the benefits were less clear in the RCT data for MG.

It is the first complement therapy investigated in MG. Fourteen patients with severe refractory generalized MG (gMG) were studied for 16 weeks with a crossover in a phase 2 randomized, double blind placebo-controlled trial. A clinically meaningful response was produced with 86% achieving primary end point of three-point reduction in QMG score and a significant overall QMG score reduction between treatment and placebo group ($p = 0.0144$) (54).

Encouraging results from phase 2 RCT led to a multinational, randomized, placebo-controlled, double-blind phase 3 study (REGAIN, safety and efficacy of eculizumab in AChR positive refractory generalized myasthenia gravis; NCT01997229) in a similar population as the phase 2 study (55). One hundred twenty-five AChR-antibody positive refractory severe generalized MG patients from North America, Latin America, Europe and Asia were enrolled into the study. Eligibility criteria were myasthenia gravis-activities of daily living (MG-ADL) score of 6 or more, myasthenia gravis foundation of America (MGFA) class II–IV disease, vaccination against *Neisseria meningitides*, and previous treatment with at least two immunosuppressive therapies (IST) or one immunosuppressive therapy and chronic IVIg or plasma exchange (PLEX) for 12 months without symptom control. Exclusion criteria were history of thymoma or thymic neoplasms, thymectomy within 12 months before screening, or use of IVIg or PLEX within 4 weeks before randomization, or rituximab within 6 months before screening. Patients had either intravenous (IV) eculizumab or placebo 900 mg on day 1, weeks 1, 2 and 3 and 1,200 mg in week 4 for induction phase, and thereafter maintenance dose of 1,200 mg every 2 weeks up to 26 weeks.

Primary endpoint was the change in MG-ADL score from baseline to week 26 using worst-rank ANCOVA (analysis of covariance) and REGAIN failed to reach a statistical significance ($p = 0.0698$). It was likely attributed by the use of worst-rank analysis that assigned patients who discontinued eculizumab regardless of the reason to the lowest ranks. Three out of seven patients who discontinued eculizumab due to adverse events rather than worsening of myasthenia were given the lowest ranks despite a clinically meaningful benefit. However, pre-specified secondary efficacy endpoints showed statistically significant benefits. The changes in quantitative myasthenia gravis (QMG) and myasthenia gravis quality of life 15-item scale (MGQoL-15) scores using worst-rank ANCOVA from the baseline met statistical significances at $p = 0.0129$ and $p = 0.0281$ but the changes in myasthenia gravis composite (MGC) scores did not. Prespecified responder analyses of MG-ADL and QMG showed that eculizumab group had a higher proportion of patients with a clinically meaningful improvement than the placebo group. In prespecified sensitivity analysis of all 4 scores (MG-ADL, QMG, MGQoL-15 and MGC), treatment group had significantly lower scores than the placebo group, which were sustained throughout from week 1 to 26. Hence the authors inferred that the use of derived rank rather than the actual change in the scores affected the primary end point outcome negatively and all the evidence from both primary and

secondary endpoint analyses should be considered in the interpretation of REGAIN trial outcome (55).

The commonest adverse events identified were headache, upper respiratory infection and nasopharyngitis which were of mild to moderate severity. The commonest serious adverse events were infections. There were no statistically significant differences between the treatment and placebo groups in terms of the adverse events. Complement system plays a major role in killing *Neisseria meningitidis* and hence meningococcemia is one serious risk considered with eculizumab, although no patients in REGAIN developed Meningococcal infection. Fewer patients in the eculizumab group experienced exacerbations and needed rescue therapy (55).

One hundred seventeen patients from the double-blind phase of REGAIN 56 from the blinded eculizumab group (eculizumab/eculizumab) and 61 from the blinded placebo group (placebo/eculizumab) were enrolled into the open label extension (OLE) phase for a maximum of 4 years. After a blinded induction phase (active drug provided as 1,200 mg every 2 weeks for previous eculizumab group and 900 mg on day 1 and weekly for 3 weeks for the previous placebo group), all patients were administered 1,200 mg every 2 weeks. Compared with the pre-REGAIN baseline, overall myasthenic exacerbations were reduced by 75.2% and the rate of MG related hospitalizations by over 80%. In the eculizumab/eculizumab group, the improvement in all 4 scores were sustained throughout the OLE. A rapid and significant improvement in all 4 scores was also observed in the placebo/eculizumab group from REGAIN: over 50% of improvement was seen in the first 3 months and the improvements were sustained for 30 months (56).

The safety profile from REGAIN OLE also matched with the current safety profile for generalized MG and post marketing safety profile of eculizumab in PNH and aHUS (56).

A retrospective analysis by Howard et al. (57) looked at the responder subgroup from RCT and OLE phases of REGAIN. Early and late responders were defined by clinically meaningful improvements in MG-ADL reduction by ≥3 points from the baseline or QMG reduction by ≥5 points from the baseline before week 12 or after week 12. 67.3% and 56.1% from eculizumab group of RCT phase were identified as early responders by MG-ADL and QMG improvements, respectively, but with longer duration of the treatment, more responders were increasingly identified. The response to eculizumab treatment was sustained until the end of the OLE as indicated by 84.7% and 71.4% responder proportion in MG-ADL and QMG.

Another post-hoc analysis of REGAIN looked at the proportion of patients who attained minimal symptom expression (MSE) as defined by MG-ADL scores 0–1 or MGQoL-15 scores 0–3. A significantly higher number of patients in the eculizumab group achieved MSE at week 26 of REGAIN than in the placebo group [MG-ADL: 21.4% vs. 1.7%; 95% confidence interval (CI) 8.5, 31.0; $p = 0.0007$; MGQoL-15: 16.1% vs. 1.7%; 95% CI 4.3, 24.6; $p = 0.0069$] (58). At week 130 of OLE phase, the proportion of patients with MSE in placebo/eculizumab group significantly increased (MG-ADL: 1.7 to 27.8%; MG-QoL15: 1.7 to 19.4%) and in eculizumab/eculizumab group, MSE was maintained at similar proportions to the RCT phase (58).

The response of immunosuppressive therapies in myasthenic patients are assessed with MGFA post intervention status (MGFA-PIS) and the consensus therapeutic efficacy goal is aimed at MGFA-PIS minimal manifestations (MM), which is defined as having no symptoms of functional limitations but with some weakness on examination or better. At week 26 of REGAIN RCT phase, a higher proportion of eculizumab group achieved better MGFA-PIS than the placebo group [improved (60.7% vs. 41.7%) or MM (25.0% vs. 13.3%); common OR: 2.3; 95% CI: 1.1–4.5]. In the OLE at week 130, 88% achieved improved status and 57.3%, MM status (59).

A small number of patients needed regular IVIg use for at least 12 months (17 patients) or rituximab (14 patients) prior to REGAIN and constituted the extreme spectrum of refractory MG, not dissimilar to real-world clinical practice (60, 61). A sufficient washout period of at least 4 weeks for IVIg and 6 months for rituximab was given before enrollment into REGAIN, to minimize bias.

IVIg subgroup had a higher exacerbation rate at pre-REGAIN baseline than the overall REGAIN cohort (150.0 versus 102.4 exacerbations/100 patient-years). At week 26 of REAGIN RCT phase, eculizumab treated group attained a clinically meaningful response (reduction of MG-ADL scores ≥3 points and QMG scores ≥5 points) in 75% compared with only one-fifth (in terms of QMG) to one-third (in terms of MG-ADL) improvement in the placebo group. Such improvements were also sustained in eculizumab/eculizumab group at 71% at the interim analysis at week 52 during OLE phase. Placebo/eculizumab group showed a rapid and sustained improvement at OLE phase. Compared with pre-REGAIN baseline, at 18 months, overall hospitalization rate was reduced by 68% however it was not statistically significant due to a small sample size of this subgroup. This data supported durable benefits of eculizumab in this sub-cohort (60).

In the rituximab subgroup in REGAIN, a significantly higher proportion had an exposure to ≥4 ISTs, which could reflect a super-refractory nature of this sub-cohort with the possibility of an increased cumulative risk from several ISTs. However, the safety profile and the efficacy of eculizumab use in this subgroup were comparable to the non-rituximab group (61).

A rapid and significant clinical improvement with the use of eculizumab was also reported in a case series of ventilator-dependent AChR-MG patients, who had been refractory to 3 or 4 immunotherapies. Two patients achieved MGFA-PIS MM within 4 to 6 weeks from the initiation of eculizumab with a sustained improvement. The third patient had a slow and partial but sustained improvement, and he was able to remain on intermittent non-invasive ventilation at week 40 of eculizumab therapy (62). Severe bulbar weakness in a refractory myasthenic crisis rapidly improved as early as 1 week with a complete resolution of bulbar symptoms within 10 weeks of eculizumab initiation (63).

The benefits and tolerability of eculizumab from REGAIN was also reflected in the evidence from real-world studies (64, 65). Fifteen treatment-refractory AChR-MG patients treated with eculizumab in a real-world study showed that a clinically meaningful reduction of mean MG-ADL score was seen as early as 3 months with further reduction at 6 and 12 months. Mean exacerbations per patient per year was reduced by 2.33 from the baseline. Burden from concomitant use of ISTs was also reduced with use of eculizumab: mean prednisolone dose was reduced by 23.33 mg/day and all 6 patients on IVIg were able to wean off from IVIg successfully. Nine out of fifteen patients also discontinued pyridostigmine at 12 months of eculizumab therapy (65).

Rituximab is a monoclonal anti-CD20 B-cell depletion therapy, and it is considered in a multi-IST refractory AChR-MG in a case-by-case basis as the response rates were not as consistent as in

anti-MuSK MG. Nelke et al. (65) compared the responses in 57 MG patients treated with rituximab with those in 20 with eculizumab in a real-world retrospective 24 months observational study. A better treatment outcome was associated with eculizumab than with rituximab in terms of QMG score reduction from the baseline and MGFA-PIS MM state were more frequently achieved by the eculizumab cohort, although the risk of myasthenic crisis did not differ in both groups.

Successful uses of eculizumab in seronegative, paediatric and thymoma associated MG patients have also been reported (66–69).

REGAIN (RCT, OLE and various post-hoc subgroup analyses), real-world data and case reports mentioned above have consistently shown the rapid and sustained clinical improvement by eculizumab but its role as a first-line agent and duration of therapy are yet to be investigated with further studies. Eculizumab is currently approved for use in generalized AChR-MG (USA, 2017), refractory AChR-MG (EU) and AChR-MG unresponsive to IVIg/PLEX (Japan). Currently, it remains one of the most expensive medicines in the world and an estimated annual cost of over half a million U.S. dollars has thus been a major hindrance for a wider use (70).

2.2.5.2.2. Ravulizumab

Ravulizumab is another recombinant human monoclonal antibody against C5 with a similar mechanism of action to eculizumab but with a longer half-life hence intravenous infusion are less frequent for maintenance (8 weekly as opposed to 2 weekly for eculizumab).

In phase 3 CHAMPION-MG randomized, placebo-controlled study (NCT03920293), 175 patients with AChR antibody positive gMG were randomly assigned 1:1 to receive ravulizumab versus placebo. A single loading dose on day one was administered at weight-dependent dosage of 2,400 mg for ≥40 to <60 kg, 2,700 mg for ≥60 to <100 kg and 3,000 mg for ≥100 kg, followed by maintenance doses of 3,000 mg, 3,300 mg and 3,600 mg respectively, every 8 weeks starting from day 15. The primary endpoint of significant mean change in MG-ADL in treatment vs. placebo groups was achieved (−3.1 vs. −1.4; $p < 0.001$) (71). A significantly higher proportion in the treatment group than in the placebo group was observed to attain a clinically meaningful response as defined by reduction of QMG scores by ≥5 points (30.0% vs. 11.3%, $p = 0.005$) (71). A rapid improvement was seen within 1 week of treatment initiation in ravulizumab group (71). Adverse events rates were comparable between the two groups. The open label extension phase of this study up to 60 weeks showed a sustained improvement (72). U.S. FDA approved Ravulizumab for use in MG in April 2022.

2.2.5.3. Other anti-C5 therapies, which have just finished phase 3 clinical trial or on ongoing phase 3 trial

2.2.5.3.1. Zilucoplan

Zilucoplan, a subcutaneously (SC) administered small macrocyclic peptide is another terminal complement inhibitor acting via two mechanisms. Its binding to C5 prevents its cleavage and the binding to the existing C5b prevents C5b's attachment to C6. The advantages of zilucoplan are (1) patients can self-administer and thus it can be more convenient, (2) a good NMJ penetration is more likely due to its small size and (3) as it is not an antibody like eculizumab or ravulizumab, it can be co-administered either with IVIg or neonatal Fc receptor (FcRN) inhibitors (73).

Safety and efficacy of zilucoplan in patients with generalized myasthenia gravis (RAISE; NCT04115293) is a multinational randomized placebo-controlled phase 3 trial. One hundred seventy-four patients with anti-AChR positive gMG were assigned 1:1 to treatment and placebo groups and participants self-administered either zilucoplan 0.3 mg/kg or matched placebo once daily for 12 weeks. Primary efficacy endpoint was met with a significant improvement in the change of MG-ADL scores from the baseline to week 12 in zilucoplan group compared with the placebo group [least squares mean change −4·39 (95% CI −5·28 to −3·50) vs. −2·30 (−3·17 to −1·43); least squares mean difference −2·09 (−3·24 to −0·95); $p = 0·0004$] (74). Treatment emergent adverse events (TEAEs) were comparable in both groups and the commonest TEAE was injection site bruising (74). An OLE phase is currently ongoing.

2.2.5.3.2. Pozelimab and/or cemdisiran

Pozelimab is a fully humanized IgG4 monoclonal antibody targeting C5 and cemdisiran is a small interfering ribonucleic acid (siRNA) which interferes with mRNA for C5 and decreases its hepatic synthesis and hence the circulating level of C5. With a loading dose of 15 mg/kg IV followed by four SC doses of 400 mg once weekly, pozelimab was able to inhibit complement activation in healthy volunteers (75). Co-administration of pozelimab with cemdisiran allowed lower and less frequent doses as compared to individual agents given separately in animal studies (76). A phase 3 randomized controlled trial of the combination (intravenous pozelimab loading followed by 4 weekly subcutaneous injections along with cemdisiran subcutaneous 4 weekly) versus placebo in generalized MG is ongoing (NCT05070858).

2.2.5.4. Future investigational complement inhibition therapies

Currently, several newer therapies are being investigated at different phases of clinical trials for MG and other complement mediated disorders. These include ANX005 (anti-C1q), sutimlimab (anti-C1s), cinryze, berinert and ruconest (C1-esterase inhibitors), compstatins (a group of cyclic peptides, which bind and interfere with the function of C3), tesidolumab, crovalimab, zimura, gefurulimab and nomacopan (all target C5), SKY59 (anti-C5 as well as FcRn inhibitor), avacopan (anti-C5aR1) and danicopan (anti-factor D) (77, 78). Avacopan and danicopan are orally administered. A summary of the main complement therapies in MG is given in Table 1.

2.2.6. Safety of complement therapies

Eculizumab is the first U.S. FDA approved complement therapy for its use in PNH since 2007 and has been in the market for the longest duration among all complement therapies. It is generally well tolerated with the commonest side effects being headache, upper respiratory tract infections and nasopharyngitis. Membrane Attack Complex is primarily responsible for killing gram negative bacteria especially *Neisseria* species and the use of eculizumab is associated with 1,000 to 2,000 times increased risk of meningococcal disease (79). Thus U.S. FDA approved prescribing information includes a boxed warning with regards to an increased risk of meningococcal infection in patients on eculizumab (79). Meningococcal vaccinations are recommended at least 2 weeks before eculizumab is initiated. The Advisory Committee on Immunization Practices (ACIP) recommends that eculizumab recipients receive both quadrivalent meningococcal

TABLE 1 Complement therapies: U.S. FDA approved or on ongoing clinical trials for myasthenia gravi.

Drug	Specific targets in the complement system	Studied group	Regimen	RCT evidence
Eculizumab	Recombinant humanized IgG2/4 monoclonal antibody against C5	AChR+ gMG	IV; induction of 900 mg weekly for 4 weeks followed by 1,200 mg maintenance every 2 weeks	Phase 3 RCT results: QMG: eculizumab vs. placebo = 54.7 vs. 70.7 ($p = 0.0129$); MG-QoL-15: eculizumab vs. placebo = 55.5 vs. 69.7 ($p = 0.0281$) (REGAIN, NCT01997229) U.S. FDA approved for treatment of adults with AChR+ gMG
Ravulizumab	Long-acting recombinant humanized monoclonal antibody against C5	AChR+ gMG	IV; weight-based dose. A single loading dose of 2,400–3,000 mg followed by maintenance doses of 3,000–3,600 mg every 8 weeks	Phase 3 RCT results: MG-ADL from baseline in treatment vs. placebo = −3.1 vs. −1.4 ($p < 0.001$) (CHAMPION MG, NCT03920293) U.S. FDA approved for treatment of adults with AChR+ gMG
Zilucoplan	Macrocyclic peptide binding C5 and C5b	AChR+ gMG	SC; once daily dose of 0.3 mg/kg	Phase 3 study showed positive results. MG-ADL from baseline in treatment vs. placebo = least squares mean change −4·39 (95% CI −5·28 to −3·50) vs. −2·30 (−3·17 to −1·43); least squares mean difference −2·09 (−3·24 to −0·95) ($p = 0·0004$) (RAISE, NCT04115293)
Pozelimab	Fully humanized IgG4 monoclonal antibody inhibiting C5 complement	AChR+ or LRP4+ gMG	SC; alone or in combination with cemdisiran	Phase 3 study is ongoing (NCT05070858)
Cemdisiran	siRNA suppressing hepatic C5 synthesis	AChR+ or LRP4+ gMG	SC; alone or in combination with pozelimab	Phase 3 study is ongoing (NCT05070858)
Gefurulimab (ALXN1720)	Anti-C5 humanized bi-specific VHH antibody (nanobody)	AChR+ gMG	SC; weight-based dose once weekly	Phase 3 study is ongoing (NCT05556096)
Danicopan (ALXN2050)	Small molecule complement pathway factor D inhibitor	AChR + gMG	Oral; 120 mg or 180 mg	Phase 2 study is ongoing (NCT05218096)

AChR, acetylcholine receptor; MG, myasthenia gravis; MG-ADL, myasthenia gravis activities of daily living score; MGC, myasthenia gravis composite score; MG-QoL-15, myasthenia gravis quality of life 15-item scale; gMG, generalized myasthenia gravis; LRP-4, low-density lipoprotein receptor related protein 4.

conjugate (MenACWY) and serogroup B (MenB) meningococcal vaccines (80). Despite prior meningococcal vaccination, there have been reports of invasive and even fatal meningococcal diseases in patients on eculizumab therapy (81–83). Thus anti-microbial prophylaxis is also recommended by clinicians and public health agencies while the patient is on eculizumab therapy and for 3 months after discontinuation (81). However, neither vaccination nor anti-microbial prophylaxis cannot eliminate the risk of severe meningococcal infections and in addition, patients may not show typical meningitis features. Thus, it is essential that health care providers and patients have a high index of suspicion for meningococcal infection. Fluoroquinolones and macrolides can block neuromuscular transmission and clinicians should avoid them to minimize the risk of myasthenic exacerbations.

Data on less than 300 pregnancy outcomes showed no increased risk of foetal malformation or foetal-neonatal toxicity. However, as a human IgG, it may cross the placenta and appear in foetal circulation. The level of eculizumab in the breast milk is undetectable or negligible. However, due to the limited data, European medicines agency recommended an individual risk benefit analysis before eculizumab is used during pregnancy or lactation (84). If complement therapy is used in children in the future, ACIP guidelines recommended additional vaccinations against *Streptococcus pneumoniae* and *Haemophilus infleunzae* type B (84). Clinically significant neutralizing antibodies have not been reported so far.

2.2.7. Prediction of complement therapy responders

Unlike some conditions, validated biomarkers are currently unavailable for myasthenia gravis to assess the disease severity. Nature of the disease makes it difficult to develop such markers for myasthenia. Some investigational assays are also being developed to assess anti-AChR mediated complement activation. Obaid et al. (85) developed an assay where an HEK293T cell line with modified expression of the complement regulator genes was used to measure AChR autoantibody-mediated MAC formation through flow cytometry. Although it was rather specific, the sensitivity was not strong enough with 59.7% detection of MAC (83 out of 139 anti-AChR positive patients) and mean fluorescence intensity of MAC and clinical severity also showed a modest positive association (85). Using humanized mouse anti-AChR antibody, mouse diaphragm and normal human serum, Plomp et al. (86) were able to visualize anti-AChR driven NMJ-restricted complement damage, complement deposition at NMJ, which correlated with electrophysiological findings.

RCT data showed that not every patient who received complement therapy achieved the desirable response and hence the biomarkers

either to predict or monitor the treatment response are much needed for a personalized medicine approach for cost effectiveness and minimization of unnecessary drug exposure and the related side effects in patients. Serological analysis of complement components and activation products such as C3a, C5a and soluble C5b9 and *in vitro* complement function assay such as haemolytic assays CH50 for classical pathway could reveal evidence of complement consumption, abnormal activation of the complement system and patient specific complement activation status and can be useful to monitor complement function during disease exacerbation (87). C3 levels were reported to be inversely correlated with disease severity in terms of QMG in AChR-MG patients (31). Combinations of drug levels, C5 function and complement haemolytic CH50 can be potential therapeutic monitoring assays for eculizumab in PNH and aHUS. In an aHUS study, a composite marker C3:CH50 changes significantly during induction and maintenance phases of eculizumab and correlates with disease markers (88) CH50 assay has been adopted in generalized myasthenia gravis studies. A case report described that eculizumab administration in an AChR gMG patient was able to decrease CH50 levels in line with a clinical improvement (89). CH50 assay was also used to define zilucoplan dose for optimal complement inhibition in a phase 2 trial (90). *In vitro* complement activity assays such as CH50 are haemolytic assays and the optimal assay for myasthenia gravis should be looking at the *in-vitro* direct neuromuscular junction damage by the complement. In fact, Fichtner et al. (91) showed that there was no correlation between CH50 and AChR antibody levels or disease severity in AChR antibody-positive patients. Hence, MG researchers have yet to fully explore and develop *in vitro* complement functional assays specific to MG.

Complotypes are genetic variants affecting complement activity and hence responsible for complement mediated diseases, differences between disease severities between individuals and treatment response (92). A few rare variants have been identified in PNH patients who did not respond to eculizumab. (Missense C5 heterozygous variants preventing its binding to eculizumab, HindIII polymorphism of the complement regulatory gene CR1) (92, 93).

3. Conclusion

We have described the available evidence of the complement system involvement in the pathogenesis of AChR-MG although the direct evidence of how complement system could be initiated specifically by anti-AChR IgGs is not available yet. We have summarized approved complement therapies backed with RCT, OLE and real-world experience data on efficacy and safety and briefly mentioned developing complement therapies in the pipeline. Biomarkers are still needed to be able to ultra-stratify MG patients into potential specific complement therapies responder group or groups so that a personalized approach could be provided to the patients in future.

Author contributions

PS: Conceptualization, Software, Writing – original draft, Writing – review & editing. SJ: Conceptualization, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

The authors are grateful to Myaware Charity UK and University of Birmingham for funding APC. Figures were drawn using www.biorender.com.

Conflict of interest

PS is currently doing a PhD and Research and Clinical Fellowship with the funding from UK myasthenia patient charity, Myaware and has received travel grants from MGFA and Myaware. SJ has served as an international advisory board member for Alexion, Alnylam, Argenx, Regeneron, Immunovant and UCB pharmaceuticals, is currently an expert panel member of Myasthenia Gravis consortium for Argenx pharmaceuticals and has received speaker fees from Argenx, Eisai, Terumo BCT and UCB pharmaceuticals. SJ is also a board member (trustee) of the UK myasthenia patient charity, Myaware.

Publisher's note

References

1. Robertson NP, Deans J, Compston DAS. Myasthenia gravis: a population based epidemiological study in Cambridgeshire, England. *J Neurol Neurosurg Psychiatry*. (1998) 65:492–6. doi: 10.1136/jnnp.65.4.492

2. Carr AS, Cardwell CR, Mccarron PO, Mcconville J. A systematic review of population based epidemiological studies in myasthenia gravis. *BMC Neurol.* (2010) 10:46. doi: 10.1186/1471-2377-10-46

3. Grob D, Brunner N, Namba T, Pagala M. Lifetime course of myasthenia gravis. *Muscle Nerve.* (2008) 37:141. doi: 10.1002/mus.20950

4. Hoch W, McConville J, Helms S, Newsom-Davis J, Melms A, Vincent A. Auto-antibodies to the receptor tyrosine kinase MuSK in patients with myasthenia gravis without acetylcholine receptor antibodies. *Nat Med.* (2001) 7:365–8. doi: 10.1038/85520

5. Higuchi O, Hamuro J, Motomura M, Yamanashi Y. Autoantibodies to low-density lipoprotein receptor-related protein 4 in myasthenia gravis. *Ann Neurol.* (2011) 69:418–22. doi: 10.1002/ana.22312

6. Pevzner A, Schoser B, Peters K, Cosma NC, Karakatsani A, Schalke B, et al. Anti-LRP4 autoantibodies in AChR- and MuSK-antibody-negative myasthenia gravis. *J Neurol.* (2012) 259:427–35. doi: 10.1007/s00415-011-6194-7

7. Tzartos JS. Autoantibodies to lipoprotein-related protein 4 in patients with double-seronegative myasthenia gravis. *Arch Neurol.* (2012) 69:445–51. doi: 10.1001/archneurol.2011.2393

8. Tzartos SJ, Barkas T, Cung MT, Mamalaki A, Marraud M, Orlewski P, et al. Anatomy of the antigenic structure of a large memberane autoantigen, the muscle-type

nicotinic acetylcholine receptor. *Immunol Rev*. (1998) 163:89–120. doi: 10.1111/j.1600-065X.1998.tb01190.x

9. Vincent A. Experimental myasthenia gravis—a new autoimmune model. *Trends Biochem Sci*. (1976) 1:289–91. doi: 10.1016/S0968-0004(76)80137-5

10. Lindstrom J, Shelton D, Fujii Y. Myasthenia gravis. *Adv Immunol*. (1988) 42:233–84. doi: 10.1016/S0065-2776(08)60847-0

11. Tzartos SJ, Lindstrom JM. Monoclonal antibodies used to probe acetylcholine receptor structure: localization of the main immunogenic region and detection of similarities between subunits. *Proc Natl Acad Sci U S A*. (1980) 77:755–9. doi: 10.1073/pnas.77.2.755

12. Luo J, Lindstrom J. Antigenic structure of the human muscle nicotinic acetylcholine receptor main immunogenic region. *J Mol Neurosci*. (2010) 40:217–20. doi: 10.1007/s12031-009-9271-y

13. Cetin H, Webster R, Liu WW, Nagaishi A, Koneczny I, Zimprich F, et al. Myasthenia gravis AChR antibodies inhibit function of rapsyn-clustered AChRs. *J Neurol Neurosurg Psychiatry*. (2020) 91:526–32. doi: 10.1136/jnnp-2019-322640

14. Matthews-Bellinger J, Salpeter M. Fine structural distribution of acetylcholine receptors at developing mouse neuromuscular junctions. *J Neurosci*. (1983) 3:644–57. doi: 10.1523/JNEUROSCI.03-03-00644.1983

15. Engel AG, Sahashi K, Fumagalli G. The immunopathology of acquired myasthenia gravis. *Ann N Y Acad Sci*. (1981) 377:158–74. doi: 10.1111/j.1749-6632.1981.tb33730.x

16. Martinez-Pena Y, Valenzuela I, Akaaboune M. The metabolic stability of the nicotinic acetylcholine receptor at the neuromuscular junction. *Cells*. (2021) 10:358. doi: 10.3390/cells10020358

17. Heinemann S, Bevan S, Kullberg R, Lindstrom J, Rice J. Modulation of acetylcholine receptor by antibody against the receptor. *Proc Natl Acad Sci U S A*. (1977) 74:3090–4. doi: 10.1073/pnas.74.7.3090

18. Drachman DB, Adams RN, Josifek LF, Self SG. Functional activities of autoantibodies to acetylcholine receptors and the clinical severity of myasthenia gravis. *N Engl J Med*. (1982) 307:769–75. doi: 10.1056/NEJM198209233071301

19. Drachman DB, Angus CW, Adams RN, Michelson JD, Hoffman GJ. Myasthenic antibodies cross-link acetylcholine receptors to accelerate degradation. *N Engl J Med*. (1978) 298:1116–22. doi: 10.1056/NEJM197805182982004

20. Sophianos D, Tzartos SJ. Fab fragments of monoclonal antibodies protect the human acetylcholine receptor against antigenic modulation caused by myasthenic sera. *J Autoimmun*. (1989) 2:777–89. doi: 10.1016/0896-8411(89)90004-8

21. Bergman RA, Johns RJ. Ultrastructural alterations in muscle from patients with myasthenia gravis and Eaton–Lumbert syndrome. *Ann N Y Acad Sci*. (1971) 183:88–122. doi: 10.1111/j.1749-6632.1971.tb30744.x

22. Rash JE, Albuquerque EX, Hudson CS, Mayer RF, Satterfield JR. Studies of human myasthenia gravis: electrophysiological and ultrastructural evidence compatible with antibody attachment to acetylcholine receptor complex. *Proc Natl Acad Sci U S A*. (1976) 73:4584–8. doi: 10.1073/pnas.73.12.4584

23. Kemper C, Atkinson JP. T-cell regulation: with complements from innate immunity. *Nat Rev Immunol*. (2007) 7:9–18. doi: 10.1038/nri1994

24. Botto M, Walport MJ. C1q, autoimmunity and apoptosis. *Immunobiology*. (2002) 205:395–406. doi: 10.1078/0171-2985-00141

25. Dunkelberger JR, Song WC. Complement and its role in innate and adaptive immune responses. *Cell Res*. (2010) 20:34–50. doi: 10.1038/cr.2009.139

26. Nastuk WL, Plescia OJ, Osserman KE. Changes in serum complement activity in patients with myasthenia gravis. *Exp Biol Med*. (1960) 105:177–84. doi: 10.3181/00379727-105-26050

27. Engel AG, Arahata K. The membrane attack complex of complement at the endplate in myasthenia gravis. *Ann N Y Acad Sci*. (1987) 505:326–32. doi: 10.1111/j.1749-6632.1987.tb51301.x

28. Tsujihata M, Yoshimura T, Satoh A, Kinoshita I, Matsuo H, Mori M, et al. Diagnostic significance of IgG, C3, and C9 at the limb muscle motor end-plate in minimal myasthenia gravis. *Neurology*. (1989) 39:1359–63. doi: 10.1212/WNL.39.10.1359

29. Sahashi K, Engel AG, Linstrom JM, Lambert EH, Lennon VA. Ultrastructural localization of immune complexes (IgG and C3) at the end-plate in experimental autoimmune myasthenia gravis. *J Neuropathol Exp Neurol*. (1978) 37:212–23. doi: 10.1097/00005072-197803000-00008

30. Romi F, Kristoffersen EK, Aarli JA, Gilhus NE. The role of complement in myasthenia gravis: serological evidence of complement consumption *in vivo*. *J Neuroimmunol*. (2005) 158:191–4. doi: 10.1016/j.jneuroim.2004.08.002

31. Liu A, Lin H, Liu Y, Cao X, Wang X, Li Z. Correlation of C3 level with severity of generalized myasthenia gravis. *Muscle Nerve*. (2009) 40:801–8. doi: 10.1002/mus.21398

32. Jacob S, Viegas S, Leite MI, Webster R, Cossins J, Kennett R, et al. Presence and pathogenic relevance of antibodies to clustered acetylcholine receptor in ocular and generalized myasthenia gravis. *Arch Neurol*. (2012) 69:994–1001. doi: 10.1001/archneurol.2012.437

33. Lennon VA, Seybold ME, Lindstrom JM, Cochrane C, Ulevitch R. Role of complement in the pathogenesis of experimental autoimmune myasthenia gravis. *J Exp Med*. (1978) 147:973–83. doi: 10.1084/jem.147.4.973

34. Tüzün E, Scott BG, Goluszko E, Higgs S, Christadoss P. Genetic evidence for involvement of classical complement pathway in induction of experimental autoimmune myasthenia gravis. *J Immunol*. (2003) 171:3847–54. doi: 10.4049/jimmunol.171.7.3847

35. Christadoss P. C5 gene influences the development of murine myasthenia gravis. *J Immunol*. (1988) 140:2589–92. doi: 10.4049/jimmunol.140.8.2589

36. Chamberlain-Banoub J, Neal JW, Mizuno M, Harris CL, Morgan BP. Complement membrane attack is required for endplate damage and clinical disease in passive experimental myasthenia gravis in Lewis rats. *Clin Exp Immunol*. (2006) 146:278–86. doi: 10.1111/j.1365-2249.2006.03198.x

37. Hughes-Jones NC, Gardner B. Reaction between the isolated globular sub-units of the complement component C1q and IgG-complexes. *Mol Immunol*. (1979) 16:697–701. doi: 10.1016/0161-5890(79)90010-5

38. Burton DR. Antibody: the flexible adaptor molecule. *Trends Biochem Sci*. (1990) 15:64–9. doi: 10.1016/0968-0004(90)90178-E

39. Diebolder CA, Beurskens FJ, de Jong RN, Koning RI, Strumane K, Lindorfer MA, et al. Complement is activated by IgG hexamers assembled at the cell surface. *Science*. (2014) 343:1260–3. doi: 10.1126/science.1248943

40. Wang G, de Jong RN, van den Bremer ETJ, Beurskens FJ, Labrijn AF, Ugurlar D, et al. Molecular basis of assembly and activation of complement component C1 in complex with immunoglobulin G1 and antigen. *Mol Cell*. (2016) 63:135–45. doi: 10.1016/j.molcel.2016.05.016

41. Strasser J, de Jong RN, Beurskens FJ, Wang G, Heck AJR, Schuurman J, et al. Unraveling the macromolecular pathways of IgG oligomerization and complement activation on antigenic surfaces. *Nano Lett*. (2019) 19:4787–96. doi: 10.1021/acs.nanolett.9b02220

42. Miwa T, Song WC. Membrane complement regulatory proteins: insight from animal studies and relevance to human diseases. *Int Immunopharmacol*. (2001) 1:445–59. doi: 10.1016/S1567-5769(00)00043-6

43. Lublin DM, Atkinson JP. Decay-accelerating factor: biochemistry, molecular biology, and function. *Annu Rev Immunol*. (1989) 7:35–58. doi: 10.1146/annurev.iy.07.040189.000343

44. Liszewski MK, Post TW, Atkinson JP. Membrane cofactor protein (MCP or CD46): newest member of the regulators of complement activation gene cluster. *Annu Rev Immunol*. (1991) 9:431–55. doi: 10.1146/annurev.iy.09.040191.002243

45. Ahearn JM, Fearon DT. Structure and function of the complement receptors, CR1 (CD35) and CR2 (CD21). *Adv Immunol*. (1989) 46:183–219. doi: 10.1016/S0065-2776(08)60654-9

46. Meri S, Morgan BP, Davies A, Daniels RH, Olavesen MG, Waldmann H, et al. Human protectin (CD59), an 18,000–20,000 MW complement lysis restricting factor, inhibits C5b-8 catalysed insertion of C9 into lipid bilayers. *Immunology*. (1990) 71:1–9.

47. Rollins SA, Sims PJ. The complement-inhibitory activity of CD59 resides in its capacity to block incorporation of C9 into membrane C5b-9. *J Immunol*. (1990) 144:3478–83. doi: 10.4049/jimmunol.144.9.3478

48. Kaminski HJ, Kusner LL, Richmonds C, Medof ME, Lin F. Deficiency of decay accelerating factor and CD59 leads to crisis in experimental myasthenia. *Exp Neurol*. (2006) 202:287–93. doi: 10.1016/j.expneurol.2006.06.003

49. Lin F, Kaminski HJ, Conti-Fine BM, Wang W, Richmonds C, Medof ME. Markedly enhanced susceptibility to experimental autoimmune myasthenia gravis in the absence of decay-accelerating factor protection. *J Clin Invest*. (2002) 110:1269–74. doi: 10.1172/JCI0216086

50. Kaminski H, Li Z, Richmonds C, Lin F, Medof M. Complement regulators in extraocular muscle and experimental autoimmune myasthenia gravis. *Exp Neurol*. (2004) 189:333–42. doi: 10.1016/j.expneurol.2004.06.005

51. Basta M, Kirshbom P, Frank MM, Fries LF. Mechanism of therapeutic effect of high-dose intravenous immunoglobulin. Attenuation of acute, complement-dependent immune damage in a guinea pig model. *J Clin Invest*. (1989) 84:1974–81. doi: 10.1172/JCI114387

52. Lünemann JD, Nimmerjahn F, Dalakas MC. Intravenous immunoglobulin in neurology—mode of action and clinical efficacy. *Nat Rev Neurol*. (2015) 11:80–9. doi: 10.1038/nrneurol.2014.253

53. Pittock SJ, Berthele A, Fujihara K, Kim HJ, Levy M, Palace J, et al. Eculizumab in aquaporin-4-positive neuromyelitis optica spectrum disorder. *N Engl J Med*. (2019) 381:614–25. doi: 10.1056/NEJMoa1900866

54. Howard JF, Barohn RJ, Cutter GR, Freimer M, Juel VC, Mozaffar T, et al. A randomized, double-blind, placebo-controlled phase II study of eculizumab in patients with refractory generalized myasthenia gravis. *Muscle Nerve*. (2013) 48:76–84. doi: 10.1002/mus.23839

55. Howard JF, Utsugisawa K, Benatar M, Murai H, Barohn RJ, Illa I, et al. Safety and efficacy of eculizumab in anti-acetylcholine receptor antibody-positive refractory generalised myasthenia gravis (REGAIN): a phase 3, randomised, double-blind, placebo-controlled, multicentre study. *Lancet Neurol*. (2017) 16:976–86. doi: 10.1016/S1474-4422(17)30369-1

56. Muppidi S, Utsugisawa K, Benatar M, Murai H, Barohn RJ, Illa I, et al. Long-term safety and efficacy of eculizumab in generalized myasthenia gravis. *Muscle Nerve*. (2019) 60:14–24. doi: 10.1002/mus.26447

57. Howard JF, Karam C, Yountz M, O'Brien FL, Mozaffar TREGAIN Study Group. Long-term efficacy of eculizumab in refractory generalized myasthenia gravis: responder analyses. *Ann Clin Transl Neurol*. (2021) 8:1398–407. doi: 10.1002/acn3.51376

58. Vissing J, Jacob S, Fujita KP, O'Brien F, Howard JFREGAIN Study Group. "Minimal symptom expression" in patients with acetylcholine receptor antibody-positive refractory generalized myasthenia gravis treated with eculizumab. *J Neurol*. (2020) 267:1991–2001. doi: 10.1007/s00415-020-09770-y

59. Mantegazza R, Wolfe GI, Muppidi S, Wiendl H, Fujita KP, O'Brien FL, et al. Post-intervention status in patients with refractory myasthenia gravis treated with eculizumab during REGAIN and its open-label extension. *Neurology*. (2021) 96:e610–8. doi: 10.1212/WNL.0000000000011207

60. Jacob S, Murai H, Utsugisawa K, Nowak RJ, Wiendl H, Fujita KP, et al. Response to eculizumab in patients with myasthenia gravis recently treated with chronic IVIg: a subgroup analysis of REGAIN and its open-label extension study. *Ther Adv Neurol Disord*. (2020) 13:175628642091178. doi: 10.1177/1756286420911784

61. Siddiqi ZA, Nowak RJ, Mozaffar T, O'Brien F, Yountz M, Patti F, et al. Eculizumab in refractory generalized myasthenia gravis previously treated with rituximab: subgroup analysis of REGAIN and its extension study. *Muscle Nerve*. (2021) 64:662–9. doi: 10.1002/mus.27422

62. Usman U, Chrisman C, Houston D, Haws CC, Wang A, Muley S. The use of eculizumab in ventilator-dependent myasthenia gravis patients. *Muscle Nerve*. (2021) 64:212–5. doi: 10.1002/mus.27326

63. Hofstadt-van Oy U, Stankovic S, Kelbel C, Oswald D, Larrosa-Lombardi S, Barchfeld T, et al. Complement inhibition initiated recovery of a severe myasthenic crisis with COVID-19. *J Neurol*. (2021) 268:3125–8. doi: 10.1007/s00415-021-10428-6

64. Katyal N, Narula N, Govindarajan R. Clinical experience with eculizumab in treatment-refractory acetylcholine receptor antibody-positive generalized myasthenia gravis. *J Neuromuscul Dis*. (2021) 8:287–94. doi: 10.3233/JND-200584

65. Nelke C, Schroeter CB, Stascheit F, Pawlitzki M, Regner-Nelke L, Huntemann N, et al. Eculizumab versus rituximab in generalised myasthenia gravis. *J Neurol Neurosurg Psychiatry*. (2022) 93:548–54. doi: 10.1136/jnnp-2021-328665

66. Datta S, Singh S, Govindarajan R. Retrospective analysis of eculizumab in patients with acetylcholine receptor antibody-negative myasthenia gravis: a case series. *J Neuromuscul Dis*. (2020) 7:269–77. doi: 10.3233/JND-190464

67. Vélez-Santamaría V, Nedkova V, Díez L, Homedes C, Alberti MA, Casasnovas C. Eculizumab as a promising treatment in thymoma-associated myasthenia gravis. *Ther Adv Neurol Disord*. (2020) 13:175628642093203. doi: 10.1177/1756286420932035

68. Amano E, Otsu S, Suzuki S, Machida A. Eculizumab improved weakness and taste disorder in thymoma-associated generalized myasthenia gravis with anti-striational antibodies: a case report. *eNeurologicalSci*. (2019) 14:72–3. doi: 10.1016/j.ensci.2019.01.006

69. Greenwood GT, Lynch Z. Successful transition from plasma exchange to eculizumab in acetylcholine receptor antibody- and muscle-specific kinase (MuSK) antibody-negative myasthenia gravis: a case report. *Am J Case Rep*. (2020) 21:e921431. doi: 10.12659/AJCR.921431

70. Tice JA, Touchette DR, Lien PW, Agboola F, Nikitin D, Pearson SD. The effectiveness and value of eculizumab and efgartigimod for generalized myasthenia gravis. *J Manag Care Spec Pharm*. (2022) 28:119–24. doi: 10.18553/jmcp.2022.28.1.119

71. Vu T, Meisel A, Mantegazza R, Annane D, Katsuno M, Aguzzi R, et al. Terminal complement inhibitor ravulizumab in generalized myasthenia gravis. *NEJM Evid*. (2022) 1. doi: 10.1056/EVIDoa2100066

72. Meisel A, Annane D, Vu T, Mantegazza R, Katsuno M, Aguzzi R, et al. Long-term efficacy and safety of ravulizumab in adults with anti-acetylcholine receptor antibody-positive generalized myasthenia gravis: results from the phase 3 CHAMPION MG open-label extension. *J Neurol*. (2023) 270:3862–75. doi: 10.1007/s00415-023-11699-x

73. Howard JF, Vissing J, Gilhus NE, Leite MI, Utsugisawa K, Duda PW, et al. Zilucoplan: an investigational complement C5 inhibitor for the treatment of acetylcholine receptor autoantibody-positive generalized myasthenia gravis. *Expert Opin Investig Drugs*. (2021) 30:483–93. doi: 10.1080/13543784.2021.1897567

74. Howard JF, Bresch S, Genge A, Hewamadduma C, Hinton J, Hussain Y, et al. Safety and efficacy of zilucoplan in patients with generalised myasthenia gravis (RAISE): a randomised, double-blind, placebo-controlled, phase 3 study. *Lancet Neurol*. (2023) 22:395–406. doi: 10.1016/S1474-4422(23)00080-7

75. Weyne J, Ni Y, DelGizzi R, Godin S, Morton L, Prasad S, et al. A randomized, double-blind, placebo-controlled phase 1 study of the pharmacokinetics and pharmacodynamics of REGN3918, a human antibody against complement factor C5, in healthy volunteers. *Blood*. (2018) 132:1039. doi: 10.1182/blood-2018-99-112262

76. Devalaraja-Narashimha K, Huang C, Cao M, Chen YP, Borodovsky A, Olson WC, et al. Pharmacokinetics and pharmacodynamics of pozelimab alone or in combination with cemdisiran in non-human primates. *PLoS One*. (2022) 17:e0269749. doi: 10.1371/journal.pone.0269749

77. Zelek WM, Xie L, Morgan BP, Harris CL. Compendium of current complement therapeutics. *Mol Immunol*. (2019) 114:341–52. doi: 10.1016/j.molimm.2019.07.030

78. Dalakas MC. Role of complement, anti-complement therapeutics, and other targeted immunotherapies in myasthenia gravis. *Expert Rev Clin Immunol*. (2022) 18:691–701. doi: 10.1080/1744666X.2022.2082946

79. Food and Drug Administration. *Food and Drug Administration. Soliris product insert*. Silver Spring, MD: US Department of Health and Human Services, Food and Drug Administration (2017, 2017) Available at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/125166s417lbl.pdf.

80. CDC. *Meningococcal ACIP recommendations*. Atlanta, GA: US Department of Health and Human Services, CDC (2017) Available at: https://www.cdc.gov/vaccines/hcp/acip-recs/vacc-specific/mening.html.

81. McNamara LA, Topaz N, Wang X, Hariri S, Fox L, MacNeil JR. High risk for invasive meningococcal disease among patients receiving eculizumab (soliris) despite receipt of meningococcal vaccine. *MMWR Morb Mortal Wkly Rep*. (2017) 66:734–7. doi: 10.15585/mmwr.mm6627e1

82. Matsumura Y. Risk analysis of eculizumab-related meningococcal disease in Japan using the Japanese adverse drug event report database. *Drug Healthc Patient Saf*. (2020) 12:207–15. doi: 10.2147/DHPS.S257009

83. Langereis JD, van den Broek B, Franssen S, Joosten I, Blijlevens NMA, de Jonge MI, et al. Eculizumab impairs *Neisseria meningitidis* serogroup B killing in whole blood despite 4CMenB vaccination of PNH patients. *Blood Adv*. (2020) 4:3615–20. doi: 10.1182/bloodadvances.2020002497

84. EMA. *Soliris-EPAR-product information*. Available at: https://www.ema.europa.eu/en/documents/product-information/soliris-epar-product-information_en.pdf.

85. Obaid AH, Zografou C, Vadysirisack DD, Munro-Sheldon B, Fichtner ML, Roy B, et al. Heterogeneity of acetylcholine receptor autoantibody–mediated complement activity in patients with myasthenia gravis. *Neurol Neuroimmunol Neuroinflamm*. (2022) 9:e1169. doi: 10.1212/NXI.0000000000001169

86. Plomp JJ, Huijbers MGM, Verschuuren JJGM, Borodovsky A. A bioassay for neuromuscular junction-restricted complement activation by myasthenia gravis acetylcholine receptor antibodies. *J Neurosci Methods*. (2022) 373:109551. doi: 10.1016/j.jneumeth.2022.109551

87. Mantegazza R, Vanoli F, Frangiamore R, Cavalcante P. Complement inhibition for the treatment of myasthenia gravis. *Immunotargets Ther*. (2020) 9:317–31. doi: 10.2147/ITT.S261414

88. Wijnsma KL, Ter Heine R, Moes DJAR, Langemeijer S, Schols SEM, Volokhina EB, et al. Pharmacology, pharmacokinetics and pharmacodynamics of eculizumab, and possibilities for an individualized approach to eculizumab. *Clin Pharmacokinet*. (2019) 58:859–74. doi: 10.1007/s40262-019-00742-8

89. Yanagidaira M, Nishida Y, Yokota T. Temporal correlation between serum CH50 level and symptom severity of myasthenia gravis during eculizumab therapy. *Clin Neurol Neurosurg*. (2020) 189:105630. doi: 10.1016/j.clineuro.2019.105630

90. Howard JF, Nowak RJ, Wolfe GI, Freimer ML, Vu TH, Hinton JL, et al. Clinical effects of the self-administered subcutaneous complement inhibitor zilucoplan in patients with moderate to severe generalized myasthenia gravis. *JAMA Neurol*. (2020) 77:582–92. doi: 10.1001/jamaneurol.2019.5125

91. Fichtner ML, Hoarty MD, Vadysirisack DD, Munro-Sheldon B, Nowak RJ, O'Connor KC. Myasthenia gravis complement activity is independent of autoantibody titer and disease severity. *PLoS One*. (2022) 17:e0264489. doi: 10.1371/journal.pone.0264489

92. Nishimura J, Ichi YM, Hayashi S, Ohyashiki K, Ando K, Brodsky AL, et al. Genetic variants in C5 and poor response to eculizumab. *N Engl J Med*. (2014) 370:632–9. doi: 10.1056/NEJMoa1311084

93. Schatz-Jakobsen JA, Zhang Y, Johnson K, Neill A, Sheridan D, Andersen GR. Structural basis for eculizumab-mediated inhibition of the complement terminal pathway. *J Immunol*. (2016) 197:337–44. doi: 10.4049/jimmunol.1600280

frontiers | Frontiers in Neurology

TYPE Original Research
PUBLISHED 28 December 2023
DOI 10.3389/fneur.2023.1320344

OPEN ACCESS

EDITED BY
Francesco Saccà,
University of Naples Federico II, Italy

REVIEWED BY
Wladimir Bocca Vieira De Rezende Pinto,
Federal University of São Paulo, Brazil
Fasheng Li,
Dalian Medical University, China

*CORRESPONDENCE
Wenzeng Zhu
✉ zhuwenzeng2630@gamyy.cn

†These authors have contributed equally to this work and share first authorship

RECEIVED 12 October 2023
ACCEPTED 15 December 2023
PUBLISHED 28 December 2023

CITATION
Tian Y, Shen Q, Peng S, Meng L, Fang R, Xiong A, Li S, Yang Y, Chang W, Ni J and Zhu W (2023) Mapping current trends and hotspots in myasthenia gravis from 2003 to 2022: a bibliometric analysis.
Front. Neurol. 14:1320344.
doi: 10.3389/fneur.2023.1320344

Mapping current trends and hotspots in myasthenia gravis from 2003 to 2022: a bibliometric analysis

Yukun Tian[1†], Qiqi Shen[1†], Siyang Peng[1], Linghao Meng[1], Ruiying Fang[1], Anni Xiong[1], Shaohong Li[2], Yajing Yang[3], Weiqian Chang[4], Jinxia Ni[5] and Wenzeng Zhu[1*]

[1]Department of Acupuncture, Guang'anmen Hospital, China Academy of Chinese Medical Sciences, Beijing, China, [2]Treatment Center of Traditional Chinese Medicine Bo'ai Hospital, China Rehabilitation Research Center, Beijing, China, [3]Department of Traditional Chinese Medicine, Yuyuantan Community Health Center, Beijing, China, [4]Department of Acupuncture, Guang'anmen Hospital, Chinese Academy of Traditional Chinese Medicine Ji'nan Hospital (Ji'nan Hospital of Traditional Chinese Medicine), Jinan, Shandong, China, [5]Department of Acupuncture, Dongzhimen Hospital of Beijing University of Traditional Chinese Medicine, Beijing, China

Introduction: Research on myasthenia gravis (MG) has undergone rapid development in recent years. This article aimed to elucidate the characteristics of MG publications over the past 20 years and analyze emerging trends using bibliometric methods.

Methods: Information on MG articles was obtained from the Web of Science Core Collection and stored in Excel for quantitative analyses. Bibliometric analyses were performed using CiteSpace and VOSviewer to visualize publications according to countries/regions, institutions, journals, and authors.

Results: A total of 3,610 publications were included in the analysis. The USA had the highest number of publications (NP) and H-index. Among the institutions, the University of Oxford had the highest NP, followed by the University of Toronto and Duke University. Close cooperation was observed among countries and institutions. The most productive author was Renato Mantegazza, followed by Jan J. Verschuuren, and Amelia Evoli. *Muscle & Nerve* published the most articles on MG, followed by the *Journal of Neuroimmunology* and *Neuromuscular Disorders*. The keyword with the highest strength is "neuromuscular transmission," followed by "safety" and "rituximab." Co-citation analysis includes 103 publications cited at least 65 times, categorized into four clusters. Additionally, 123 keywords cited more than 40 times were analyzed and divided into five clusters.

Conclusion: This bibliometric analysis shows the framework of research over the past 20 years by mapping the scholarly contributions of various countries or regions, institutions, journals, and authors in MG. The analysis also explores future trends and prospective directions, emphasizing individualized treatment based on subtypes, novel immunotherapeutic approaches, and thymectomy.

KEYWORDS

myasthenia gravis, bibliometric analysis, VOSviewer, CiteSpace, citations, keywords

1 Introduction

Myasthenia gravis (MG) is an autoimmune disease characterized by skeletal muscle weakness and fatigability. It is caused by antibodies directed against proteins at the neuromuscular junction, including the acetylcholine receptor (AChR), muscle-specific kinase (MuSK), lipoprotein-related protein 4 (LRP4), and the postsynaptic membrane (1). The prevalence of MG is estimated to be 100 to 350 cases per 1 million people, with increasing trends likely due to improved diagnostics, aging populations, and the number of systematic epidemiological studies conducted over the past decade (2–4).

Bibliometric analysis has become an internationally recognized tool since 1958 that not only contributes to important decisions about government budgets (5), but more significantly, also helps understand new research areas in rapidly advancing fields. By analyzing the relationships between journals, citations, keywords, and scholars, it enables the creation of visual maps to represent publications and academic networks. MG research has expanded substantially in recent years. The aim of this study was to use bibliometric techniques to characterize patterns in MG publications over the past 20 years and elucidate trends in this growing area of inquiry.

2 Methods

2.1 Data acquisition and search strategy

Data were collected from the Web of Science Core Collection, and the search period was set between 1 January 2003 and 31 December 2022. A literature search was conducted on 9 June 2023. The search terms were as follows: ts = "myasthenia gravis" (ts = topic), and 6,898 publications were identified. The articles and reviews in English were analyzed after removing the meeting abstracts, letters, editorial materials, proceedings, corrections, early accesses, news items, retractions, biographical items, book chapters, and retracted publications. A total of 3,130 articles and 480 reviews were included in the analysis. The collected data included the journal titles, authors, keywords, countries or regions, institutions, and cited references, and the downloaded data were screened by S.Y. Peng to identify the relationships on the topic. The strategy for accessing and searching for articles is shown in Figure 1.

2.2 Data analysis

A quantitative analysis of the number of publications (NP) and citations (NC) in the most productive countries, authors, journals, and affiliations was conducted using Microsoft Office Excel 2013. VOSviewer (Centre for Science and Technology Studies, Leiden University, Leiden, the Netherlands) is a computer program used to create scientific maps for co-citation analysis, co-occurrence of keywords, and co-authorship analysis of authors, affiliations, and countries (6). CiteSpace was used to visualize and analyze bursts of references and keywords (7).

Abbreviations: NC, Number of citations; NP, Number of publications.

3 Results

3.1 Annual trends in the number of publications

An annual analysis of NP directly reflects trends in a specific research field over a given period. The annual publications of MG generally showed a steady growth trend, peaking in 2021, as shown in Figure 2.

3.2 National research status and international cooperation

Figure 3 displays the world distribution map based on the NP in different countries. In this figure, the depth of the color represents the magnitude of NP. The top 10 countries in NP are presented in Table 1. It is evident that the USA had the highest NP over the past two decades in MG research, followed by China, Japan, the UK, and Italy. The USA had the highest number of total citations after removing self-citations, followed by the UK, Italy, China, and Japan, with H-indices of 68, 49, 45, 37, and 38, respectively. H-indices reflect the general quality of a country's literature. Figure 4 present a bubble chart of the yearly publications of the top 10 countries in NP is shown in Figure 4, demonstrating an annual increase from 2003 to 2022. The USA led in yearly publications until 2014, after which China took the lead and continued until 2022.

In the co-authorship analysis (Figure 5), 55 countries with a minimum of five publications were included. Countries were divided into eight clusters of different colors. Bold lines between countries indicate closer collaborations, and the node size reflects the centrality and influence of the country on associations. Owing to the diversity of scientific research foundations and resources in other countries or regions, a specific collaborative network structure was observed at the national or regional scale. According to this map, cooperation between most countries was close. The USA, China, Japan, the UK, Italy, France, and Canada were the central countries in cooperation, with the USA being the most influential.

3.3 Analysis of institutions

The top 10 institutions in the total NP in this research area are listed in Table 2. Oxford University had the highest NP, with 94 publications, followed by the University of Toronto (62) and Duke University (61). One hundred sixty institutions with 10 or more publications were included in VOSviewer for co-authorship analysis. As shown in Figure 6A, the affiliations were divided into nine clusters. Close collaborations among Oxford University, Leiden University, and Duke University were apparent. Meanwhile, Fudan University, Istanbul University, University of Toronto, University of Bergen, and Chiba University were the centers of the collaboration clusters. However, because to geographical distance, a lack of cooperation between several institutions from different countries was observed, whereas cooperation was closer within each cluster because the institutions in a cluster were from the same countries. We also visualized the time overlay for the co-authorship institutional analysis using VOSviewer. As shown in Figure 6B, the institutions were divided

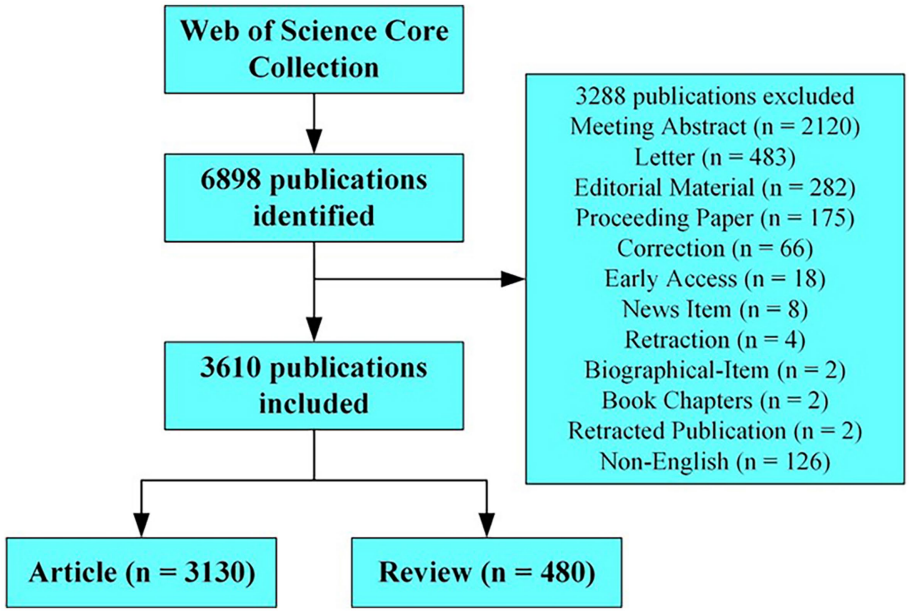


FIGURE 1
Flowchart of literature screening.

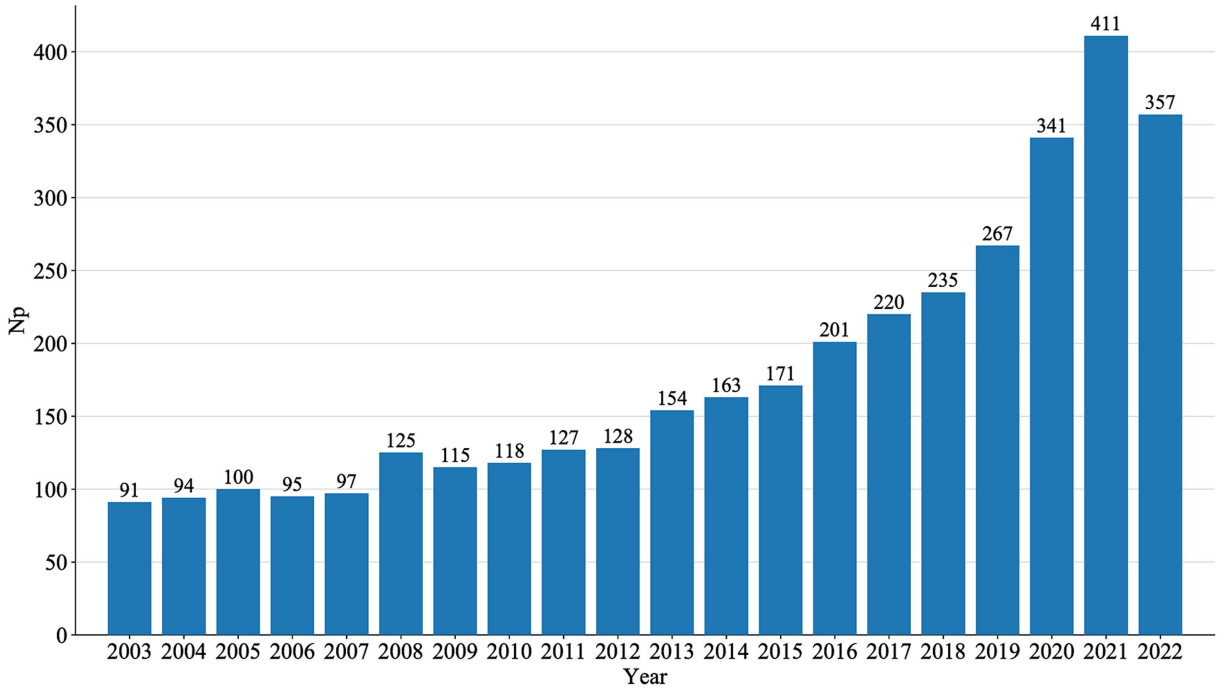


FIGURE 2
Current status of global publications.

into different colors according to the average year of publication; the institutions in yellow were active later than those in blue. The University of Toronto, Capital Medical University, and Shandong University recently appeared more in cooperation, indicating that they were more active in MG research and full of potential in international cooperation.

3.4 Analysis of journals

The influence of a journal is expressed not only by the NP, but also by the NC. In Table 3, we summarized the top 10 journals with the highest NP in MG research. The journal with the highest NP was *Muscle & Nerve* ($n = 224$), followed by the *Journal of Neuroimmunology*

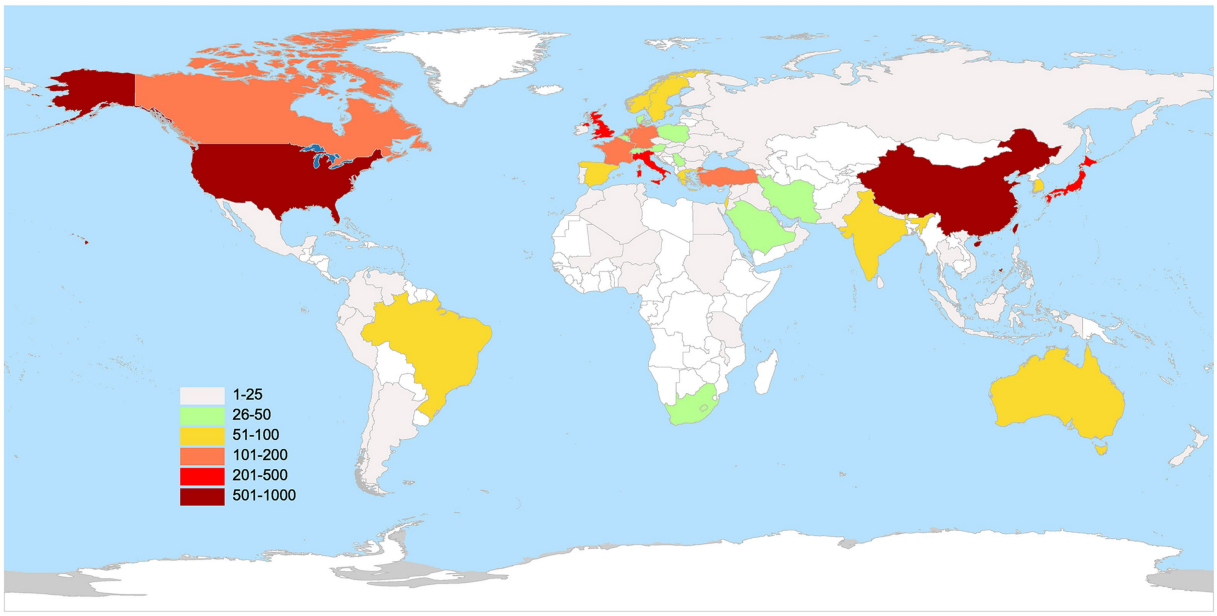


FIGURE 3
The geography distribution map manifested the number of publications in distinct countries.

TABLE 1 The top 10 most productive countries.

Rank	Country	Np	Nc	H-index	Average citations
1	China	747	7,736	37	10.36
2	the United States	767	20,097	68	26.20
3	Japan	375	7,369	38	19.65
4	Italy	269	8,236	45	30.62
5	England	271	9,220	49	34.02
6	Germany	169	5,503	38	32.56
7	France	142	4,898	40	34.49
8	Canada	135	3,939	29	29.18
9	Turkey	142	1787	21	12.58
10	Netherlands	116	5,607	38	48.34

(*n* = 117) and *Neuromuscular Disorders* (*n* = 98). Nine of the top 10 journals had an impact factor exceeding 3 and were mostly focused on neuromuscular diseases, neuroimmunology, and neurology.

3.5 Author analysis

One hundred five authors with at least 13 publications were included in the co-authorship analysis. The authors were divided into 10 clusters based on their co-authorship, and cooperation among authors was close, as shown in Figure 7A. Based on density visualization (Figure 7B), the central authors were Amelia Evoli, Renato Mantegazza, Vera Bril, Henry Kaminski, Sonia Berrih-Aknin, and Huan Yang. As shown in Table 4, the author with the highest NP was Renato Mantegazza (64), followed by Jan J. Verschuuren (63), and Amelia Evoli (61). Renato Mantegazza is an Italian researcher who has studied a wide range of myopathies and participated in a randomized clinical trial of efgartigimod for the treatment of generalized MG (8). Notably, the author with the highest NC was Amelia Evoli (4,216), followed by Jan J. Verschuuren (3,925) and Angela Vincent (3,865). Amelia Evoli is also from Italy, and by assessing cognitive dysfunction in muscle-specific tyrosine kinase antibody seropositive (MuSK+) passive transfer MG mice, she suggested that recognition memory in the perirhinal cortex of MuSK+ patients with MG could be affected (9). Her team also focuses on immunotherapy in MG (10) and detection methodologies of antibodies in MG (11).

3.6 Co-citation analysis

The co-cited references were analyzed in VOSviewer, with a threshold of at least 65 co-citation counts, incorporating 103 publications (Figure 8). The NC serves as the most objective and direct measure of a publication's significance (12). In the figure, the highly

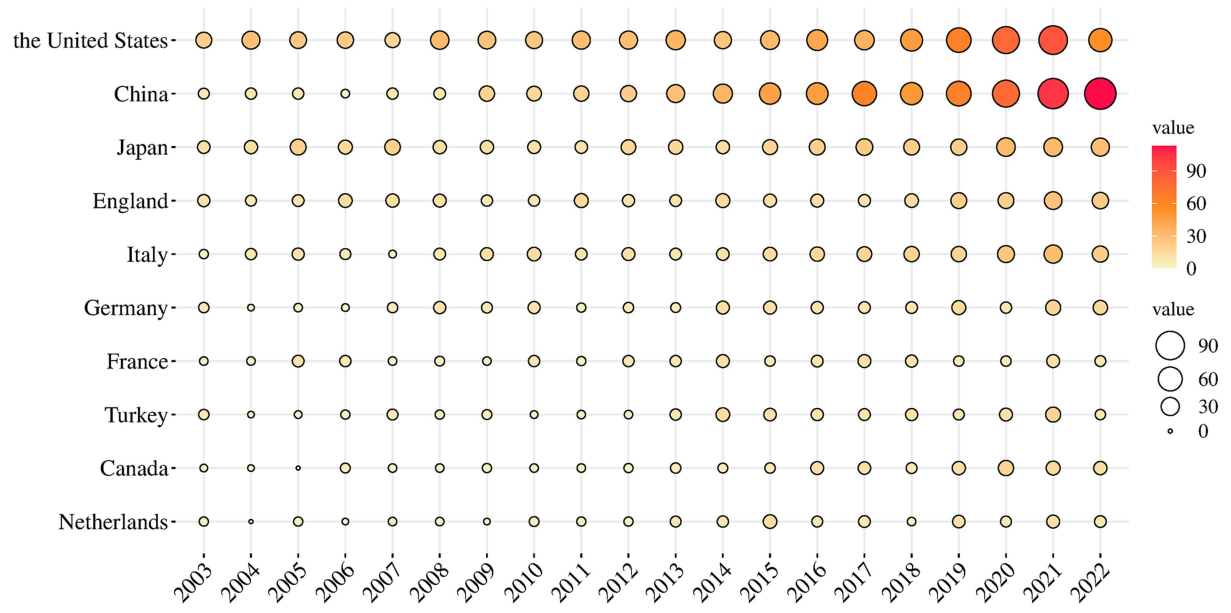


FIGURE 4
The bubble chart of yearly publications of countries.

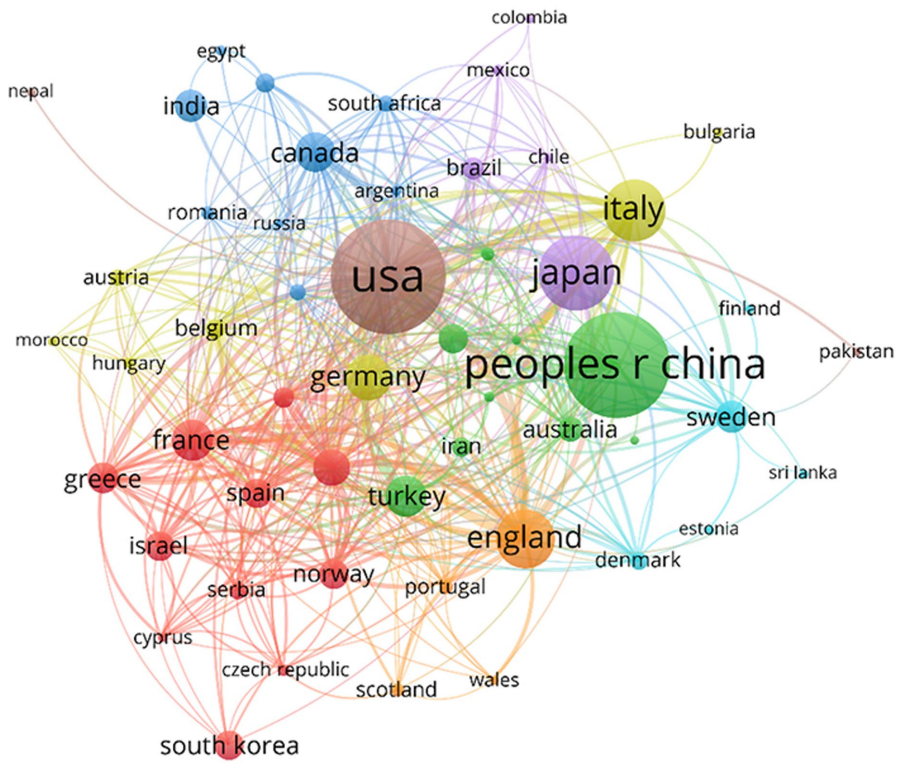


FIGURE 5
Cooperations among countries or regions.

cited literature is divided into four clusters corresponding to four colors: red, yellow, blue, and green. The red cluster consists of 34 publications, primarily reviews, that laid the foundation for theory and were conducted before 2010. These contributions standardized the clinical treatment and scientific research of MG (13–15). The green category consists of 27 publications focused on the pathogenic autoimmune antibodies in patients with MG and the immunopathogenesis of MG, providing ideas for therapeutic drug

TABLE 2 The top 10 most productive institutions.

Rank	Affiliations	Country	Np	Nc	H-index	Average citations
1	University of Oxford	UK	120	6,073	38	50.61
2	Udice—French Research Universities	France	109	4,123	36	37.83
3	University of Texas System	the US	84	2,250	23	26.79
4	Catholic University of the Sacred Heart	Italy	78	4,144	28	53.13
5	IRCCS Policinivo Gemelli	Italy	77	4,127	28	53.60
6	Institut National de la Sante et de la Recherche Medicale Inserm	France	72	3,018	29	41.92
7	Sorbonne University	France	70	2,559	29	36.56
8	University of Toronto	Canada	70	1,668	21	23.83
9	Leiden University	Netherlands	67	3,893	27	58.10
10	Duke University	the US	64	4,615	34	72.11
11	IRCCS Istituto Neurologico Besta	Italy	64	2,647	28	41.36
12	University of Bergen	Norway	64	3,937	32	61.52
13	University of California system	the US	64	2,872	23	44.88

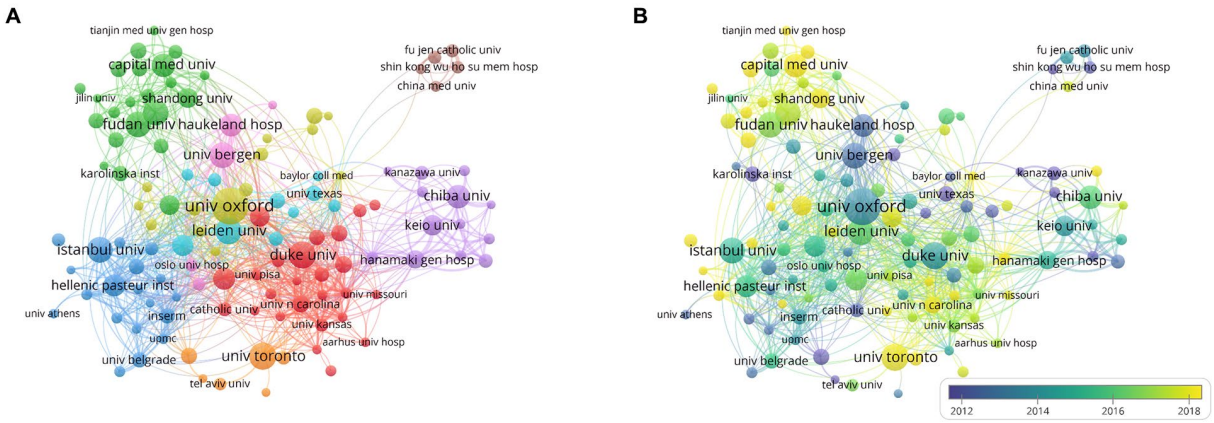


FIGURE 6
Network visualization of cooperations among institutions. **(A)** Visualization of institutions divided into nine clusters. **(B)** Visualization of the institutions network according to the average years of publication.

development (16–18). The blue category consists of 24 publications focused on introducing clinical treatment protocols for MG and standardizing management of patients with MG (19–21). The yellow category consists of 18 publications focused on assessing the conditions of patients with MG, such as the design and application of scales, and establishing the clinical assessment and diagnostic criteria of MG (22–24).

3.7 Analysis of keywords

The co-occurrence analysis of keywords within the publications was performed using VOSviewer. A minimum occurrence of 40 was set, resulting in 123 keywords for visualization and analysis out of a total of 7,857 keywords (Figure 9A). Cluster analysis of keywords can identify popular research topics that can guide future directions. In the figure, larger circles represent a higher number of keyword occurrences. The lines connecting the circles indicate the frequency of co-occurrence, with different cluster colors signifying distinct research directions. The yellow cluster mainly focuses on thymectomy, featuring keywords such as "thymoma," "thymectomy," and "surgery." The red cluster is associated with the pathogenesis of MG, including keywords such as "acetylcholine receptor," "b-cells," and "thymus." The blue cluster, with keywords such as "autoantibodies," "musk," and "protein 4," explores autoantibodies in patients with MG, revealing the relationship between autoantibodies and MG diagnosis. The green cluster is dominated by therapeutic aspects of MG, featuring keywords such as "rituximab," "patient," and "efficacy." The purple cluster, with keywords such as "prevalence," "epidemiology," and "classification," reflects its theme as epidemiology in MG. Representative terms in MG research, such as "myasthenia gravis," "thymectomy," "autoantibodies," "acetylcholine-receptor," and "disease," constitute larger circles in each cluster. Figure 9B depicts the temporal characteristics of keyword co-occurrence from blue to yellow, representing the chronology from 2003 to 2022, with "rituximab," "safety," "classification," "COVID-19," and "clinical characteristics" as recent research hotspots.

TABLE 3 The top 10 most productive journals.

Rank	Journals	IF (2022)	Np	Nc	H-index	Average citations
1	Muscle & Nerve	3.852	224	5,755	39	25.69
2	Journal of Neuroimmunology	3.221	177	3,252	32	18.37
3	Neuromuscular Disorders	3.538	98	1,253	18	12.79
4	Frontiers in Neurology	4.086	90	637	13	7.08
5	Journal of the Neurological Sciences	4.553	82	1,520	22	18.54
6	European Journal of Neurology	6.288	72	1,697	24	23.57
8	Neurological Sciences	3.830	68	649	15	9.54
7	Neurology	11.800	67	4,383	35	65.42
9	Journal of Neurology	6.682	64	1746	24	27.28
10	Journal of Clinical Neuroscience	2.116	45	461	12	10.24

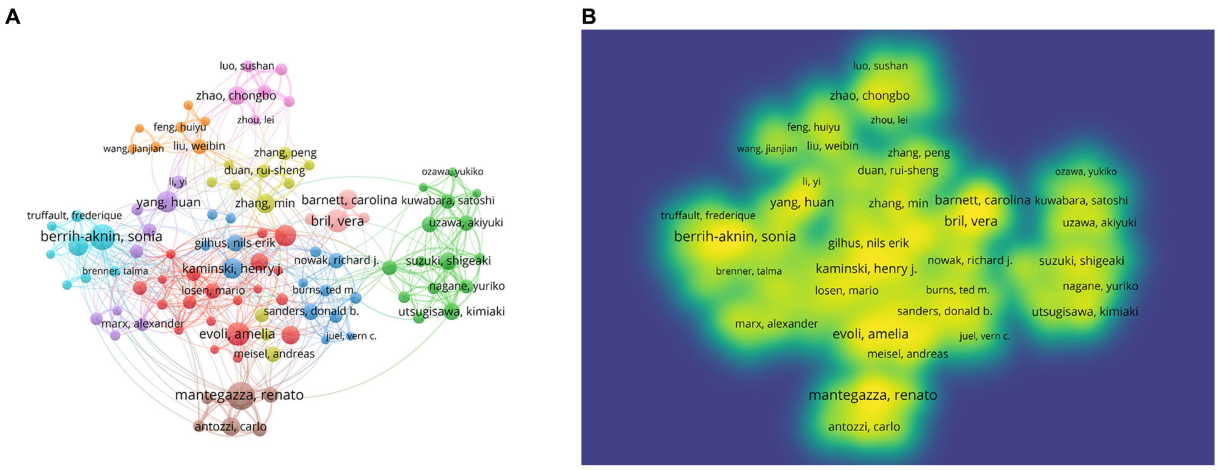


FIGURE 7
Network visualization of authors. **(A)** Visualization of authors divided into 10 clusters. **(B)** Density overlay visualization of the authors network.

TABLE 4 The top 10 most productive authors.

Rank	Author	Country	Np	Nc	H-index	Average citations
1	Mantegazza, Renato	Italy	64	2,628	28	41.06
2	Verschuuren, Jan J.	Netherlands	63	3,925	27	62.30
3	Evoli, Amelia	Italy	61	4,216	28	69.11
4	Vincent, Angela	England	59	3,865	33	65.51
5	Berrih-aknin, Sonia	France	56	3,044	32	54.36
6	Bril, Vera	Canada	53	1,671	20	31.53
7	Kaminski, Henry J.	the United States	48	2,232	21	46.50
8	Punga, Anna Rostedt	Sweden	41	772	18	18.83
9	Barnett, Carolina	Canada	41	737	16	17.98
10	Utsugisawa, K.	Japan	40	1,438	19	35.95
11	Antozzi, Carlo	Italy	40	1,628	22	40.70
12	Le Panse, Rozen	France	40	1,536	24	38.40

The keyword "outbreak analysis" highlights a sudden growth in keyword citations during a specific period, indicating research hotspots. Figure 10 presents the top 25 keywords with the strongest citation analysis. The red line indicates the time of the outbreak, whereas the blue line indicates the period. Keywords that burst earlier included "acetylcholine receptor antibody," "IFN

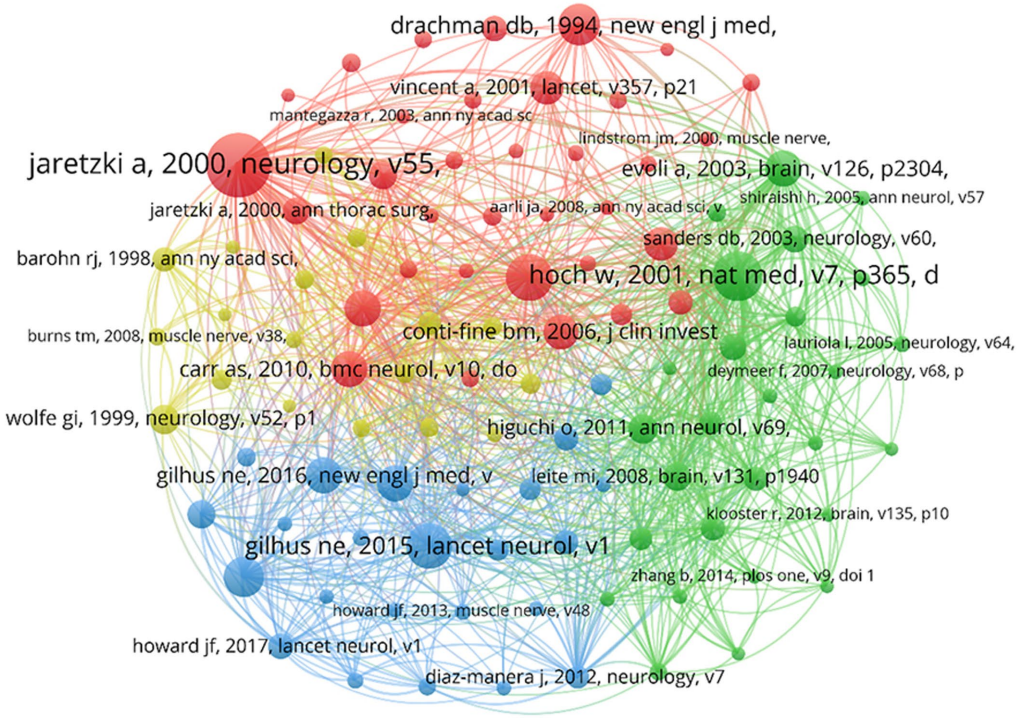


FIGURE 8
Network visualization of co-cited references.

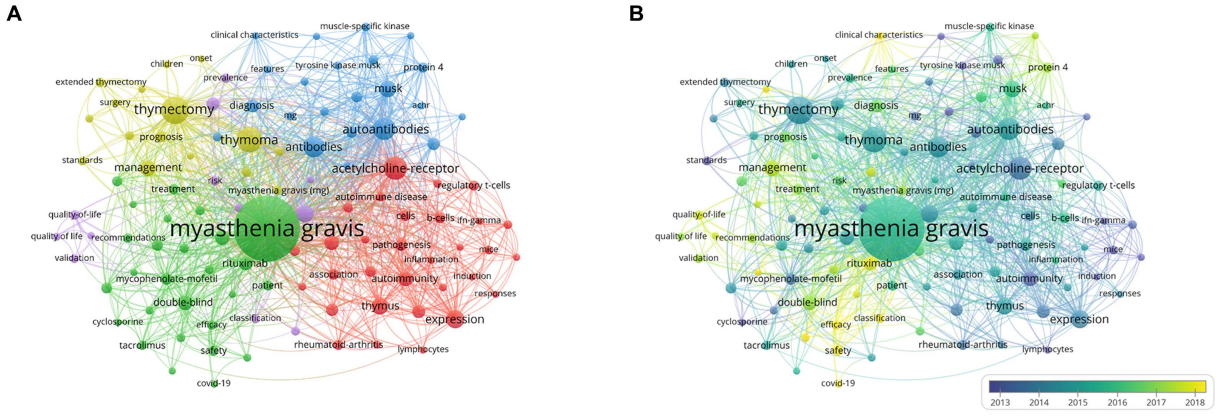


FIGURE 9
Network visualization of co-occurrence keywords. **(A)** Visualization of keywords divided into five clusters. **(B)** Time overlay visualization of the keywords network.

gamma," "lymphocytes," "alpha subunit," and "neuromuscular transmission." Keyword bursts in 2010–2015 that remained in the outbreak period were "safety," "rituximab," "efficacy," "classification," "eculizumab," "case report," "generalized myasthenia gravis," "neuromuscular disease," and "prognosis." The shift in the burst keywords of MG reflects changing research interests in recent years, with "neuromuscular transmission" being the keyword with the highest strength (13.51), followed by "safety" (12.65) and "rituximab" (11.05). These represent current research hotspots.

3.8 Citation burst analysis

Figure 11 shows the citation burst analysis of the top 25 cited papers using CiteSpace. This analysis aimed to identify heightened interest during specific periods within the research area, accomplished by examining the temporal characteristics of the cited articles. Over the past two decades, the earliest articles contributing to the burst period were written by W. Hoch, A. Vincent, and A. Jaretzki. Hoch et al. explored the role of MuSK antibodies in MG pathogenesis (16), whereas Vincent et al.

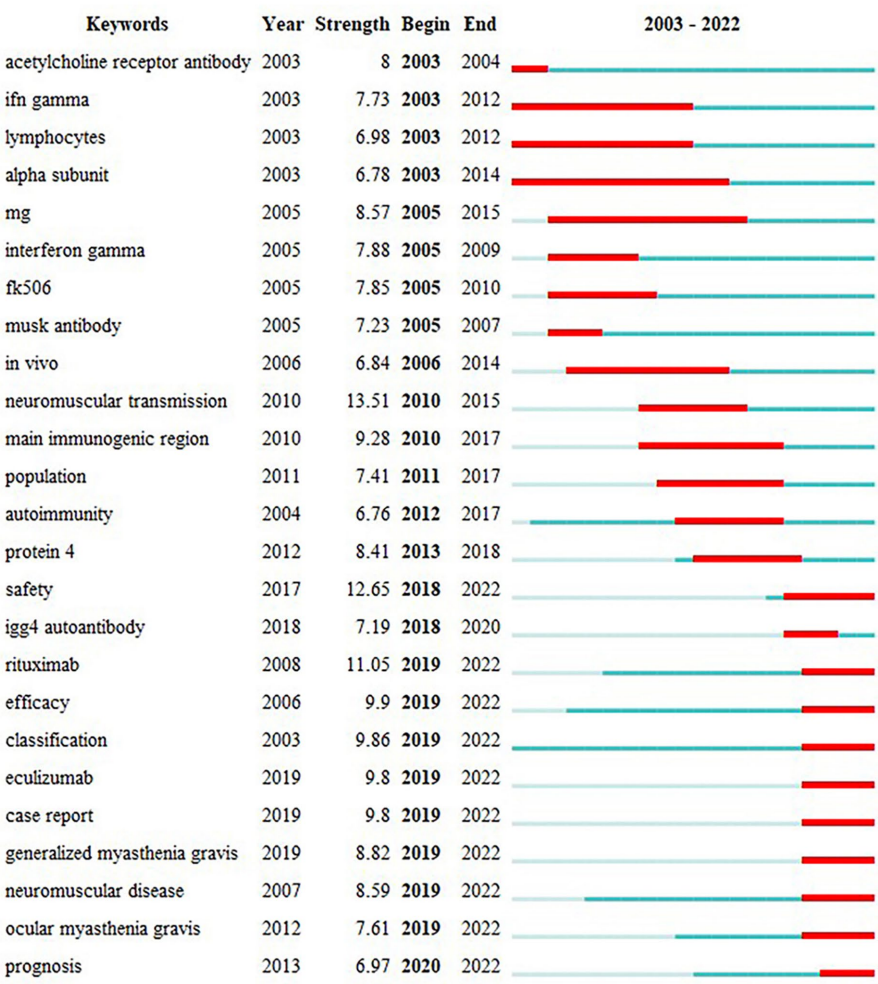


FIGURE 10
The top 25 keywords with the strongest citation bursts.

provided an overview of the diagnosis and treatment of MG (25). Jaretzki et al. suggested the standardization of clinical trials for MG (13). Publications during the burst phase included articles by G.I. Wolfe, D.B. Sanders, J.F. Howard, and N.E. Gilhus. Sanders et al. made significant contributions to the management of patients with MG (21). Gilhus and colleagues conducted an updated systematic review of MG (26, 27). Additionally, both G.I. Wolfe (28) and J.F. Howard (29) conducted randomized controlled clinical trials that provided substantial evidence supporting thymectomy and eculizumab as effective interventions for MG, respectively.

4 Discussion

In this study, we used bibliometrics to visualize publication trends in MG research from 2003 to 2022. We analyzed 3,610 eligible publications using VOSviewer and CiteSpace, exploring patterns in citations, contributing countries and regions, institutions, authors, journals, keywords, and co-citations. Specific bibliometric techniques, including burst hotspot, cluster, and keyword analyses, were conducted to determine the research status and future directions of MG research.

4.1 Knowledge base

The analysis of the change in annual NP indicated an overall upward trend. The analysis of the top 10 countries in terms of NP revealed that the USA had the highest NP and H-index, which indicates a higher quantity and quality of research in this field. The annual NP in China increased rapidly and exceeded that of the USA in 2014, indicating China's rapid development in MG research. However, China's lower citation ranking suggests a need for improved research quality and global impact. Intercountry cooperation is crucial, as seen in the visualization, with most countries collaborating closely. Countries at the periphery face challenges such as geographic distance, and they must strengthen cooperation with other countries, enhance research capacity, and delve into key scientific issues in the field.

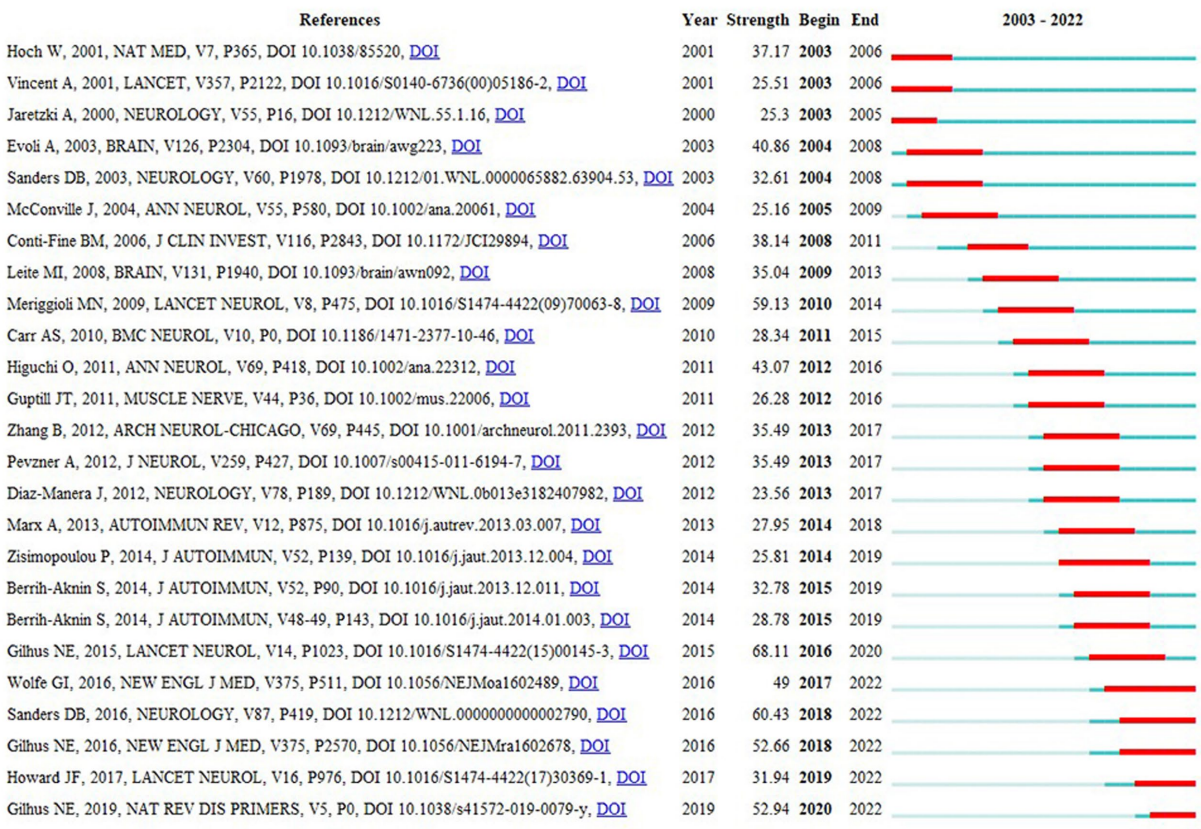


FIGURE 11
The top 25 references with the strongest citation bursts.

Identifying institutions with a solid research base influences the selection of long-term work and cooperation among researchers. In a visual analysis of publishers and institutional collaborations, Oxford University and Duke University stand out, and research institutions must keep pace with these leaders to enhance capacity and explore deeper research. The time overlay analysis revealed that the University of Toronto, Capital Medical University, and Shandong University are up-and-coming institutions in MG research. Hence, it is prudent for government sectors to consider increasing the financial support for these institutions.

According to the visualization of co-authorship, cooperation among authors was close, enhancing research quality. Moreover, authors who play a crucial part in cooperations may have higher quality of research. The core authors of this cooperation were Amelia Evoli, Renato Mantegazza, Vera Bril, Henry Kaminski, Sonia Berrih-Aknin, and Huan Yang. Renato Mantegazza and Amelia Evoli are the most productive and most cited authors, respectively, showing significant influence of their study and outstanding contribution to this field of research. Researchers should read and refer to the publications of these scholars to identify the pivotal and renewed points of MG.

Muscle & Nerve, the *Journal of Neuroimmunology*, and *Neuromuscular Disorders* published the most articles, making them suitable outlets for the publication and dissemination of research in this field. Researchers could consider submitting their articles to these journals, and scholars can consult publications from these journals to obtain the latest information on MG.

In the co-citation science map, larger circles indicate articles with more citations, pointing to greater influence. The largest circle features Jaretzki et al.'s article, titled "Myasthenia Gravis: Recommendations for Clinical Research Standards" (13), published in *Neurology* and cited 918 times. This article addressed challenges in MG clinical trials, including refining the Myasthenia Gravis Foundation of America (MGFA) clinical classification and establishing the Quantitative Myasthenia Gravis (QMG) score, which remains a global scientific standard. Their efforts systematically organized critical details, facilitating uniformity in MG's clinical research and management processes, explaining the enduring recognition and referencing of this article. The understanding of the MG knowledge base unfolds through the exploration of relevant research domains visualized in the science map generated by the VOSviewer. The four clusters in Figure 7 include topics ranging from the underlying pathogenesis to intricate clinical management and from foundational theoretical aspects to their practical applications. This comprehensive development is reflective of the extensive research conducted on MG from 2003 to 2022.

4.2 Research hotspot

Upon reviewing citations and keywords, the continuous improvement of precise therapeutic approaches for diverse patient profiles emerged as the hotspot of MG research. This can be categorized into three aspects: MG antibody-related subtype therapy, novel immunotherapeutic approaches, and thymectomy.

4.2.1 Treatment strategies for different MG subtypes

The treatment options for MG include cholinesterase inhibitors, thymectomy, immunosuppressive or immunomodulatory medications, and plasma exchange. However, the increasing prevalence of MG demands more effective therapeutic approaches. The keyword "classification" stands out in recent research, especially during the burst period. Notably, an article published in 2015 in *Lancet Neurology* (19) covering autoantibodies, epidemiology, clinical presentation, and comorbidities holds the highest burst strength (68.11) in reference citation burst detection. It summarizes potential treatments for MG and suggests a future research direction of exploring new immunosuppressive drugs and drug combinations tailored to MG subgroups. This article plays a crucial role in subgroup classification and provides valuable insights for future research in MG. The emphasis on burst keywords and references underscores researchers' keen interest in MG with different antibodies, emphasizing the crucial role of autoantibodies in MG diagnosis, understanding patient features, and personalizing treatment approaches. Most patients with MG have antibodies targeting AChRs, with fewer having antibodies against MuSK or LRP4 (30). A multicenter study showed that patients with antibodies against LRP4 and/or agrin exhibit more generalized symptoms (69%) than antibody-negative patients, but most of them responded favorably to standard MG therapy (31). This showcases how studying the clinical characteristics of MG with specific antibodies can significantly improve diagnosis and management. MuSK, as an antibody, differs from classic AChR antibodies, impacting clinical manifestations and treatment responses, thus posing challenges to accurate diagnosis and management. MuSK antibody-positive MG is prevalent among females (32), affecting muscles not typically weakened in non-MuSK antibody-positive MG, with increased respiratory weakness in this subgroup (33). Cholinesterase inhibitors often yield unsatisfactory results; therefore, early rituximab administration is recommended (34, 35). Future research will likely focus on treatments for patients with MG who have other specific autoimmune antibodies.

4.2.2 Novel immunotherapeutic approaches

In 1996, a soluble recombinant form of human complement receptor 1 was demonstrated as an additional therapeutic approach for MG (36), suggesting that complement inhibition may be a potential therapeutic approach for MG. A phase 3, randomized, double-blind, placebo-controlled, multicenter study evaluating the efficacy and safety of eculizumab in AChR-positive refractory generalized MG was conducted during the burst period (29). Eculizumab, the first complement-specific drug clinically used for the treatment of paroxysmal nocturnal orphan disease and hemoglobinuria, has bolstered confidence in therapeutic complement inhibition (37) and has gained market approval in countries such as the USA, Japan, and China. As reported in a retrospective study, patients treated with eculizumab were more likely to achieve minimal manifestations than those treated with rituximab, but the risk of crisis was not reduced (38). Eculizumab has also been confirmed to benefit refractory AChR-MG in a real-world experience (39), but one patient reported acute worsening after discontinuation of eculizumab (40). Moreover, the optimal duration of treatment remains unclear. Therefore, further studies are needed on safety and treatment duration. Research to identify biomarkers predicting the response to eculizumab is also necessary. Eculizumab was also a keyword in the burst period, indicating attention to selective immunosuppressants in MG treatment. Ravulizumab is a monoclonal antibody complement inhibitor approved by the Food and Drug Administration (FDA) in April 2022 for AChR-Ab-generalized MG. A phase 3, randomized, double-blind, placebo-controlled, multicenter trial in patients with AChR-Ab-generalized MG indicated improved clinical outcomes for patients treated with ravulizumab, and the drug was well tolerated (41). The latest pharmacokinetics and pharmacodynamics research based on the data from this phase 3 study supports dosing every 8 weeks for immediate, complete, and sustained inhibition of terminal complement C5, and it could reduce the burden on patients (42).

Targeting the neonatal fragment crystallizable receptor (FcRn) is another novel therapeutic approach considered for generalized MG that has failed standard treatment. Meanwhile, efgartigimod, a human IgG1 antibody that reduces IgG recycling and increases IgG degradation by outcompeting endogenous IgG binding (43), was approved by the FDA in July 2022 for the treatment of AChR-Ab-generalized MG in adult patients. A phase 3, randomized, double-blind, placebo-controlled trial in generalized MG revealed better clinical improvement in the efgartigimod arm than in the placebo arm, whereas most patients had AChR-MG (44). A meta-analysis found that anti-FcRn has an advantage in improving the QMG score than complement treatments in patients with generalized MG, and they also proposed the high probability of efgartigimod and rozanolixizumab being the most effective treatment in generalized MG (45).

Rituximab, a keyword with high citation strength and more recent citations, is a monoclonal antibody directed against CD20 antigen on B cells that has been used for years. The clear benefit of refractory MuSK antibody-positive MG has been demonstrated; however, its efficacy in AChR-MG remains controversial (46). A systematic review of 13 studies proposed that the small number of patients with AChR-MG in previous studies may have caused bias in efficacy evaluation and suggested that dosages for different subtypes of MG should be considered in future studies (47). In addition, due to the off-label use of rituximab and the inherent risk of infection associated with continuous B-cell depletion, safety is a crucial factor and should be the focus. For example, infection is the most common side effect of this treatment. A retrospective study of adverse events in treatment of refractory MG with rituximab found that infection was associated with hypogammaglobulinemia and proposed that a standardized monitoring scheme of IgG is necessary (48). To ensure the safety of rituximab in patients with MG and enhance risk control, high-quality randomized controlled trials with large samples should be conducted.

4.2.3 Thymectomy

Thymectomy is inevitable in thymomatous MG (49). Moreover, a multicenter, randomized, single-blind trial comparing thymectomy combined with prednisone to prednisone alone, published in the *New England Journal of Medicine* in 2016 (28), provided a conclusive

outcome that thymectomy benefits patients with MGFA clinical class II to IV disease with non-thymomatous AChR-MG. These authors extended the study up to 2 years to investigate the durability of the treatment associated with thymectomy, published in 2019, further affirming the advantages of thymectomy and reversing the treatment decision trends for this procedure (50). Both aforementioned studies were still in the burst period, indicating sustained interest in thymectomy studies. However, the prognosis of thymectomy in certain outcomes can be observed in the worsening or relapse of MG, even after thymectomy. This may be associated with patient heterogeneity, autoantibody profiles, thymic pathology, operation-associated factors, perioperative care, and disease conditions, among other factors (51). To better guide the decisions between conservative treatments or surgery, the pivotal mechanisms of clinical thymectomy in MG need to be elucidated, which may be a promising topic for future research.

4.3 Advantages and limitations

The present research has some novel contributions to this field. First, this is the first bibliometric study that not only systematically analyzed research of MG but also paid special attention to research hotpots, especially MG treatment. Therefore, it will be beneficial to scholars who are interested in this subject as well as neurologists who want to catch up with recent advances in a visual manner. Second, based on our study, we proposed some rational recommendations for potential project sponsor and related government sector. Resource integration could be further enhanced, which may stimulate the development of MG research.

This bibliometric analysis has several limitations. First, the NC of recently published articles may not fully reflect their quality, potentially introducing bias into our qualitative assertions (52). Second, as this study exclusively included publications in English, articles in other languages were omitted, potentially introducing bias.

4.4 Conclusion

Using VOSviewer and CiteSpace, this study has dynamically clarified the trajectory of MG research, revealing its developmental nuances, hotspots, and future trends. Notably, the USA had the highest NP and NC. Nevertheless, there is potential for more collaboration among countries and institutions. Current MG research shows enthusiasm, especially for individualized treatments based on subtypes, novel immunotherapeutic approaches, and thymectomy. These facets collectively shape ongoing and future research into the intricacies of MG.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

YT: Data curation, Writing – original draft, Writing – review & editing, Formal Analysis, Investigation, Visualization. QS: Data curation, Writing – original draft. SP: Data curation, Writing – review & editing. LM: Writing – review & editing. RF: Data curation, Writing – review & editing. AX: Investigation, Writing – review & editing. SL: Writing – review & editing. YY: Writing – review & editing. WC: Writing – review & editing. JN: Conceptualization, Writing – review & editing. WZ: Conceptualization, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This study was supported by the Innovation Fund of China Academy of Chinese Medical Sciences (CI2021A01309).

Acknowledgments

The authors acknowledge gratitude to all the staff who participated in this study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Drachman DB. Myasthenia gravis. *Semin Neurol.* (2016) 36:419–24. doi: 10.1055/s-0036-1586265

2. Santos E, Coutinho E, Moreira I, Silva AM, Lopes D, Costa H, et al. Epidemiology of myasthenia gravis in northern Portugal: frequency estimates and clinical epidemiological distribution of cases. *Muscle Nerve.* (2016) 54:413–21. doi: 10.1002/mus.25068

3. Gattellari M, Goumas C, Worthington JM. A national epidemiological study of myasthenia gravis in Australia. *Eur J Neurol.* (2012) 19:1413–20. doi: 10.1111/j.1468-1331.2012.03698.x

4. Park S-Y, Lee JY, Lim NG, Hong Y-H. Incidence and prevalence of myasthenia gravis in Korea: A population-based study using the National Health Insurance Claims Database. *J Clin Neurol.* (2016) 12:340–4. doi: 10.3988/jcn.2016.12.3.340

5. Thelwall M. Bibliometrics to webometrics. *J Inf Sci.* (2008) 34:605–21. doi: 10.1177/0165551507087238

6. van Eck NJ, Waltman L. Software survey: VOSviewer, a computer program for bibliometric mapping. *Scientometrics.* (2010) 84:523–38. doi: 10.1007/s11192-009-0146-3

7. Chen C. Searching for intellectual turning points: progressive knowledge domain visualization. *Proc Natl Acad Sci.* (2004) 101:5303–10. doi: 10.1073/pnas.0307513100

8. Howard JF Jr, Bril V, Burns TM, Mantegazza R, Bilinska M, Szczudlik A, et al. Randomized phase 2 study of FcRn antagonist efgartigimod in generalized myasthenia gravis. *Neurology*. (2019) 92:e2661–73. doi: 10.1212/WNL.0000000000007600

9. Sabre L, Evoli A, Punga AR. Cognitive dysfunction in mice with passively induced MuSK antibody seropositive myasthenia gravis. *J Neurol Sci*. (2019) 399:15–21. doi: 10.1016/j.jns.2019.02.001

10. Rodolico C, Nicocia G, Damato V, Antonini G, Liguori R, Evoli A. Benefit and danger from immunotherapy in myasthenia gravis. *Neurol Sci*. (2021) 42:1367–75. doi: 10.1007/s10072-021-05077-6

11. Spagni G, Gastaldi M, Businaro P, Chemkhi Z, Carrozza C, Mascagna G, et al. Comparison of fixed and live cell-based assay for the detection of AChR and MuSK antibodies in myasthenia gravis. *Neurol Neuroimmunol Neuroinflam*. (2023) 10:38. doi: 10.1212/NXI.0000000000200038

12. Pieters R, Baumgartner H. Who talks to whom? Intra-and interdisciplinary communication of economics journals. *J Econ Lit*. (2002) 40:483–509. doi: 10.1257/jel.40.2.483

13. Jaretzki A, Barohn RJ, Ernstoff RM, Kaminski HJ, Keesey JC, Penn AS, et al. Myasthenia gravis: recommendations for clinical research standards. *Neurology*. (2000) 55:16–23. doi: 10.1212/WNL.55.1.16

14. Meriggioli MN, Sanders DB. Autoimmune myasthenia gravis: emerging clinical and biological heterogeneity. *Lancet Neurol*. (2009) 8:475–90. doi: 10.1016/S1474-4422(09)70063-8

15. Carr AS, Cardwell CR, McCarron PO, McConville J. A systematic review of population based epidemiological studies in myasthenia gravis. *BMC Neurol*. (2010) 10:46. doi: 10.1186/1471-2377-10-46

16. Hoch W, McConville J, Helms S, Newsom-Davis J, Melms A, Vincent A. Auto-antibodies to the receptor tyrosine kinase MuSK in patients with myasthenia gravis without acetylcholine receptor antibodies. *Nat Med*. (2001) 7:365–8. doi: 10.1038/85520

17. Higuchi O, Hamuro J, Motomura M, Yamanashi Y. Autoantibodies to low-density lipoprotein receptor–related protein 4 in myasthenia gravis. *Ann Neurol*. (2011) 69:418–22. doi: 10.1002/ana.22312

18. Leite MI, Jacob S, Viegas S, Cossins J, Clover L, Morgan BP, et al. IgG1 antibodies to acetylcholine receptors in 'seronegative' myasthenia gravis†. *Brain*. (2008) 131:1940–52. doi: 10.1093/brain/awn092

19. Gilhus NE, Verschuuren JJ. Myasthenia gravis: subgroup classification and therapeutic strategies. *Lancet Neurol*. (2015) 14:1023–36. doi: 10.1016/S1474-4422(15)00145-3

20. Gilhus NE, Skeie GO, Romi F, Lazaridis K, Zisimopoulou P, Tzartos S. Myasthenia gravis — autoantibody characteristics and their implications for therapy. *Nat Rev Neurol*. (2016) 12:259–68. doi: 10.1038/nrneurol.2016.44

21. Sanders DB, Wolfe GI, Benatar M, Evoli A, Gilhus NE, Illa I, et al. International consensus guidance for management of myasthenia gravis: executive summary. *Neurology*. (2016) 87:419–25. doi: 10.1212/WNL.0000000000002790

22. Wolfe GI, Herbelin L, Nations SP, Foster B, Bryan WW, Barohn RJ. Myasthenia gravis activities of daily living profile. *Neurology*. (1999) 52:1487–7. doi: 10.1212/WNL.52.7.1487

23. Burns TM, Conaway MR, Cutter GR, Sanders DBGroup TMS. Less is more, or almost as much: A 15-item quality-of-life instrument for myasthenia gravis. *Muscle Nerve*. (2008) 38:957–63. doi: 10.1002/mus.21053

24. Pascuzzi RM, Coslett HB, Johns TR. Long-term corticosteriod treatment of myasthenia gravis: report of 116 patients. *Ann Neurol*. (1984) 15:291–8. doi: 10.1002/ana.410150316

25. Vincent A, Palace J, Hilton-Jones D. Myasthenia gravis. *Lancet*. (2001) 357:2122–8. doi: 10.1016/S0140-6736(00)05186-2

26. Gilhus NE. Myasthenia gravis. *N Engl J Med*. (2016) 375:2570–81. doi: 10.1056/NEJMra1602678

27. Gilhus NE, Tzartos S, Evoli A, Palace J, Burns TM, Verschuuren JJGM. Myasthenia gravis. *Nat Rev Dis Primers*. (2019) 5:1–19. doi: 10.1038/s41572-019-0079-y

28. Wolfe GI, Kaminski HJ, Aban IB, Minisman G, Kuo H-C, Marx A, et al. Randomized trial of Thymectomy in myasthenia gravis. *N Engl J Med*. (2016) 375:511–22. doi: 10.1056/NEJMoa1602489

29. Howard JF, Utsugisawa K, Benatar M, Murai H, Barohn RJ, Illa I, et al. Safety and efficacy of eculizumab in anti-acetylcholine receptor antibody-positive refractory generalised myasthenia gravis (REGAIN): a phase 3, randomised, double-blind, placebo-controlled, multicentre study. *Lancet Neurol*. (2017) 16:976–86. doi: 10.1016/S1474-4422(17)30369-1

30. Vincent A, Huda S, Cao M, Cetin H, Koneczny I, Rodriguez-Cruz P, et al. Serological and experimental studies in different forms of myasthenia gravis. *Ann N Y Acad Sci*. (2018) 1413:143–53. doi: 10.1111/nyas.13592

31. Rivner MH, Quarles BM, Pan J-X, Yu Z, Howard JF Jr, Corse A, et al. Clinical features of LRP4/agrin-antibody–positive myasthenia gravis: A multicenter study. *Muscle Nerve*. (2020) 62:333–43. doi: 10.1002/mus.26985

32. Chang T, Leiite MI, Senanayake S, Gunaratne PS, Gamage R, Riffsy MTM, et al. FP59-FR-06 clinical and serological study of myasthenia gravis in a south Asian population using both radioimmunoprecipitation and cell-based assays. *J Neurol Sci*. (2009) 285:S152. doi: 10.1016/S0022-510X(09)70586-6

33. Guptill JT, Sanders DB, Evoli A. Anti-musk antibody myasthenia gravis: clinical findings and response to treatment in two large cohorts. *Muscle Nerve*. (2011) 44:36–40. doi: 10.1002/mus.22006

34. Anderson D, Phan C, Johnston WS, Siddiqi ZA. Rituximab in refractory myasthenia gravis: a prospective, open-label study with long-term follow-up. *Ann Clin Transl Neurol*. (2016) 3:552–5. doi: 10.1002/acn3.314

35. Beecher G, Anderson D, Siddiqi ZA. Rituximab in refractory myasthenia gravis: extended prospective study results. *Muscle Nerve*. (2018) 58:452–5. doi: 10.1002/mus.26156

36. Piddlesden SJ, Jiang S, Levin JL, Vincent A, Morgan BP. Soluble complement receptor 1 (sCR1) protects against experimental autoimmune myasthenia gravis. *J Neuroimmunol*. (1996) 71:173–7. doi: 10.1016/S0165-5728(96)00144-0

37. Ricklin D, Mastellos DC, Reis ES, Lambris JD. The renaissance of complement therapeutics. *Nat Rev Nephrol*. (2018) 14:26–47. doi: 10.1038/nrneph.2017.156

38. Nelke C, Schroeter CB, Stascheit F, Pawlitzki M, Regner-Nelke L, Huntemann N, et al. Eculizumab versus rituximab in generalised myasthenia gravis. *J Neurol Neurosurg Psychiatry*. (2022) 93:548–54. doi: 10.1136/jnnp-2021-328665

39. Oyama M, Okada K, Masuda M, Shimizu Y, Yokoyama K, Uzawa A, et al. Suitable indications of eculizumab for patients with refractory generalized myasthenia gravis. *Ther Adv Neurol Disord*. (2020) 13:1756286420904207. doi: 10.1177/1756286420904207

40. Uzawa A, Ozawa Y, Yasuda M, Kuwabara S. Severe worsening of myasthenic symptoms after the eculizumab discontinuation. *J Neuroimmunol*. (2020) 349:577424. doi: 10.1016/j.jneuroim.2020.577424

41. Meisel A, Annane D, Vu T, Mantegazza R, Katsuno M, Aguzzi R, et al. Long-term efficacy and safety of ravulizumab in adults with anti-acetylcholine receptor antibody-positive generalized myasthenia gravis: results from the phase 3 CHAMPION MG open-label extension. *J Neurol*. (2023) 270:3862–75. doi: 10.1007/s00415-023-11699-x

42. Vu T, Ortiz S, Katsuno M, Annane D, Mantegazza R, Beasley KN, et al. Ravulizumab pharmacokinetics and pharmacodynamics in patients with generalized myasthenia gravis. *J Neurol*. (2023) 270:3129–37. doi: 10.1007/s00415-023-11617-1

43. Ulrichts P, Guglietta A, Dreier T, Bragt T van, Hanssens V, Hofman E, Vankerckhoven B, Verheesen P, Ongenae N, Lykhopiy V, et al. Neonatal fc receptor antagonist efgartigimod safely and sustainably reduces IgGs in humans. *J Clin Invest* (2018) 128:4372–4386. doi: 10.1172/JCI97911

44. Howard JF, Bril V, Vu T, Karam C, Peric S, Margania T, et al. Safety, efficacy, and tolerability of efgartigimod in patients with generalised myasthenia gravis (ADAPT): a multicentre, randomised, placebo-controlled, phase 3 trial. *Lancet Neurol*. (2021) 20:526–36. doi: 10.1016/S1474-4422(21)00159-9

45. Saccà F, Pane C, Espinosa PE, Sormani MP, Signori A. Efficacy of innovative therapies in myasthenia gravis: A systematic review, meta-analysis and network meta-analysis. *Eur J Neurol*. (2023) 30:3854–67. doi: 10.1111/ene.15872

46. Zebardast N, Patwa HS, Novella SP, Goldstein JM. Rituximab in the management of refractory myasthenia gravis. *Muscle Nerve*. (2010) 41:375–8. doi: 10.1002/mus.21521

47. Di Stefano V, Lupica A, Rispoli MG, Di Muzio A, Brighina F, Rodolico C. Rituximab in AChR subtype of myasthenia gravis: systematic review. *J Neurol Neurosurg Psychiatry*. (2020) 91:392–5. doi: 10.1136/jnnp-2019-322606

48. Caballero-Ávila M, Álvarez-Velasco R, Moga E, Rojas-Garcia R, Turon-Sans J, Querol L, et al. Rituximab in myasthenia gravis: efficacy, associated infections and risk of induced hypogammaglobulinemia. *Neuromuscul Disord*. (2022) 32:664–71. doi: 10.1016/j.nmd.2022.06.006

49. Drachman DB. Myasthenia gravis. *N Engl J Med*. (1994) 330:1797–810. doi: 10.1056/NEJM199406233302507

50. Wolfe GI, Kaminski HJ, Aban IB, Minisman G, Kuo H-C, Marx A, et al. Long-term effect of thymectomy plus prednisone versus prednisone alone in patients with non-thymomatous myasthenia gravis: 2-year extension of the MGTX randomised trial. *Lancet Neurol*. (2019) 18:259–68. doi: 10.1016/S1474-4422(18)30392-2

51. Chen K, Li Y, Yang H. Poor responses and adverse outcomes of myasthenia gravis after thymectomy: predicting factors and immunological implications. *J Autoimmun*. (2022) 132:102895. doi: 10.1016/j.jaut.2022.102895

52. Roldan-Valadez E, Salazar-Ruiz SY, Ibarra-Contreras R, Rios C. Current concepts on bibliometrics: a brief review about impact factor, Eigenfactor score, CiteScore, SCImago journal rank, source-normalised impact per paper, H-index, and alternative metrics. *Ir J Med Sci*. (2019) 188:939–51. doi: 10.1007/s11845-018-1936-5

frontiers | Frontiers in Neurology

TYPE Case Report
PUBLISHED 22 January 2024
DOI 10.3389/fneur.2024.1321058

OPEN ACCESS

EDITED BY
German Moris,
SESPA, Spain

REVIEWED BY
Yuri Matteo Falzone,
San Raffaele Scientific Institute (IRCCS), Italy
Vincenzo Di Stefano,
University of Palermo, Italy

*CORRESPONDENCE
Ryuta Kinno
✉ kinno@med.showa-u.ac.jp

RECEIVED 13 October 2023
ACCEPTED 08 January 2024
PUBLISHED 22 January 2024

CITATION
Watanabe K, Ohashi S, Watanabe T, Kakinuma Y and Kinno R (2024) Case report: Recovery from refractory myasthenic crisis to minimal symptom expression after add-on treatment with efgartigimod.
Front. Neurol. 15:1321058.
doi: 10.3389/fneur.2024.1321058

Case report: Recovery from refractory myasthenic crisis to minimal symptom expression after add-on treatment with efgartigimod

Keiko Watanabe[1], Shinichi Ohashi[2], Takuya Watanabe[1], Yuki Kakinuma[1] and Ryuta Kinno[1]*

[1]Division of Neurology, Department of Internal Medicine, Showa University Northern Yokohama Hospital, Yokohama, Japan, [2]Respiratory Disease Center, Showa University Northern Yokohama Hospital, Yokohama, Japan

Myasthenic crisis, a life-threatening exacerbation of myasthenia gravis, is a significant clinical challenge, particularly when refractory to standard therapies. Here, we described a case of myasthenic crisis in which the patient transitioned from refractory myasthenic crisis to minimal symptom expression after receiving add-on treatment with efgartigimod, a novel neonatal Fc receptor antagonist. A 54 years-old woman who was diagnosed with anti-acetylcholine receptor antibody-positive myasthenia gravis experienced respiratory failure necessitating mechanical ventilation. Despite aggressive treatment with plasmapheresis, intravenous immunoglobulins, and high-dose corticosteroids, her condition continued to deteriorate, culminating in persistent myasthenic crisis. Efgartigimod was administered as salvage therapy. Remarkable improvement in neuromuscular function was observed within days, allowing for successful weaning from mechanical ventilation. Over the subsequent weeks, the patient's symptoms continued to ameliorate, ultimately reaching a state of minimal symptom expression. Serial assessments of her serum anti-acetylcholine receptor antibody titer showed a consistent decline in parallel with this clinical improvement. This case highlights efgartigimod's potential as an effective therapeutic option for refractory myasthenic crisis, offering new hope for patients facing this life-threatening condition.

KEYWORDS

myasthenic crisis, efgartigimod, minimal symptom expression, intravenous immunoglobulins, plasmapheresis

Introduction

Myasthenic crisis (MC) is the most severe, life-threatening manifestation of myasthenia gravis (MG), often requiring noninvasive and/or mechanical ventilation, supportive enteral feeding, and intensive care unit management (1). Disease-modifying treatments such as plasmapheresis and intravenous immunoglobulins (IVIG) can be administered in the management of MC, but these treatments are not effective in all patients. IVIG and plasmapheresis are more likely to be combined sequentially for the refractory MC (2).

In recent years, the pathogenesis of MG has become clearer, and more targeted therapies are being developed (3). Monoclonal antibodies (mAbs) now offer a very attractive therapeutic

approach to MG because they can specifically and effectively target several immunopathological pathways, including the complement cascade, B-cell-associated differentiation group proteins, and human neonatal Fc receptors (FcRn). To date, the C5-directed mAb eculizumab and the FcRn inhibitor efgartigimod have been approved for the chronic treatment of anti-acetylcholine receptor (AChR) antibody-positive MG. However, the efficacy of these agents in MC remains unknown. We present a case of MC in which the patient transitioned from refractory MC to minimal symptom expression (MSE), defined as a MG activities of daily living (MG-ADL) scale of 0 or 1, after she receiving add-on treatment with efgartigimod. This case suggests that efgartigimod may be a viable treatment option for MC.

Case presentation

At 5 months prior to the present event, the patient (a 54 years-old Japanese woman) had been diagnosed with anti-AChR antibody-positive MG. She had a history of lumbar disc herniation but was not receiving treatment for it. Her initial presenting symptoms were drooping eyelids and double vision. A thymectomy was performed for her non-invasive thymoma (Type B1, Masaoka stage II) 2 months prior to her presentation. Treatment with oral steroid therapy (prednisolone 5 mg/day), tacrolimus (3 mg/day), and pyridostigmine (60 mg 3×/day) resulted in complete resolution of her clinical symptoms [MG-ADL scale: 0; MG composite (MGC) scale: 0]. Serum anti-glutamic acid decarboxylase (GAD) antibody was measured for possible comorbid stiff-person syndrome (SPS) but was negative (<5.0 U/mL).

During her routine clinic follow-up, she noticed weakness in her neck muscles, along with a recurrence of drooping eyelids and double vision. She was admitted to our hospital due to progressive neck-muscle weakness (day 0). On admission, percutaneous oxygen saturation was recorded at 97% (room air); all other vital signs were normal. She exhibited drooping eyelids, double vision, dysarthria, dysphagia, and hypernasality. Manual muscle test scores were 2 for neck flexion and extension, and 3 for shoulder abduction. The MG-ADL and MGC scale results were 17 and 32, respectively. Blood sample testing was positive for anti-AChR antibodies (28.0 nmol/L) and negative for anti-muscle-specific tyrosine kinase (MuSK) antibodies (<0.02 nmol/L). Blood gas analysis showed normal findings (PaO_2: 74.3 mmHg; $PaCO_2$: 39.0 mmHg).

After admission (Figures 1, 2), the patient required tube feeding due to severe dysphagia. Unfortunately, there was no way to administer tacrolimus via a feeding tube in our setting, and the patient's tacrolimus medication was discontinued. In accord with the recommendation of the Japanese guidelines (4), we decided to perform the first-acting treatments. We initiated plasma exchange therapy. Following the first plasma exchange, the patient experienced anaphylactic shock, leading to the implementation of immunoadsorption plasmapheresis. However, after six plasmapheresis sessions the patient's clinical symptoms showed no improvement, and limb muscle weakness had developed. On day 15, we also administered IVIG therapy (0.4 g/kg/day × 5 days), but the patient's clinical symptoms worsened, resulting in respiratory failure (MG-ADL scale: 20; MGC scale: 32).

On day 24, we commenced the first cycle of intravenous efgartigimod (10 mg/kg/week, 4 infusions per cycle) as immunosuppressive therapy. To intensify the immunosuppressive therapy, we suggested increasing the dose of oral prednisone, but the patient refused to increase the dose beyond 10 mg. On day 29 (15 days after the IVIG), her respiratory failure worsened (PaO_2: 93.0 mmHg; $PaCO_2$: 73.6 mmHg), necessitating mechanical ventilation. The first cycle of efgartigimod was limited to three infusions due to ventilator-associated pneumonia. Following the intravenous efgartigimod therapy, the patient's limb muscle weakness and neck muscle weakness gradually resolved on days 42 and 48, respectively.

On day 66, the second cycle of intravenous efgartigimod was administered. The patient's dyspnea gradually improved after the second cycle of treatment, and on day 84, she was successfully weaned from mechanical ventilation. She was able to walk approx. 50 meters on her own. On day 105 (39 days after the second cycle of intravenous efgartigimod), she noticed muscle weakness in her neck and upper limbs. Considering the possibility of prolonged effects of the initial IVIG for her initial improvement, we initiated the second IVIG treatment (0.4 g/kg/day × 5 days), which proved ineffective. Intravenous corticosteroids (1 g/day × 5 days), which was standard therapy for MC, were also ineffective. Based on the above data, we considered for the first time that efgartigimod could be effective for improving the clinical symptoms of MC. We thus initiated the third cycle of intravenous efgartigimod on day 143. After completing three cycles of intravenous efgartigimod, the patient's dysphagia gradually improved, and she was able to tolerate oral intake very well.

She was discharged on day 182. Serial assessments of her serum anti-AChR antibody titers showed a consistent decline in parallel with her clinical improvement. In addition, her serum IgG concentration was decreased after both plasmapheresis and intravenous efgartigimod, whereas anti-AChR antibodies were decreased after intravenous efgartigimod only (Figure 3). Based on this clinical course, we consider this a case of refractory MC that responded well to efgartigimod. At the first outpatient visit after discharge (on day 196), the patient had maintained the MSE status. Follow-up chest computed tomography (~6 months after the tumor removal) showed no evidence of recurrence of the thymoma.

Discussion

This patient, a 54 years-old woman, presented with severe weakness and promptly underwent the disease-modifying therapies considered standard first-line treatments for MG exacerbation, including plasmapheresis and IVIG (5). However, her clinical symptoms did not resolve following these interventions. A response to treatment is typically observed within 2 days of plasmapheresis and within 4–5 days of IVIG (6). In the case of IVIG, it has been noted that the therapeutic effect may appear over a relatively long period of time — as long as 28 days (7). In our patient's case, MG exacerbation persisted even after 2 weeks of IVIG, ultimately leading to MC. Moreover, her ventilator management continued until day 85. These clinical observations suggest that (i) the patient had refractory MC, and (ii) standard first-line therapies alone were inadequate for her treatment.

Intravenous efgartigimod is the first FcRn antagonist therapy approved in several countries worldwide for the chronic management of MG (8, 9). FcRn plays a central role in IgG homeostasis by rescuing IgGs from lysosomal degradation. Efgartigimod disrupts this IgG

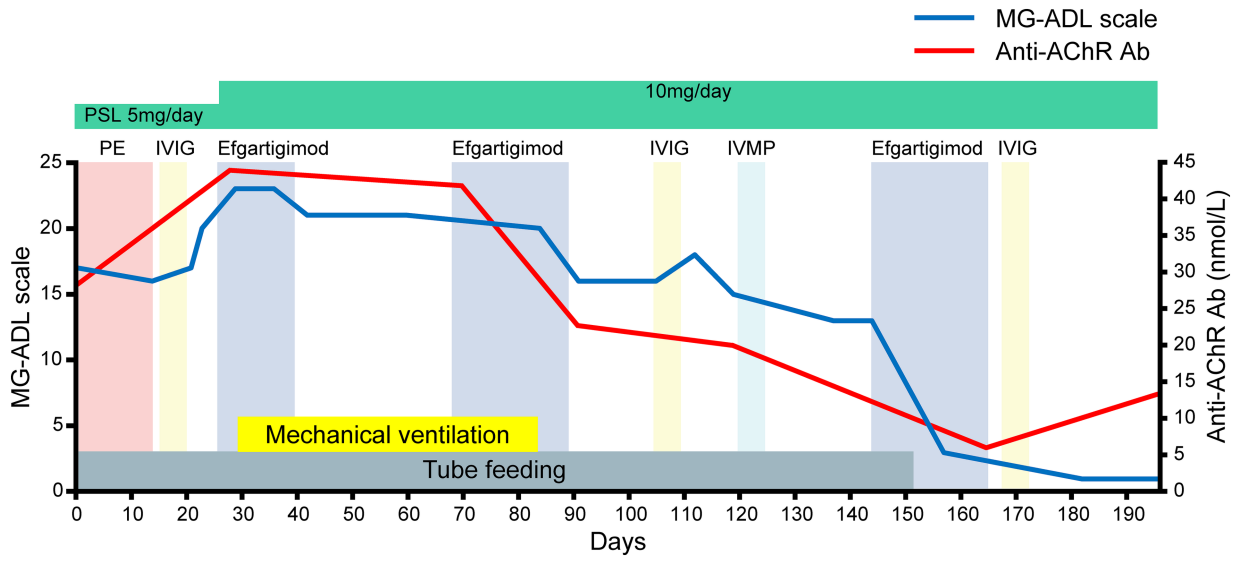


FIGURE 1
The patient's clinical course after admission as assessed by the myasthenia gravis-activities of daily living (MG-ADL) scale. The timing of each therapy, the MG-ADL results, and the titer of anti-AChR antibody (anti-AChR Ab) are shown. Day 0: the day of hospital admission. Note that serial assessments of the patient's serum anti-AChR antibody titers paralleled the clinical improvement assessed by the MG-ADL scale after efgartigimod administration. IVIG, intravenous immunoglobulin; IVMP, intravenous methylprednisolone; PE, plasmapheresis; PSL, prednisolone.

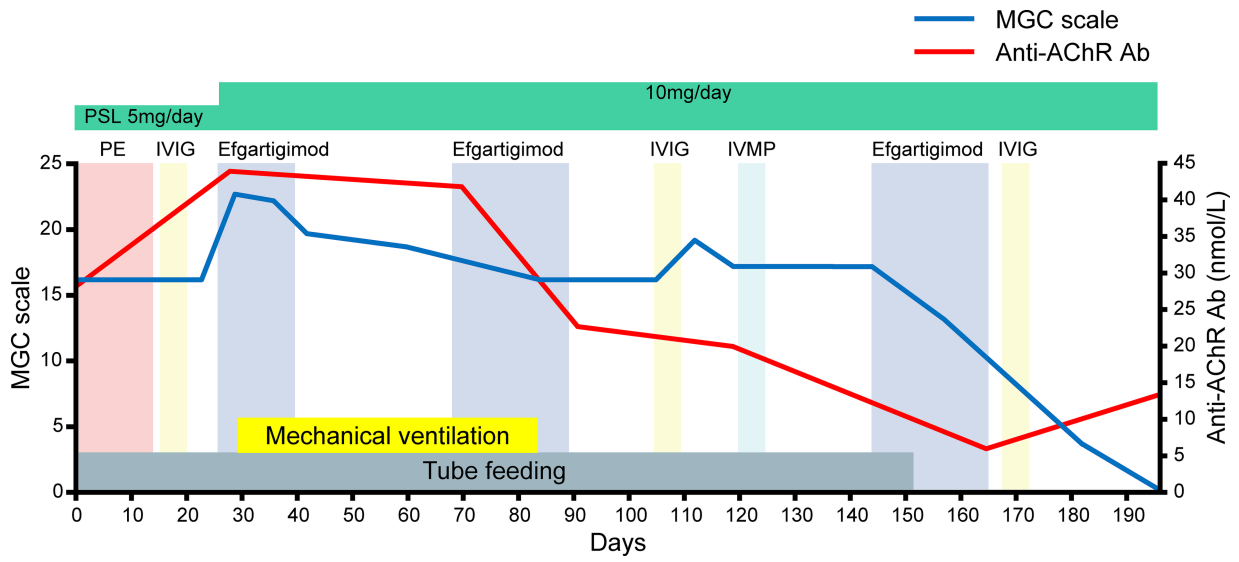


FIGURE 2
The patient's clinical course after admission as assessed by the myasthenia gravis composite (MGC) scale. Note that the serial assessments of the patient's serum anti-AChR antibody titers paralleled the clinical improvements assessed by the MGC scale after efgartigimod administration.

recycling process by binding to the FcRn, thereby reducing the levels of IgG, including anti-AChR antibodies, in the blood. Improvement can typically be observed within the first 1 to 2 weeks after injection. Because of this relatively immediate effect, it is thought that efgartigimod may be effective against MC (1). Improvement in our patient's MC was noted about 12 days after the first intravenous efgartigimod administration, suggesting the effectiveness of this therapy for MC. This clinical progress aligned with the serial assessments of the patient's serum anti-AChR antibody titers, further supporting the efficacy of this therapy for her case. Moreover, her status transitioned from MC to MSE after three cycles of intravenous efgartigimod, indicating the potential effectiveness of multi-cycle therapy for the treatment of MC. The interval before the third cycle was slightly longer because it took time to determine whether efgartigimod was effective for improving the symptoms of MC. The reduced time in the cycle may improve the efficacy of the drug.

The patient's serum anti-AChR levels decreased after the intravenous efgartigimod treatment, in contrast to the standard first-line treatment (Figure 3), and this correlated with the improvement in her clinical symptoms (Figures 1, 2). These findings suggest that the addition of intravenous efgartigimod was effective and rapid in reducing IgG in this patient compared to the standard first-line

treatment alone, such as plasmapheresis or IVIG. Regarding the patient's plasmapheresis, it is surprising that the plasmapheresis was ineffective while efgartigimod was effective, despite their similar mechanisms of action.

Therapeutically, plasmapheresis works by filtering circulating proteins and antibodies, whereas efgartigimod accelerates the catabolism of IgG (10). The anti-AChR antibodies belong to the IgG1–IgG3 class (11), and the depletion of IgG constitutes the primary objective of therapies designed to mitigate the pathophysiological repercussions of IgG autoantibodies binding to their targets. The efficacy of plasmapheresis in successfully depleting pathogenic targets hinges on various factors, including the size and half-life of the target molecule, its distribution within compartments (intravascular vs. extravascular), and the volume and frequency of plasma exchange (12). Due to the diminutive size and protracted half-life of IgG, multiple plasmapheresis procedures are imperative to eliminate IgG from the circulation and the extravascular space, with an estimated requirement of approx. six procedures to achieve a reduction in circulating IgG levels by ~60%–70% (12). Such procedures induce a precipitous decline in circulating IgG autoantibodies, potentially triggering an augmented production of autoantibodies (i.e., an anti-AChR antibody overshoot) following therapeutic plasmapheresis (13), leading to a resurgence of disease activity.

It is established that the increase in antibody levels observed after plasmapheresis likely reflects a reduction in catabolism, coupled with an unchanged rate of synthesis occurring in the extravascular space (e.g., spleen) (14). Moreover, when performing plasmapheresis, an invasive procedure is required for vascular access, and there are non-negligible problems associated with venous access, such as cardiovascular adverse events and infections (15). In contrast, treatment with FcRn inhibitors requires minimally invasive intervention and may be less contingent on the compartmental localization of IgG, effectively targeting both intravascular and extravascular IgG for lysosomal degradation, due to the nearly ubiquitous expression of FcRn (16). In addition, since the plasma concentrations of therapeutic antibodies persist for long periods of time (17), IgG depletion may be more sustained than the short-term effects of plasmapheresis. Another possibility is that only the anti-AChR antibodies in the intravascular space are removed by plasmapheresis, leading to an overshoot in the production of anti-AChR antibodies. In our patient's case, the intravenous efgartigimod may have affected the extravascular space, resulting in a comprehensive reduction of serum anti-AChR antibody levels, which in turn contributed to the clinical improvements.

IVIG requires a minimally invasive procedure, similar to efgartigimod. However, a problem with IVIG is the lack of volunteer blood donors, since IVIG is a product of plasma processing. IVIG has limited availability and relies on blood donors, which contributes to supply challenges (18). The limited availability is also because IVIG is used for many other indications (chronic inflammatory demyelinating polyneuropathy, hematology, etc.). In this respect, efgartigimod has an advantage over IVIG because it does not require blood from a donor.

A recent study described excellent results regarding MG complicated by SPSs successfully treated with efgartigimod; these patients experienced very large reductions in MG-ADL scores (19). This evidence opens the possibility of treating patients affected by anti-GAD antibody-related diseases, which are also often found in SPS. Since our patient also showed a decrease in the MG-ADL scale (Figure 1) and MGC scale (Figure 2), the coexistence of autoimmune diseases may need to be considered. Since our patient did not have anti-GAD antibodies and did not have the typical clinical manifestations of SPS such as progressive and fluctuating muscle rigidity (20), the possibility that SPS was at least a complication is negative. Nevertheless, clinicians should suspect SPS when a patient improves rapidly with efgartigimod, as in the present case, because of the risk of the underdiagnosis of SPS in patients with MG.

It should be noted that the effect of efgartigimod observed in our patient's case may be an "add-on" effect to other conventional therapies. Especially considering the duration of the effect onset of IVIG (7), it is difficult to interpret the course of our patient's case as an effect of efgartigimod alone. In addition, it must be emphasized

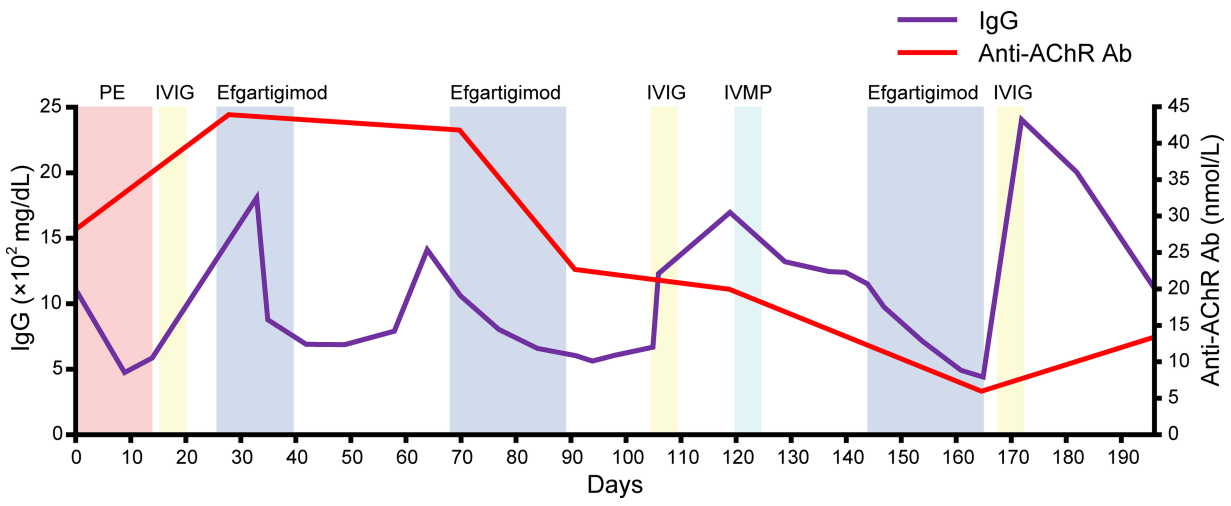


FIGURE 3
The serial data of the IgG concentration and anti-AChR antibody. Note that the serum IgG concentration was decreased after both plasmapheresis and intravenous efgartigimod, whereas anti-AChR antibodies were decreased after the intravenous efgartigimod only.

that the report presented here is a single case study; comprehensive investigations involving larger patient cohorts and experimental studies are needed to elucidate the underlying mechanisms and to develop this potential new therapeutic strategy for managing MC. Nonetheless, the observations from this case have promising implications for clinicians encountering cases of refractory MC.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical approval was not required for the studies involving humans because this is a case report and no experimental procedures were performed. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

KW: Conceptualization, Data curation, Investigation, Writing – original draft, Writing – review & editing. SO: Data curation, Writing – review & editing. TW: Data curation, Writing – review & editing. YK: Data curation, Writing – review & editing. RK: Conceptualization, Data curation, Funding acquisition, Supervision, Validation, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was partly supported by a grant from the Japan Society for the Promotion of Science (JSPS) KAKENHI, No. 23K00487.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Claytor B, Cho SM, Li Y. Myasthenic crisis. *Muscle Nerve*. (2023) 68:8–19. doi: 10.1002/mus.27832

2. Neumann B, Angstwurm K, Mergenthaler P, Kohler S, Schonenberger S, Bosel J, et al. Myasthenic crisis demanding mechanical ventilation: a multicenter analysis of 250 cases. *Neurology*. (2020) 94:e299–313. doi: 10.1212/WNL.0000000000008688

3. Vanoli F, Mantegazza R. Antibody therapies in autoimmune neuromuscular junction disorders: approach to myasthenic crisis and chronic management. *Neurotherapeutics*. (2022) 19:897–910. doi: 10.1007/s13311-022-01181-3

4. Murai H, Utsugisawa K, Motomura M, Imai T, Uzawa A, Suzuki S. The Japanese clinical guidelines 2022 for myasthenia gravis and Lambert–Eaton myasthenic syndrome. *Clin Exp Neuroimmunol*. (2023) 14:19–27. doi: 10.1111/cen3.12739

5. Gajdos P, Chevret S, Clair B, Tranchant C, Chastang C. Clinical trial of plasma exchange and high-dose intravenous immunoglobulin in myasthenia gravis. Myasthenia Gravis Clinical Study Group. *Ann Neurol*. (1997) 41:789–96. doi: 10.1002/ana.410410615

6. Gold R, Schneider-Gold C. Current and future standards in treatment of myasthenia gravis. *Neurotherapeutics*. (2008) 5:535–41. doi: 10.1016/j.nurt.2008.08.011

7. Zinman L, Bril V. IVIG treatment for myasthenia gravis: effectiveness, limitations, and novel therapeutic strategies. *Ann N Y Acad Sci*. (2008) 1132:264–70. doi: 10.1196/annals.1405.038

8. Howard JF Jr, Bril V, Vu T, Karam C, Peric S, Margania T, et al. Safety, efficacy, and tolerability of efgartigimod in patients with generalised myasthenia gravis (ADAPT): a multicentre, randomised, placebo-controlled, phase 3 trial. *Lancet Neurol*. (2021) 20:526–36. doi: 10.1016/S1474-4422(21)00159-9

9. Bhandari V, Bril V. FcRN receptor antagonists in the management of myasthenia gravis. *Front Neurol*. (2023) 14:1229112. doi: 10.3389/fneur.2023.1229112

10. Mina-Osorio P, Tran M-H, Habib AA. Therapeutic plasma exchange versus FcRn inhibition in autoimmune disease. *Transfus Med Rev*. (2023) 38:150767. doi: 10.1016/j.tmrv.2023.150767

11. Rødgaard A, Nielsen F, Djurup R, Somnier F, Gammeltoft S. Acetylcholine receptor antibody in myasthenia gravis: predominance of IgG subclasses 1 and 3. *Clin Exp Immunol*. (1987) 67:82–8.

12. Rossing N. Intra- and extravascular distribution of albumin and immunoglobulin in man. *Lymphology*. (1978) 11:138–42.

13. Ching J, Richards D, Lewis RA, Li Y. Myasthenia gravis exacerbation in association with antibody overshoot following plasmapheresis. *Muscle Nerve*. (2021) 64:483–7. doi: 10.1002/mus.27341

14. Charlton B, Schindhelm K. The effect of extracorporeal antibody removal on antibody synthesis and catabolism in immunized rabbits. *Clin Exp Immunol*. (1985) 60:457–64.

15. Ipe TS, Davis AR, Raval JS. Therapeutic plasma exchange in myasthenia gravis: a systematic literature review and meta-analysis of comparative evidence. *Front Neurol*. (2021) 12:662856. doi: 10.3389/fneur.2021.662856

16. Kim J, Hayton WL, Robinson JM, Anderson CL. Kinetics of FcRn-mediated recycling of IgG and albumin in human: pathophysiology and therapeutic implications using a simplified mechanism-based model. *Clin Immunol*. (2007) 122:146–55. doi: 10.1016/j.clim.2006.09.001

17. Pyzik M, Sand KMK, Hubbard JJ, Andersen JT, Sandlie I, Blumberg RS. The neonatal Fc receptor (FcRn): a misnomer? *Front Immunol*. (2019) 10:1540. doi: 10.3389/fimmu.2019.01540

18. Pavlekovics M, Engh MA, Lugosi K, Szabo L, Hegyi P, Terebessy T, et al. Plasma exchange versus intravenous immunoglobulin in worsening myasthenia gravis: a systematic review and meta-analysis with special attention to faster relapse control. *Biomedicine*. (2023) 11:3180. doi: 10.3390/biomedicines11123180

19. Di Stefano V, Alonge P, Rini N, Militello M, Lupica A, Torrente A, et al. Efgartigimod beyond myasthenia gravis: the role of FcRn-targeting therapies in stiff-person syndrome. *J Neurol*. (2023) 271:254–62. doi: 10.1007/s00415-023-11970-1

20. Toro C, Jacobowitz DM, Hallett M. Stiff-man syndrome. *Semin. Neurol*. (1994). 14:54–8. doi: 10.1055/s-2008-1041073

frontiers | Frontiers in Neurology

TYPE Original Research
PUBLISHED 01 February 2024
DOI 10.3389/fneur.2024.1309173

OPEN ACCESS

EDITED BY
Francesco Saccà,
University of Naples Federico II, Italy

REVIEWED BY
Xuemei Chen,
Shanghai Jiao Tong University, China
Frauke Stascheit,
Charité University Medicine Berlin, Germany

*CORRESPONDENCE
Juan Carlos Trujillo Reyes
✉ jtrujillo@santpau.cat

RECEIVED 07 October 2023
ACCEPTED 04 January 2024
PUBLISHED 01 February 2024

CITATION
Trujillo Reyes JC, Martinez Tellez E, Belda Sanchis J, Planas Canovas G, Libreros Niño A, Guarino M, Hernández Ferrandez J and Moral Duarte A (2024) Are the minimally invasive techniques the new gold standard in thymus surgery for myasthenia gravis? Experience of a reference single-site in VATS thymectomy.
Front. Neurol. 15:1309173.
doi: 10.3389/fneur.2024.1309173

Are the minimally invasive techniques the new gold standard in thymus surgery for myasthenia gravis? Experience of a reference single-site in VATS thymectomy

Juan Carlos Trujillo Reyes[1,2]*, Elisabeth Martinez Tellez[1,2], Josep Belda Sanchis[1,2], Georgina Planas Canovas[1], Alejandra Libreros Niño[1], Mauro Guarino[1], Jorge Hernández Ferrandez[1] and Antonio Moral Duarte[2,3]

[1]Department of Thoracic Surgery, Hospital de la Santa Creu I Sant Pau, Barcelona, Spain, [2]Department of Surgery, Faculty of Medicine, Autonomous University of Barcelona, Bellaterra, Spain, [3]Department of General Surgery, Hospital de la Santa Creu I Sant Pau, Barcelona, Spain

The thymus is the primary lymphoid organ responsible for the maturation and proliferation of T lymphocytes. During the first years of our lives, the activation and inactivation of T lymphocytes occur within the thymus, facilitating the correct maturation of central immunity. Alterations in the positive and negative selection of T lymphocytes have been studied as the possible origins of autoimmune diseases, with Myasthenia Gravis (MG) being the most representative example. Structural alterations in the thymus appear to be involved in the initial autoimmune response observed in MG, leading to the consideration of thymectomy as part of the treatment for the disease. However, the role of thymectomy in MG has been a subject of controversy for many years. Several publications raised doubts about the lack of evidence justifying thymectomy's role in MG until 2016 when a randomized study comparing thymectomy via sternotomy plus prednisone versus prednisone alone was published in the New England Journal of Medicine (NEJM). The results clearly favored the group of patients who underwent surgery, showing improvements in symptoms, reduced corticosteroid requirements, and fewer recurrences over 3 years of follow-up. In recent years, the emergence of less invasive surgical techniques has made video-assisted or robotic-assisted thoracoscopic (VATS/RATS) thymectomy more common, replacing the traditional sternotomy approach. Despite the increasing use of VATS, it has not been validated as a technique with lower morbidity compared to sternotomy in the treatment of MG. The results of the 2016 trial highlighted the benefits of thymectomy, but all the patients underwent surgery via sternotomy. Our hypothesis is that VATS thymectomy is a technique with lower morbidity, reduced postoperative pain, and shorter postoperative hospital stays than sternotomy. Additionally, VATS offers better clinical improvement in patients with MG. The primary objective of this study is to validate the VATS technique as the preferred approach for thymectomy. Furthermore, we aim to analyze the impact of VATS thymectomy on symptoms and corticosteroid dosage in patients with MG, identifying factors that may predict a better response to surgery.

10.3389/fneur.2024.1309173

KEYWORDS

thoracoscopy, myasthenia gravis, video-assisted thoracic surgery, MG treatment, thymic hyperplasia, thymic tumors

1 Introduction and objectives

The thymus gland, also known as the thymus, is the primary lymphoid organ responsible for the maturation of T lymphocytes, the proliferation of mature T lymphocyte clones, and the control of self-reactive T lymphocytes. During the early years of our lives, within the gland, the activation and, most importantly, the inactivation of T lymphocytes occur, leading to the correct maturation of central immunity (1, 2).

Surgical removal of the thymus, known as thymectomy, is a classic technique first described in 1912 by Ferdinand Sauerbruch, who performed a transcervical thymectomy along with thyroidectomy in a patient with hyperthyroidism and MG. Years later, in 1936, Blalock recommended the exploration of the mediastinum and the performance of a thymectomy in patients with severe MG.

One of the most significant aspects of thymus function is its relationship with autoimmunity. The theories attempting to explain the changes or alterations that cause autoimmune disorders appear too simplistic, especially considering the complexity of the maturation process that takes place in the thymic cortex (3). Currently, there are two scenarios in which thymectomy may be indicated:

- The first scenario is when a thymic tumor is diagnosed. Surgery is the primary treatment, whether or not the tumor is associated with the presence of MG. At the end of 2015, the World Health Organization (WHO) published the 4th edition of the classification of thoracic tumors, where, for the first time, all thymic tumors of malignant etiology are considered (4). This represents a significant advancement in our understanding of thymic tumors and their classification. Fundamental fact when considering treatment, with surgical resection indicated even in advanced stages.
- A second scenario in which thymectomy may be indicated involves selected patients with MG who do not have an associated thymic tumor. The role of thymic surgery in MG has been a subject of debate for many years. A publication by Jaretzki and Sonett in 2008 highlighted the lack of randomized trials supporting the role of thymectomy in patients with MG (5). Another example of this debate occurred in 2010 (6) after the publication of guidelines on the treatment of neuromuscular pathologies, which pointed out the lack of consistent selection criteria and assessments of response after thymectomy.

This controversy surrounding the role of surgery led to a prospective, randomized, multicenter trial, the results of which were published in the NEJM in 2016 (7). This study compared two groups: the first group underwent transsternal thymectomy in addition to prednisone treatment, while the second group received prednisone-only treatment. The results clearly favored the group of patients who underwent surgery, showing improvements in symptoms, reduced need for corticosteroid therapy, and a lower number of recurrences at three years of follow-up.

Following the publication of these results, the role of the thoracic surgeon as part of the multidisciplinary treatment of MG regained importance. The findings from the NEJM publication and the new WHO classification reaffirmed the role of thymectomy both in the treatment of MG patients and in those diagnosed with thymic tumors.

From a surgical perspective, one of the recent advancements has been the progressive and successful implementation of minimally invasive techniques. Initially, VATS and later RATS surgery have become the preferred techniques for most thoracic procedures. However, have we truly validated these techniques in thymus surgery, and what advantages do they offer over the traditional choice of sternotomy?

1.1 The thymus and self-tolerance

1.1.1 Functions of the thymus in self-tolerance

The thymic gland plays an essential role in T cell differentiation, establishing central tolerance. While both B and T cells recognize antigens specifically, they follow different pathways. T cells are incapable of recognizing antigens in their natural form and require maturation within antigen-presenting cells. There are two types of T cells: CD4 and CD8, each with distinct functions and development. CD4 cells assist in antibody formation and are involved in delayed-type hypersensitivity, recognizing antigens strictly in association with MHC class II. Conversely, CD8 cells play a role in viral infection defense, tumor rejection, and graft rejection, recognizing antigens in association with MHC class I.

The thymus is where the T cell receptor repertoire is generated, along with T lymphocyte maturation. During this differentiation process, a critical step is immune tolerance to self, which is relevant to MG.

Within the thymic cortex, the enabling environment for T-cell differentiation is created. As T cells migrate to the deep cortical areas, they express both CD4 and CD8. During their maturation, they develop into mature CD4+, CD4-, CD8+, and CD8- cells (8).

1.1.2 The relationship between the thymus and MG

The role of the thymus in the pathogenesis of MG has been a subject of debate for many years and has undergone numerous studies. Structural changes in the thymus appear to be involved in the initial autoimmune response observed in MG. Autopsy analysis suggests that the thymus exhibits abnormalities in 80–90% of MG patients (9).

1.1.2.1 Follicular lymphoid hyperplasia

Follicular lymphoid hyperplasia may be present in up to 70% of MG patients. It is frequently observed in women with early disease onset (age at onset less than 50 years with thymic hyperplasia). The

architecture of the hyperplastic thymus remains intact. What stands out is the increased number of germinal centers in the medulla, similar to those observed in lymph nodes.

In follicular lymphoid hyperplasia, we can find B and T cells, plasma cells, and myoid cells. Myoid cells are the only cells capable of expressing antigenic determinants of the main immunogenic region of RACh (10). Thymocytes in culture can generate anti-RACh antibodies (AChR-Abs), supporting the disease's pathogenesis (11).

1.1.2.2 Normal thymus or thymic remnants

These are very common in cases of MG with late onset (age at onset greater than 50 years with thymic atrophy. Thymoma-associated MG. MG with anti-MuSK antibodies) and do not have a clear causal relationship or mechanism.

1.1.2.3 Thymic tumor and thymoma

Approximately 10% of MG patients will present with a thymoma. They typically have AChR-Abs and exhibit more severe clinical manifestations but a similar prognosis to other MG patients (12).

In addition to MG, thymic tumors are associated with other autoimmune disorders. This can be explained by the fact that tumor cells present autoantigens, leading to the disruption of lymphocyte selection (13). A deficiency in AIRE gene expression and the selective loss of regulatory T cells have been described as the causes of negative selection and the upregulation of autoreactive T cells (14).

As previously mentioned, thymic tumors have been classified as malignant since the 2015 WHO classification (4). The treatment of thymic tumors should be individualized and approached from a multidisciplinary standpoint. Thymic tumor follow-up should be conducted for at least 10 years due to the tumor's biology. This follow-up should run concurrently with that of MG, as MG exacerbations can sometimes result from tumor recurrence and should always be ruled out.

1.1.3 Current evidence for surgery in MG

The ongoing controversy regarding the role of surgery led to a prospective, randomized, multicenter trial, the results of which were published in the NEJM journal in 2016 (7). The results were decidedly favorable in the group of patients who underwent surgery, showing improvements in symptoms, reduced reliance on corticosteroid therapy, and a lower number of recurrences at the three-year follow-up.

After the publication of these results, the role of the thoracic surgeon within the multidisciplinary treatment of MG regained significance. The findings from the NEJM publication, along with the new WHO classification, further emphasize the importance of thymectomy in the management of MG patients and those diagnosed with thymic tumors.

1.2 Types of surgical approach and recommended extent of thymectomy

1.2.1 Types of surgical approach

Transcervical approach: As mentioned earlier, the inclusion of mediastinoscopy by Carlens (15) led to the resurgence of the transcervical approach in thymic gland resection. Zielinski et al. (16) have one of the largest published series of maximum thymectomies performed transcervically using the TEMLA (Transcervical Extended Mediastinal Lymphadenectomy) technique.

Trans-sternal approach: This category encompasses partial, total, or transverse sternotomy approaches. Through sternotomy, access is provided to both pleural cavities.

Cervical and trans-sternal approach: Combining both approaches allows us to explore the cervical area to rule out the presence of supernumerary thymic gland poles.

1.2.2 Recommended extent of thymectomy

It's essential to consider the anatomy of the thymic gland, being aware of its extrathoracic locations where supernumerary thymic gland poles can be found.

As mentioned previously, regardless of the approach chosen, a more extensive thymectomy yields better results, as described by Zielinski et al. (17).

Therefore, it is recommended to perform maximum thymectomy in both cases with associated thymic tumors and cases without them. A maximum extension is advised, avoiding thymomectomy, even in cases where there is no associated MG or other paraneoplastic syndromes, due to the non-negligible percentage of secondary tumor foci in other poles of the thymic gland.

1.2.3 The emergence of thoracoscopic surgery in thymus surgery for MG

The introduction of thoracoscopic surgery in thymus surgery for MG marks a significant development. From a surgical perspective, one of the notable recent advances has been the gradual and successful integration of minimally invasive techniques in the field of thoracic surgery. Initially, VATS and subsequently RATS surgery have become the preferred techniques for most thoracic procedures.

1.2.4 A critical issue in thymic surgery

One of the critical issues in thymic surgery is whether thoracoscopy can achieve the recommended resection and effectively remove most of the thymic gland.

Mantegazza (18) compared patients operated on for MG without associated thymic tumors, with 159 cases operated on using VATS versus 47 operated on with sternotomy. He focused on analyzing morbidity and mortality, observing a reduction compared to sternotomy. This study continued to address the concerns of critics of MG surgery, as the results of the 2016 study were not yet available.

Years later, in 2018, following the publication of the 2016 study in NEJM, Salim (19) presented a series of 50 patients, with 25 operated on using thoracoscopy and 25 using ministernotomy. The objective was to assess an even greater improvement in disease severity in MG in the thoracoscopy-operated group.

1.2.5 The lack of comprehensive studies on thoracoscopic thymic gland surgical procedures

The scarcity of studies involving a substantial number of thoracoscopic thymic gland surgical procedures has prompted the initiation of the present project. The aim is to analyse our series of thymectomies conducted via thoracoscopy in comparison to those

performed through sternotomy. This project seeks to contribute to the validation of the technique and to observe its impact on MG, specifically assessing how it affects symptomatology and the use of immunosuppressive treatments.

In this current study, we aim to analyse our series of thoracoscopic thymectomies and compare their outcomes with those of patients who underwent sternotomy. Additionally, we intend to assess the therapeutic outcomes on MG and thymic tumors in patients who underwent surgery, whether they had MG alone or in association with a thymic tumor.

1.3 Study hypotheses

- VATS thymectomy is a safe technique that results in reduced morbidity compared to sternotomy.
- VATS thymectomy is comparable to sternotomy in terms of improving the clinical condition and the need for corticosteroids in the medium and long term in patients with MG or other PNS.
- The presence of a concomitant thymic tumor in MG is a negative predictor for the MG's progression.

2 Methodology

To validate our hypothesis, we designed an observational study conducted at a single-center referral center for MG. This study included 113 patients who had undergone thymectomy. Data were collected as follows: From 1990 to 2016, we retrospectively collected data from 40 patients who underwent sternotomy. From 2017 to 2021, we prospectively collected data from 73 patients who underwent VATS. In each group, we analyzed the following variables: surgical complications within 30 days, postoperative pain, and the duration of hospitalization. Additionally, for patients diagnosed with MG, we assessed the occurrence of myasthenic crises after surgery, the clinical response to thymectomy based on physical examination, and the impact on corticosteroid treatment at one, six, and twelve months following surgery.

2.1 Study population

2.1.1 Selection of cases

2.1.1.1 Retrospective cases

We identified cases from the databases of the Thoracic Surgery and Pulmonary Allergy departments at Santa Creu i Sant Pau Hospital, where thymectomy had been performed for the excision of mediastinal masses.

2.1.1.2 Prospective cases

All patients diagnosed with MG who underwent VATS and those who underwent VATS or sternotomy with a diagnosis of mediastinal masses associated with MG or other paraneoplastic syndromes were prospectively included. Patients with a diagnosis of MG or other paraneoplastic syndromes not associated with thymic tumors were exclusively included in the prospective study.

Once the anatomopathological results were confirmed, and patients did not meet any exclusion criteria, they were included in the study.

2.2 Inclusion and exclusion criteria

2.2.1 Inclusion criteria

- Patients diagnosed with a thymic tumor and associated paraneoplastic syndromes (MG or other) undergoing VATS thymectomy.
- Patients diagnosed with a thymic tumor and associated paraneoplastic syndromes (MG or other) undergoing sternotomy thymectomy.
- Patients diagnosed with MG or other paraneoplastic syndromes undergoing VATS thymectomy.
- Age between 16 and 85 years.
- Patients with thymic tumors without extrathoracic metastases.
- Patients who do not meet exclusion criteria.

2.2.2 Exclusion criteria

- Patients with comorbidities that prevent them from undergoing surgical treatment.
- Patients with thymic tumors and extrathoracic metastases.
- Patients diagnosed with MG who do not meet the criteria for surgical indication.

The data search was conducted with a specific focus to achieve the established objectives.

In the primary cohort, the following data are recorded:

Preoperative characteristics: This includes information such as sex, age, the preoperative diagnosis that prompted the surgical intervention, and the surgical technique used for thymectomy. For patients diagnosed with MG or other paraneoplastic syndromes, the titration of autoantibodies in peripheral blood and whether or not corticosteroids were taken before surgery are recorded. If corticosteroids were taken, their doses in milligrams are also recorded.

Postoperative characteristics: These encompass the number of days of hospital stay and postoperative pain assessed using the VAS scale at discharge and one month after discharge.

Surgical specimen: This includes the definitive anatomopathological result. For patients with confirmed thymic tumors, the tumor stage according to the 8th TNM classification and the status of the neoplastic disease according to the last thoracic CT scan performed before the end of follow-up are also documented.

Patients with a diagnosis of MG: For these patients, data recorded include clinical worsening immediately after the intervention and the clinical assessment of the disease at the last recorded visit. For both variables, fatigue and muscle weakness were documented using the MG-ADL scale (20) described above in the study variables. The MG-ADL scale analyses the degree of difficulties that has the patient doing activities of daily living such as talking, chewing, swallowing, breathing, impairment of ability to brush teeth, or comb hair, impairment of ability to arise from a chair, double vision and eyelid droop. According to the results, the MG-ADL scale classifies the degree of MG from 0 to 3, being 0 a clinical situation with minimal symptomatology and being 3 severe symptomatology.

Postoperative complications: This section comprises details regarding mortality data, the reasons for patient exits during the entire follow-up period, the conversion rate in the VATS group, and complications associated with surgery within the initial 30 days post-surgery. Furthermore, a bivariate and multivariate analysis was conducted to identify independent predictors of complications following thymectomy, irrespective of the surgical approach used.

2.3 Analysis by subgroups

2.3.1 Subgroup of patients according to the route of approach (VATS vs. sternotomy)

In this subgroup, the following characteristics were analyzed:

- *Preoperative characteristics*: Recording the median age in both the VATS group and the sternotomy group.
- *Postoperative characteristics*: Documentation of the number of days of hospital stay, levels of postoperative pain, and any postoperative complications.

2.3.2 Subgroup of patients with a diagnosis of MG or other paraneoplastic syndromes (PNS)

Within this subgroup, the following characteristics are examined:

- *Preoperative characteristics*: Noting the surgical approach used and the titers of MG-specific autoantibodies in peripheral blood (AChR-Abs and anti-MuSK autoantibodies).
- *Postoperative characteristics*: Recording the duration of hospitalization, any changes in the dose of corticosteroid therapy if taken before surgery, any worsening of MG after surgery, and a clinical assessment at the last visit based on the MG-ADL scale.

2.3.3 Subgroup of patients with a diagnosis of MG or other PNS

In this subgroup, the analysis includes the definitive anatomopathological diagnosis according to the pathology report and its relationship with clinical improvement according to the MG-ADL scale as a postoperative variable.

2.3.4 Subgroup of patients with a diagnosis of thymic tumor

This subgroup includes the following:

- *Preoperative characteristics*: The surgical approach route used.
- *Postoperative characteristics*: The tumor stage and the status of the neoplastic disease at the last visit are documented.

The characteristics of the information source, the storage and database and the statistical analysis used are detailed below:

2.4 Sources of information

For this study, specific information in the variables section was collected from the medical records of the patients who consented to participate. This data was extracted from the SAP patient software, SAP Logon 7.40 CA ES x86N4, which has been in use at the Hospital de la Santa Creu i Sant Pau since 2011.

Patients who underwent surgery before 2011 had their medical records obtained in paper format from the hospital's documentation department.

2.5 Data storage and processing

Throughout the study and during the targeted search for patient history information, the collected data were recorded on a designated form. These data were then entered into an Excel spreadsheet for storage and evaluation. Each patient was assigned an identification code.

The data were subsequently imported from the Excel spreadsheet and stored in the IBM SPSS program, version 22.0, for further analysis.

2.6 Expected sample size

No formal sample size calculation was conducted for this study. Since it is a retrospective and prospective descriptive study, all patients who underwent thoracic surgery and met the inclusion criteria during the estimated time frame were included. The last patient was included in December 2021 to allow for a two-month follow-up period for data analysis.

2.7 Statistical analysis

The statistical analysis was conducted by *Sail Biometria – Research and Logistics Consulting Services*[1] and was funded by resources from the Thoracic Surgery Service at the *Hospital de la Santa Creu i Sant Pau* in Barcelona.

All study variables are summarized using descriptive statistics. For quantitative variables, the following statistics are presented: mean, standard deviation (SD), 95% confidence interval (95% CI) of the mean, median, range, and interquartile range. For qualitative variables, the summary includes absolute frequency (*n*) and relative frequency (%), categorized accordingly.

The distribution type of the variables was assessed, and their adherence to a Gaussian distribution was evaluated using the Kolmogorov–Smirnov test.

Homogeneity was examined among the following cohorts:

- Global sample ($N = 113$)
- VATS thymectomy vs. sternotomy
- 30-day post-surgery complications (No vs. Yes)
- Sample with MG or SPN
- Without thymic tumor (TmT) vs. with thymic tumor (TmT)
- With clinical improvement vs. without clinical improvement

1 https://www.sail-biometria.com

- Preoperative corticosteroids vs. corticosteroids at 1-year follow-up

Statistical significance (value of *p*) was calculated using the appropriate statistical tests as follows:

- Fisher's exact test for binary variables
- Chi-square test for variables with more than two categories
- Student's *t*-test for continuous variables that follow a normal distribution
- Mann–Whitney test for continuous variables that do not follow a normal distribution
- Spearman's correlation for ordinal variables

2.8 Ethical aspects

2.8.1 Benefit–risk assessment of the research

Through this project, we hope to derive indirect benefits for patients who undergo this intervention:

- o Awareness of the reduced morbidity associated with VATS thymectomy.
- o Direct impact on MG or other paraneoplastic syndromes (PNS) in terms of clinical improvement and reduced immunosuppressant usage after surgery.
- o Assessment of the therapeutic impact of VATS thymectomy at the oncologic level.

2.8.2 Ethical, subject information, and informed consent considerations

The study was conducted in strict accordance with international ethical guidelines for medical research involving human subjects. The investigator ensured that the study adhered to the principles outlined in the Declaration of Helsinki.

Before beginning the study, the Ethics Committee of the *Hospital de la Santa Creu i Sant Pau* approved the study protocol, the information provided to the subjects, and the informed consent form used.

The investigator or a designated representative explained the study's objectives, methods, and potential risks to the subject or their legal guardian or family member.

2.8.3 Considerations on the treatment of biological samples

No biological samples were collected for this study.

2.8.4 Data confidentiality

Regarding the confidentiality of study data, we adhered to the provisions outlined in Organic Law 15/1999 of December 13, 1999, concerning the "Protection of Personal Data."

2.8.5 Conflicts of interest

It is hereby declared that there are no conflicts of interest associated with the conduct of this research, whether by the principal investigator, directors, tutors, or other collaborators.

3 Results

3.1 Reference study population cohort

Between July 1990 and December 2021, a total of 113 thymectomies were performed at the Thoracic Surgery Service of the Hospital de la Santa Creu i Sant Pau. These surgeries were carried out either as treatment for mediastinal masses suspected of being tumors or as treatment for MG.

3.1.1 Preoperative characteristics

3.1.1.1 Sex and age

The majority of the patients who underwent surgery were women ($n = 64$; 56.6%). The median age of these patients was 53 years, with a range from 17 to 84 years.

3.1.1.2 Preoperative diagnosis

Out of the 113 cases operated on (Figure 1):

- MG: clinically, 68 patients (60%) had a diagnosis of MG.
- Other paraneoplastic syndromes (PNS): Nine patients (8%) had PNS other than MG, including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), bullous pemphigoid, and polyneuropathy.

3.1.1.3 Anatomical findings

From an anatomical perspective, CT imaging revealed some form of anatomical alteration in thymic cells in 100 patients (90%).

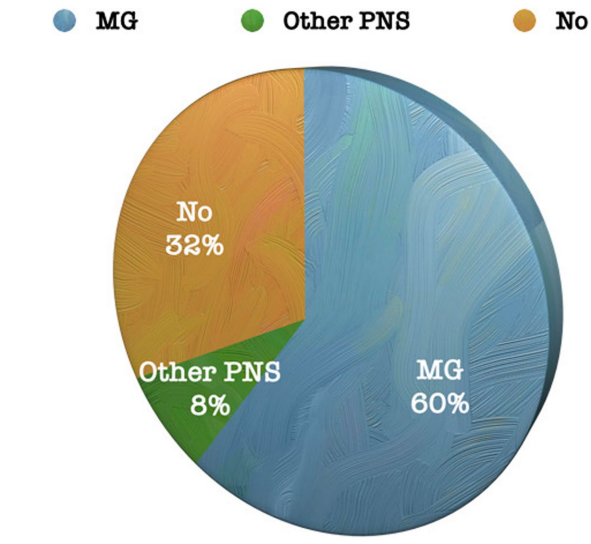


FIGURE 1
Percentage of patients diagnosed with PNS in the entire series.

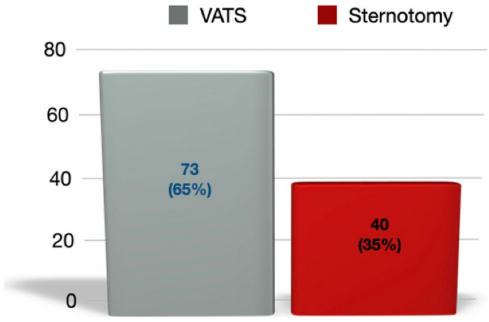


FIGURE 2
Total patients and percentage relative to the total cohort, depending on the surgical technique used.

3.1.1.4 Surgical technique

The majority of patients underwent VATS ($n = 73$; 64.6%), with the right unilateral approach being the most commonly used ($n = 52$; 71.2%) (Figure 2).

3.1.1.5 Corticosteroid treatment

Out of the group of patients with MG or other PNS, 47 patients (61%) were receiving corticosteroid treatment before surgery. Preoperative corticosteroid doses were recorded.

3.1.1.6 Peripheral blood antibodies

In patients with MG or other PNS, peripheral blood was tested for AChR-Abs and anti-MuSK antibodies. These antibodies were found to be positive in 71 out of 77 patients (92%).

3.1.2 Postoperative characteristics

3.1.2.1 Anatomopathologic diagnosis

The anatomopathologic diagnosis after surgery yielded the following results:

- *Thymic hyperplasia*: A total of 23 patients (20.4%) underwent surgery with a diagnosis of thymic hyperplasia. All of these cases were patients who had a preoperative diagnosis of MG.
- *Primary thymic tumor*: Out of the total patients, 66 (58%) had a preoperative suspicion of a thymic tumor, and this diagnosis was confirmed after surgical resection. Among the patients with thymic tumors, 37% ($n = 42$) had MG or other associated PNS.
- *Other lesions*: In 11 cases (9.7%), the anatomopathological diagnosis after surgical resection confirmed a different diagnosis, including thymolipoma, thymic cysts, and ectopic parathyroid gland.

3.1.2.2 Days of hospital stay

The median number of days of hospitalization after thymectomy in our series was 2 days, with a range from 1 to 40 days.

3.1.2.3 Postoperative pain

Postoperative pain was assessed using the Visual Analog Scale (VAS). When analyzing the entire patient cohort, 43.8% of the patients classified their pain as mild at the time of discharge, and this percentage increased to 71.4% one month after the operation. Only 4.5% of the patients ($n = 5$) rated their pain as severe (VAS 8–10) one month after surgery.

3.1.2.4 Treatment with corticosteroids after surgery

Regarding the reduction of corticosteroid treatment, approximately 50% of the patients who underwent surgery were able to progressively reduce their corticosteroid doses.

3.1.2.5 Clinical assessment in specific consultation for MG or other associated NPS

Among the patients diagnosed with MG or other NPS, the majority experienced clinical improvement ($n = 41$; 53.2%) or clinical stability ($n = 27$; 35.1%) during follow-up consultations. Only 9 patients (11.7%) showed worsening of their condition after surgery.

3.1.3 Postoperative complications

3.1.3.1 Mortality

Out of the entire series of 113 patients, the mortality rate during follow-up was 9.7% ($n = 11$). Among these, one patient (9.1%) died in the immediate postoperative period (on the 3rd day).

3.1.3.2 Conversion rate

The conversion rate was analyzed only in the VATS group. Among these patients, only 2 cases were converted to sternotomy, which corresponds to 2.7% of the VATS cases. In both cases, the cause of conversion was bleeding from a large vessel.

3.1.3.3 Complications associated with surgery at 30 days post-surgery

The complication rate in the entire series was 24.8% ($n = 28$). The most frequent complication was phrenic nerve injury ($n = 5$; 17.9%), followed by hemothorax ($n = 4$; 14.3%) and pneumothorax ($n = 4$; 14.3%). There was only one empyema in the entire series associated with surgical intervention (3.6%).

Bivariate analysis identified two predictors of complications after thymectomy: the surgical approach and the presence of a thymic tumor.

3.1.3.4 Presence of thymic tumor

Although the result was not statistically significant ($p = 0.0751$), there was a 21% increase in complications when the intervention was performed for the excision of a thymic tumor.

After multivariate analysis, it was observed that the two factors acting as predictors of complications were the surgical approach and the presence of preoperative MG, whereas the presence or absence of a thymic tumor was not statistically significant (Figure 3).

1 *Surgical approach*: Patients undergoing VATS have a lower risk of complications (OR = 0.239; $p = 0.0027$) than patients undergoing sternotomy. In other words, patients operated by sternotomy are 4.2 times more likely (=1/0.239) to have more complications than patients operated by VATS.
2 *Presence of MG*: Patients with a preoperative diagnosis of MG have 3.5 times more risk of complications than patients without MG ($p = 0.0283$).

10.3389/fneur.2024.1309173

The LOGISTIC Procedure

Response Variable Complicación 30 días
Number of Observations Read 113
Number of Observations Used 113

Ordered Value	COMP30D	Total Frequency
1	Sí	28
2	No	85

Analysis of Maximum Likelihood Estimates

Parameter		DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept		1	-1.2943	0.2816	21.1206	<.0001
TECNICA_IQ	VATS	1	-0.7162	0.2389	8.9904	0.0027
MG_SPN	Sí	1	0.6327	0.2884	4.8120	0.0283

Odds Ratio (OR) Estimates

Effect		Point Estimate	95% Wald Confidence Limits	
TECNICA	**VATS vs Sternotomy**	**0.239**	**0.094**	**0.609**
MG_SPN	**Sí vs No**	**3.545**	**1.144**	**10.981**

Association of Predicted Probabilities and Observed Responses

Percent Concordant	55.7	Somers' D	0.386
Percent Discordant	17.1	Gamma	0.530
Percent Tied	27.2	Tau-a	0.145
Pairs	2380	c	0.693

FIGURE 3
Predicators of post-surgery complications – multivariate logistic regression. In red, the higher probability of complications is marked in case of having MG or undergoing thymectomy by sternotomy.

3.2 Patient cohorts according to the route of approach (VATS vs. sternotomy)

3.2.1 Preoperative characteristics

3.2.1.1 Age

The median age in both groups is similar (VATS = 52.6 [17.5; 84.1]; sternotomy = 54.1 [28.2; 81.8]), and this difference is not statistically significant.

3.2.2 Postoperative characteristics

3.2.2.1 Days of hospital stay

In the sternotomy thymectomy group, the median number of days of hospital stay was significantly higher ($p < 0.0001$) than in VATS patients (2.0 vs. 6.5, respectively).

3.2.2.2 Postoperative pain

Patients who underwent VATS had less pain at discharge and one month after surgery, and this difference was statistically significant ($p < 0.0001$). Up to 62% of the VATS patients had mild pain at the time of discharge, whereas this percentage was only 7.7% in those who underwent sternotomy.

3.2.3 Postoperative complications

3.2.3.1 Complications associated with surgery at 30 days post-surgery

The rate of complications at 30 days post-surgery was significantly lower in patients operated by VATS ($p < 0.0112$). Among those who underwent sternotomy, 40% presented complications, with surgical wound infection ($n = 8$) being the most frequent. Forty percent of the patients in whom thymectomy was performed by sternotomy presented complications, the most frequent being surgical wound infection ($n = 8$). In the VATS group the most frequent complication was pneumothorax ($n = 4$) (Figure 4).

3.3 Cohort of patients with MG

The results of the cohort of patients with a preoperative diagnosis of MG are presented below. Of the total number of patients who underwent surgery ($n = 113$), 60% had a preoperative diagnosis of MG ($n = 68$; 60.1%).

The results of the variables that help us to assess the impact on the evolution of MG according to the type of approach by which thymectomy is performed are presented.

3.3.1 Preoperative characteristics

- *Approach*: Among the patients diagnosed with MG ($n = 68$), 45 (66%) underwent VATS, and 23 (34%) underwent sternotomy.
- *Peripheral blood antibody title (AChR-Abs and anti-MuSK Abs)*: Most of the patients operated on with a diagnosis of MG had positive antibodies in peripheral blood regardless of the approach, with no statistically significant differences in both groups.

3.3.2 Postoperative characteristics

- *Worsening of MG after surgery*: After surgery, there was a significantly greater increase in muscle weakness in patients who

	VATS	STERNOTOMY	
Complications at 30 days			
N	73	40	
NO	61 (83,6%)	24 (60%)	0,0112
YES	12 (16,4%)	16 (40%)	

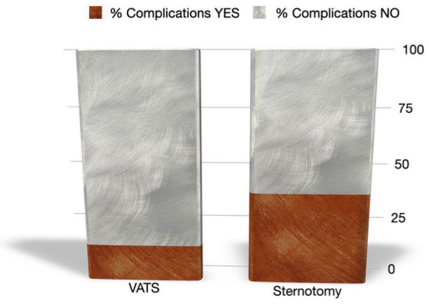


FIGURE 4
Percentage of complications depending on the surgical approach.

TABLE 1 Worsening of MG after surgery according to the surgical approach.

	VATS	Sternotomy	*p*-value
Worsening of MG after surgery			
N	45	22	
No	45 (100%)	11 (50%)	<0.0001
Yes	0 (0%)	11 (50%)	

underwent sternotomy (48%) compared to those who underwent VATS (Table 1).

- *Myasthenic crisis*: There were no cases of myasthenic crisis in either group, regardless of the approach.
- *Clinical assessment in specific neuromuscular pathology consultation*: Patients who underwent VATS thymus excision showed a greater clinical improvement in their MG compared to those who underwent sternotomy, and this difference was statistically significant ($p < 0.0044$).
- *Change in corticosteroid dose preoperatively vs. one year after surgery*: We analyzed how many of the patients who were taking corticosteroids pre-surgery reduced their corticosteroid dose at one year. We observed that there was no statistically significant difference.
- *Days of hospital stay*: The data indicates that patients with an improvement in MG symptoms after the intervention had a shorter hospital stay (median 2.0 [1.0;13.0]).

A multivariate analysis was attempted to determine which factors might be predictors of a better response after thymectomy in MG patients. However, this analysis was inconclusive due to the limitation of the sample size (*n* number).

3.4 Cohort of patients with MG or other syndrome associated with THYMIC lesion

The following are the results of the cohort of patients with a preoperative diagnosis of MG or other SPN, who also present a thymic lesion confirmed after anatomopathological study.

The results of the variables that help us to assess the impact on the evolution of MG according to the thymic lesion present are presented.

3.4.1 Postoperative characteristics

In the cohort of 77 patients with MG or other PNS, 23 cases showed The presence of thymic hyperplasia, 42 cases showed The presence of a thymic tumor, and The remaining 12 cases showed other thymic lesions or a normal thymus (Table 2).

- *Diagnosis of thymic hyperplasia*: Patients whose thymectomy confirmed the diagnosis of thymic hyperplasia had a better clinical evolution of MG.
- *Thymic tumor*: Among the patients who underwent surgery and had a thymic tumor confirmed after thymectomy, the clinical evolution of MG was worse.

3.5 Population cohort of patients diagnosed with THYMIC tumor

The results of the cohort of patients diagnosed with thymic tumors, confirmed by anatomopathologic results, are presented below. Among the analyzed cohort of patients ($n = 103$), 66 patients were diagnosed with thymic tumors.

3.5.1 Preoperative characteristics

3.5.1.1 Approach

Of the 66 patients diagnosed with thymic tumor, 26 (39%) were operated by VATS and 40 (61%) by sternotomy.

TABLE 2 Relationship between the presence of hyperplasia and the clinical evolution of MG.

MG or other PNS	Improvement: No	Improvement: Yes	*p*-value
Pathological results			
N	37	40	
No hyperplasia	3 (8%)	9 (22.5%)	0.0050
Hyperplasia	7 (19%)	16 (40%)	
Thymic tumor	27 (73%)	15 (37.5%)	

3.5.2 Postoperative characteristics

3.5.2.1 Thymic tumor staging

We observed that most of the tumors presented a pT1N0 stage, both in the group operated by VATS ($n = 29$; 63%) and those operated by sternotomy ($n = 11$; 52.4%), with no statistically significant differences.

3.5.2.2 Neoplastic disease status

There were statistically significant differences ($p < 0.0001$) in terms of local or distant recurrence depending on the approach. In patients operated by VATS with a diagnosis of thymic tumor ($n = 26$), there was no evidence of local or distant recurrence. In the cases operated by sternotomy ($n = 39$), 46.2% presented local or distant recurrence.

4 Discussion

Thymectomy is one of the most common surgical procedures in thoracic surgery departments and has been widely used for various diseases. It is likely one of the earliest procedures in thoracic surgery to adopt minimally invasive techniques. After the initial positive publications, the trend of minimally invasive surgery in thoracic procedures, including thymus surgery, has continued to grow.

However, despite the early favorable reports, some researchers have raised questions about the quality of VATS thymectomy. They have pointed to limitations in the size and heterogeneity of published series, leading to ongoing debates in the field.

These debates primarily revolve around three key issues:

- The validity of VATS thymectomy vs. sternotomy: The effectiveness and safety of VATS thymectomy compared to the traditional sternotomy approach have been a subject of contention.
- The controversy over thymectomy for MG without thymic tumors: The role of thymectomy as a treatment for MG, especially in patients without thymic tumors, remains a topic of debate.
- The oncologic radicality achieved with VATS thymectomy: Concerns have been raised about whether VATS thymectomy can provide the same level of oncologic completeness as sternotomy, particularly for thymic tumors.

In light of these ongoing debates, our study was designed to investigate these issues by examining our patient cohort and providing insights into the current state of thoracic surgery.

4.1 Representativeness

Our study cohort includes a group of patients who underwent thymectomy at our medical center. While it is a study conducted in a single medical facility, it is important to note that our center specializes in the treatment of MG. This specialization means that we receive a higher number of patients requiring thymectomy evaluation compared to other centers. However, due to the relatively low prevalence of both MG and thymic tumors, the total number of patients included in our study, collected over a span of 30 years, is only 113 cases. This highlights the challenges in recruiting a larger sample size. Nevertheless, it's worth noting that the sample size in our study is comparable to or even larger than other published reference series (19).

Another crucial aspect to consider within our series is that the cases treated with sternotomy belong to the earlier series. Consequently, the outcomes observed should not be solely attributed to the quality of the surgical technique but should also take into account the advancements in perioperative care processes that patients have undergone over time.

4.2 Validity of VATS thymectomy compared to sternotomy

In the early 2000s, numerous publications highlighted the advantages of VATS over sternotomy for thymus resection (21, 22). Due to the relatively small number of patients included in each of these series, several meta-analyses were conducted to assess the quantity and quality of the evidence available.

In the first meta-analysis by Yang et al. published in 2015, they analyzed 14 publications out of more than 200, which met the necessary quality criteria for drawing conclusions regarding the comparison of both techniques. These 14 publications encompassed a total of 1,087 patients, with 587 undergoing VATS and 400 undergoing sternotomy. Their results demonstrated that VATS led to a reduction in hospital stay, decreased intraoperative blood loss, and a lower rate of complications, all of which were statistically significant (23).

A year later, in 2016 (24), a new meta-analysis was conducted. This analysis examined 12 articles selected from a pool of 162 (with only two being prospective studies) that compared both techniques. In this meta-analysis, it is again apparent that patient recruitment is limited, with the longest series being published by Julissa et al. in 2013 (25). They conclude that in those who underwent VATS there was a reduction in intraoperative blood loss, a shorter hospital stay and an overall reduction in complications. On the other hand, no statistically significant differences were found in the rate of postoperative infections.

Although the advantages of VATS thymectomy over sternotomy seem apparent, a study by Orsini et al. (26) in 2015, involving 278 patients from the EPITHOR database in France, presented a different perspective. Among these patients, 161 underwent sternotomy, and 116 underwent VATS thymectomy. This study stands out for its relatively large sample size but reaches conclusions that emphasize the challenges of making definitive claims about the superiority of one technique over the other due to the inherent heterogeneity between the two groups. Despite the growing adoption of VATS, this study could not conclusively demonstrate its clear superiority.

Upon analyzing our own series of patients, several postoperative variables clearly favored VATS thymectomy:

- *Hospital stay:* VATS patients experienced a remarkable reduction in hospital stay, with a median of only 2 days. This figure is notably lower than in other VATS series, where the median stay, although shorter than sternotomy, typically averaged around 4 days (26).
- *Postoperative pain*: VATS was associated with reduced postoperative pain, both immediately after surgery and one month after discharge. A significant finding was the decreased need for opioid pain relief in the first 24 h post-surgery.

- *Complications at 30 days post-surgery*: The rate of complications was significantly lower in the VATS group. It's important to note that the existing literature does not consistently report a reduction in complications, which can be attributed to the heterogeneity among patient groups in different studies (23, 26, 27).

In an effort to identify potential predictors of complications, a multivariate logistic regression model was applied. This analysis revealed that, apart from the surgical approach where VATS clearly had an advantage, the presence of preoperative MG, regardless of the approach, acted as a predictor of complications. This association is logical, as many of these patients are under pharmacological immunosuppression, making them more susceptible to post-surgery infections. In our series, contrary to the findings in the meta-analysis by Kang Qi et al. (24), the infection rate was significantly higher in the sternotomy group. This was attributed not only to the higher prevalence of MG in this group (58%) but also to the increased infection risk associated with sternotomy.

To eliminate potential selection biases favoring these results, we examined whether there were significant age differences between both groups. It was found that both groups had a similar median age.

It's important to emphasize that the outcomes related to the variables mentioned above are not solely determined by the surgical technique itself. In the current medical landscape, there is a growing trend toward the integration of prehabilitation programs. These programs aim to optimize various preoperative factors, leading to a reduction in complications, improved pain management, and earlier discharge. Such programs are increasingly becoming a standard practice in the field of surgery, contributing significantly to the overall patient care and recovery process.

4.3 Thymectomy in MG

4.3.1 Indication

The use of thymectomy in the treatment of MG has been a subject of ongoing debate, particularly when there is no associated tumor. In 2000, Grohnset et al. (28) conducted an analysis of 28 non-randomized studies comparing outcomes between thymectomized and non-thymectomized MG patients. While the study concluded that thymectomy had a beneficial effect on clinical improvement in MG patients, it faced criticism due to the non-randomized nature of the trials and the heterogeneity of the groups being compared.

This controversy was reflected in the 2010 guidelines for the treatment of neuromuscular disorders, which excluded thymectomy from therapeutic recommendations due to inconsistent selection criteria and variability in response assessment criteria following the procedure.

Despite the trend toward discontinuing thymectomy in MG, Kauffman et al. (29) published the results of a retrospective series of over 1,000 MG cases in 2016, demonstrating a 47% improvement in symptoms following thymectomy.

To address ongoing uncertainties, the first prospective, randomized, multicenter trial, known as the MGTX Trial, was designed to compare two groups: one group underwent transsternal thymectomy in addition to prednisone treatment, while the second group received prednisone treatment alone. The results favored the surgical group.

The analysis of the results from our series, which included 77 patients diagnosed with MG who underwent surgery, aligns with the findings of the MGTX Trial. It appears that patients who underwent thymectomy experienced positive outcomes in terms of MG symptom improvement and remission, consistent with the trial's conclusions. Showed an improvement in MG symptoms regardless of the approach used.

On the other hand, we could not demonstrate a significant reduction in corticosteroid intake. Probably the limited number of patients treated with corticosteroids and the short follow-up time have influenced the outcome with respect to corticosteroid therapy.

4.3.2 Impact according to the approach route

Once the importance of thymectomy in treatment was reestablished, a new debate emerged regarding the surgical approach. In the MGTX Trial, all patients underwent sternotomy, leaving uncertainty about whether VATS could achieve comparable results. Concerns were based on findings from a study by Kaufman et al., which noted that only patients who underwent sternotomy showed clinical improvement.

Studies like the one published by Evoli et al. (30) indicated that, in addition to the improvements in morbidity associated with the technique, the impact of VATS thymectomy in MG was similar to results obtained with sternotomy.

In contrast, a recent study by Raja et al. (31) concluded that more information is needed to confirm that the reduced morbidity associated with VATS translates into an improvement in MG symptoms. Beyond debates about the surgical approach, Jaretzki and Sonett suggested that the crucial factor lies not in the approach itself but in the extent of resection, recommending excision of the cervical poles of the thymus to avoid leaving up to 25% of the gland behind.

Based on our results, it appears that thymectomy has a more significant impact on MG symptoms in patients undergoing VATS. Clinical assessments showed greater symptom improvement in this group. However, it's important to acknowledge potential selection bias when interpreting these results. All patients in our series who underwent sternotomy with a diagnosis of MG also had a concurrent diagnosis of another autoimmune disease, potentially influencing the outcome. Thymic tumor. When analyzing the predictors of response we will see that the presence of thymic tumor acts as an unfavorable predictor.

On the other hand, there was no significant difference in the reduction of corticosteroids one year after the intervention.

4.3.3 Predictors of MG response to thymectomy

Several predictors of a positive response to thymectomy in patients with MG have been identified. Reviewing the literature, factors associated with a better response include:

- Younger age at the time of surgery.
- Shorter duration of MG symptoms before thymectomy.
- Presence of thymic hyperplasia or thymoma as opposed to normal thymus.
- Positive AChR-Abs status.
- Lack of severe bulbar symptoms.
- Lower prednisone dose at the time of surgery.

- Preoperative Osserman stage I or II.
- Female gender.

In our series, we have identified a selection bias that prevents us from determining whether the presence of certain factors acts as predictors of a positive response or not. For instance, regarding the levels of ACs (antibodies) in peripheral blood, over 90% of our patients had positive values before surgery. This was because it was already a selection criterion for surgery. Most of the patients, whether they had MG associated with a thymic tumor or not, tested positive for these antibodies. Only two patients underwent surgery with negative ACs, and their outcomes were favorable. However, this result was not statistically significant due to the small sample size of two cases.

In terms of the age of the cohort of MG patients without associated thymic tumors, their median age was 42 years. Here, too, there is a selection bias since this criterion was considered before referral to our office.

We have been able to confirm that the presence of thymic hyperplasia, as confirmed in the definitive anatomopathological study, is a favorable factor for a positive response. This finding is statistically significant.

The presence of a thymic tumor, according to existing literature, tends to be an unfavorable factor in MG patients. This concomitance often occurs at an older age and is associated with a worse prognosis in terms of MG progression (32).

Our analysis of 42 patients who had both MG and thymoma confirms that the presence of a thymic tumor acts as an unfavorable factor. These patients tend to have a more challenging clinical course, requiring a progressive increase in immunosuppressive medication to manage their symptoms. Furthermore, when you examined the relationship between thymic tumor size and patient outcomes, you found that patients with larger pTNM tumors had a worse prognosis.

It's worth noting the association between tumor recurrence and the worsening of MG. You observed that the timing of disease recurrence coincided with MG deterioration or an increased need for corticosteroids. Therefore, in patients with thymoma who experience MG worsening, it's crucial to investigate the possibility of tumor recurrence through imaging. In some cases, this recurrence may manifest in extrathoracic locations.

4.4 Oncologic outcomes by route of approach

From the oncological point of view, the use of new minimally invasive techniques such as VATS should not reduce the guarantees of the oncological quality of the resection (33). The application of new techniques should follow the same oncological principles and also, if possible, offer a reduction in the associated morbidity.

Different authors support the fact that there may be a higher rate of recurrence in cases operated by VATS for two main reasons (34):

- Excessive manipulation of the tumor due to the reduction of the operative field, which can cause capsular rupture, especially in large tumors.
- The excision of the specimen through small incisions must be careful since it can produce compression of the tumor, capsular rupture, and as a consequence tumor dissemination.

Our results do not agree with these critical statements regarding VATS. We observed that during follow-up, more than 40% of patients who underwent sternotomy had local or distant recurrence, whereas in patients who underwent VATS the rate was 0%.

The first reason we can associate with the difference in oncologic outcomes is that the tumors operated on by VATS corresponded to smaller tumors. However, upon analyzing the results, we observed that there were no differences in terms of pathologic TNM staging in both groups.

Multiple factors may explain this difference, but we want to highlight the following:

1 After the update in the WHO classification published in 2015, all thymic tumors are now considered malignant tumors. This change led to a shift in our overall approach to the diagnosis and treatment of these tumors, particularly regarding surgical treatment. In the past, the erroneous classification of these tumors as benign meant that more extensive surgery was not always considered. Instead, suboptimal thymus resections were often offered, which may not have been oncologically ideal point of view.

Currently, we understand that one of the primary prognostic factors for thymic tumors is achieving a complete resection during surgery. En bloc resections, involving the removal of the tumor along with potentially infiltrated structures, are considered the minimum acceptable approach to avoid suboptimal treatment. Due to the low prevalence of these tumors and the evolving approach to their diagnosis and treatment, the idea of creating homogeneous, multicenter, international databases has gained momentum. In 2010, the ITMIG group proposed such a database with the goal of assisting in the reorientation of diagnostic and therapeutic protocols for thymic tumors. The ITMIG database is now recognized as the most extensive thymic tumor-specific database globally, with more than 10,800 registered cases. Until relatively recently, the assessment of thymic tumor treatment did not involve multidisciplinary committees. Instead, it was divided into surgical treatment for potentially resectable cases and oncologic treatment for unresectable cases. However, our current understanding emphasizes the importance of precise diagnosis and preoperative staging. We must also recognize that, in some cases, chemotherapy and radiotherapy will play a crucial role in treatment, in addition to surgery.

This underscores the necessity for specialized reference units equipped with specific multidisciplinary committees. These committees should be led by professionals experienced in the diagnosis and treatment of thymic tumors, as emphasized in the guidelines published by the National Comprehensive Cancer Network (NCCN) (35).

2 Despite being classified as malignant tumors, thymic tumors exhibit slow growth rates due to their unique biology. Consequently, it is recommended to extend the follow-up period to 10 years to minimize the chances of local or distant recurrence. Considering this factor, it's important to note that our prospective series has not yet reached the recommended minimum follow-up period. The first VATS thymectomy case in our series was performed in 2017. Therefore, further research will be necessary

to validate the observed recurrence rates in our VATS series. Recognizing the limitations of the population studied, the results obtained from our study lead us to conclude that, based on our experience, VATS thymectomy is a safe technique associated with a reduced incidence of postoperative complications compared to sternotomy. In our working group, we can confidently state that performing thymectomy using minimally invasive techniques is both safe and effective.

5 Conclusion

- VATS thymectomy demonstrates a lower rate of intraoperative and postoperative complications, reduced morbidity, and a shorter postoperative length of stay when compared to sternotomy thymectomy.
- VATS thymectomy leads to a favorable impact on MG symptoms.
- Patients with MG who undergo sternotomy experience greater postoperative muscle weakness than those undergoing VATS.
- Patients with MG undergoing VATS achieve better long-term control of myasthenic symptoms than those undergoing sternotomy.
- These results underscore the importance of continuing thymectomy using minimally invasive techniques due to their superiority in our series. Additionally, they emphasize the need for enhancements in perioperative care programs.
- The presence of thymic hyperplasia in the definitive pathology results has been identified as a favorable predictor of clinical improvement. Therefore, diagnosing thymic hyperplasia via CT should serve as a selection criterion when considering thymectomy for MG patients.
- Conversely, the presence of thymic tumors negatively affects the clinical course of MG.
- Patients undergoing VATS have lower rates of local and distant recurrence compared to those undergoing sternotomy.

5.1 Limitations of the study

- The study has limitations due to the low prevalence of both MG and primary thymic gland tumors.
- The non-homogeneity of the series is another limitation, attributed to the incorporation of new surgical approaches, changes in the global approach to treating MG and thymic tumors, and improvements in perioperative care in recent years. Each of these factors can independently influence the results obtained.
- Interpretation of oncologic results for VATS thymectomy should be approached cautiously, as these tumors require long-term follow-up due to their distinct biology.

5.2 Future prospects

We must increase the number of randomized trials with homogeneous groups of patients that will allow us to improve the knowledge of both pathologies within the medical society. This will lead to better diagnostic and therapeutic management at an early stage of the disease. To achieve this objective, it is necessary to create reference, multicenter and international groups that allow the creation of databases of a greater number of patients.

The lines of work should be focused on research in both the experimental and clinical (diagnostic and therapeutic) fields. The choice of surgical approach is simply a small grain of sand on a vast beach of ignorance.

Ethics statement

The studies involving humans were approved by Ethics Committee of Hospital de la Santa Creu i Sant Pau. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

JT: Data curation, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. EM: Data curation, Validation, Visualization, Writing – review & editing. JB: Formal analysis, Investigation, Methodology, Supervision, Writing – review & editing. GP: Data curation, Visualization, Writing – review & editing. AL: Writing – review & editing, Data curation. MG: Data curation, Writing – review & editing. JH: Data curation, Writing – review & editing. AM: Formal analysis, Methodology, Supervision, Visualization, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Miller JF. Immunological function of the thymus. *Lancet*. (1961) 278:748–9. doi: 10.1016/s0140-6736(61)90693-6

2. Klein L, Kyewski B, Allen PM, Hogquist KA. Positive and negative selection of the T cell repertoire: what thymocytes see (and don't see). *Nat Rev Immunol*. (2014) 14:377–91. doi: 10.1038/nri3667

3. Trujillo-Reyes JC, Martínez-Téllez E, Thymoma B-SJ. A systemic disease? *Arch Bronconeumol*. (2019) 55:235–6. doi: 10.1016/j.arbres.2018.03.014

4. Travis WD, Brambilla E, Burke AP, Marx A, Nicholson AG. *WHO classification of tumours of the lung, pleura, thymus and heart*. Lyon: International Agency for Research on Cancer (2015).

5. Sonett JR, Jaretzki A 3rd. Thymectomy for nonthymomatous myasthenia gravis: a critical analysis. *Ann N Y Acad Sci*. (2008) 1132:315–28. doi: 10.1196/annals.1405.004

6. Skeie GO, Apostolski S, Evoli A, Gilhus NE, Illa I, Harms L, et al. Guidelines for treatment of autoimmune neuromuscular transmission disorders. *Eur J Neurol*. (2010) 17:893–902. doi: 10.1111/j.1468-1331.2010.03019.x

7. Wolfe G, Kaminiski H, Aban I, Minisman G, Kuo H-C, Marx A, et al. Randomized trial of Thymectomy in myasthenia gravis. *N Engl J Med*. (2016) 375:511–22. doi: 10.1056/NEJMoa1602489

8. Von Boehmer H, Kisielow P. Lymphocyte lineage commitment: instruction versus selection. *Cell*. (1993) 73:207–8. doi: 10.1016/0092-8674(93)90220-k

9. Castleman B, Norris EH. The pathology of the thymus in myasthenia gravis; a study of 35 cases. *Medicine (Baltimore)*. (1949) 28:27–58. doi: 10.1097/00005792-194902000-00002

10. Kirchner T, Tzartos S, Hoppe F, Schalke B, Wekerle H, Müller-Hermelink HK. Pathogenesis of myasthenia gravis. Acetylcholine receptor-related antigenic determinants in tumor-free thymuses and thymic epithelial tumors. *Am J Pathol*. (1988) 130:268–80.

11. Berrih-Aknin S, Le Panse R. Myasthenia gravis: a comprehensive review of immune dysregulation and etiological mechanisms. *J Autoimmun*. (2014) 52:90–100. doi: 10.1016/j.jaut.2013.12.011

12. Romi F. Thymoma in myasthenia gravis: from diagnosis to treatment. *Autoimmune Dis*. (2011) 2011:474512. doi: 10.4061/2011/474512

13. Mikhail M, Mekhail Y, Mekhail T. Thymic neoplasms: a clinical update. *Curr Oncol Rep*. (2012) 14:350–8. doi: 10.1007/s11912-012-0246-8

14. Scarpino S, Di Napoli A, Stoppacciaro A, Antonelli M, Pilozzi E, Chiarle R, et al. Expression of autoimmune regulator gene (AIRE) and T regulatory cells in human thymomas. *Clin Exp Immunol*. (2007) 149:504–12. doi: 10.1111/j.1365-2249.2007.03442.x

15. Carlens E. Mediastinoscopy: a method for inspection and tissue biopsy in the superior mediastinum. *Dis Chest*. (1959) 36:343–52. doi: 10.1378/chest.36.4.343

16. Huang J, Detterbeck FC, Wang Z, Loehrer PJ Sr. Standard outcome measures for thymic malignancies. *J Thorac Oncol*. (2010) 5:2017–23. doi: 10.1097/JTO.0b013e3181f13682

17. Zieliński M, Kuzdzał J, Szlubowski A, Soja J. Transcervical-subxiphoid-videothoracoscopic "maximal" thymectomy--operative technique and early results. *Ann Thorac Surg*. (2004) 78:404–9. doi: 10.1016/j.athoracsur.2004.02.021

18. Mantegazza R, Baggi F, Bernasconi P, Antozzi C, Confalonieri P, Novellino L, et al. Video-assisted thoracoscopic extended thymectomy and extended transsternal thymectomy (T-3b) in non-thymomatous myasthenia gravis patients: remission after 6 years of follow-up. *J Neurol Sci*. (2003) 212:31–6. doi: 10.1016/s0022-510x(03)00087-x

19. Salim EF. Role of VATS in thymectomy for non-thymomatous myasthenia gravis. *J Egypt Soc Cardiothorac Surg*. (2018) 26:205–11. doi: 10.1016/j.jescts.2018.05.001

20. Muppidi S, Wolfe GI, Conaway M, Burns TMMG Composite and MG-QOL15 Study Group. Mg-ADL: still a relevant outcome measure. *Muscle Nerve*. (2011) 44:727–31. doi: 10.1002/mus.22140

21. Ye B, Tantai JC, Ge XX, Li W, Feng J, Cheng M, et al. Surgical techniques for early-stage thymoma: video-assisted thoracoscopic thymectomy versus transsternal thymectomy. *J Thorac Cardiovasc Surg*. (2014) 147:1599–603. doi: 10.1016/j.jtcvs.2013.10.053

22. Liu TJ, Lin M-W, Hsieh M-S, Kao M-W, Chen K-C, Chang C-C, et al. Video-assisted Thoracoscopic surgical Thymectomy to treat early Thymoma: a comparison with the conventional Transsternal approach. *Ann Surg Oncol*. (2014) 21:322–8. doi: 10.1245/s10434-013-3228-7

23. Yang Y, Dong J, Huang Y. Thoracoscopic thymectomy versus open thymectomy for the treatment of thymoma: a meta-analysis. *Eur J Surg Oncol*. (2016) 42:1720–8. doi: 10.1016/j.ejso.2016.03.029

24. Qi K, Wang B, Wang B, Zhang LB, Chu XY. Video-assisted thoracoscopic surgery thymectomy versus open thymectomy in patients with myasthenia gravis: a meta-analysis. *Acta Chir Belg*. (2016) 116:282–8. doi: 10.1080/00015458.2016.1176419

25. Jurado J, Javidfar J, Newmark A, Lavelle M, Bacchetta M, Gorenstein L, et al. Minimally invasive thymectomy and open thymectomy: outcome analysis of 263 patients. *Ann Thorac Surg*. (2012) 94:974–82. doi: 10.1016/j.athoracsur.2012.04.097

26. Orsini B, Santelmo N, Pages PB, Baste JM, Dahan M, Bernard A, et al. Comparative study for surgical management of thymectomy for non-thymomatous myasthenia gravis from the French national database EPITHOR. *Eur J Cardiothorac Surg*. (2016) 50:418–22. doi: 10.1093/ejcts/ezw064

27. Bachmann K, Burkhardt D, Schreiter I, Kaifi J, Busch C, Thayssen G, et al. Long-term outcome and quality of life after open and thoracoscopic thymectomy for myasthenia gravis: analysis of 131 patients. *Surg Endosc*. (2008) 22:2470–7. doi: 10.1007/s00464-008-9794-2

28. Gronseth GS, Barohn RJ. Practice parameter: thymectomy for autoimmune myasthenia gravis (an evidence-based review): report of the quality standards Subcommittee of the American Academy of neurology. *Neurology*. (2000) 55:7–15. doi: 10.1212/wnl.55.1.7

29. Kaufman AJ, Palatt J, Sivak M, Raimondi P, Lee DS, Wolf A, et al. Thymectomy for myasthenia gravis: complete stable remission and associated prognostic factors in over 1000 cases. *Semin Thorac Cardiovasc Surg*. (2016) 28:561–8. doi: 10.1053/j.semtcvs.2016.04.002

30. Evoli A, Meacci E. An update on thymectomy in myasthenia gravis. *Expert Rev Neurother*. (2019) 19:823–33. doi: 10.1080/14737175.2019.1600404

31. Raja SM, Guptill JT, McConnell A, Al-Khalidi HR, Hartwig MG, Klapper JA. Perioperative outcomes of Thymectomy in myasthenia gravis: a thoracic surgery database analysis. *Ann Thorac Surg*. (2022) 113:904–10. doi: 10.1016/j.athoracsur.2021.06.071

32. Liu W, Tong T, Ji Z, Zhang Z. Long-term prognostic analysis of thymectomized patients with myasthenia gravis. *Chin Med J*. (2002) 115:235–7. doi: 10.3901/jme.2002.supp.235

33. Rakovich G, Deslauriers J. Video-assisted and minimally-invasive open chest surgery for the treatment of mediastinal tumors and masses. *J Vis Surg*. (2017) 3:25. doi: 10.21037/jovs.2017.01.01

34. Chao YK, Liu YH, Hsieh MJ, Wu YC, Chen TP, Lu MS, et al. Long-term outcomes after thoracoscopic resection of stage I and II thymoma: a propensity-matched study. *Ann Surg Oncol*. (2015) 22:1371–6. doi: 10.1245/s10434-014-4068-9

35. National Comprehensive Cancer Network. (2022) Thymomas and thymic carcinomas (version 1.2022). Available at: https://www.nccn.org/guidelines/guidelines detail?category=1&id=1469 (Accessed September 2023).

frontiers | Frontiers in Neurology

TYPE Original Research
PUBLISHED 26 February 2024
DOI 10.3389/fneur.2024.1297997

OPEN ACCESS

EDITED BY
Francesco Saccà,
University of Naples Federico II, Italy

REVIEWED BY
Jana Zschüntzsch,
University of Göttingen, Germany
Renato Mantegazza,
IRCCS Carlo Besta Neurological Institute Foundation, Italy

*CORRESPONDENCE
Philipp Mergenthaler
philipp.mergenthaler@charite.de

†These authors have contributed equally to this work

RECEIVED 20 September 2023
ACCEPTED 29 January 2024
PUBLISHED 26 February 2024

CITATION
Mehnert A, Bershan S, Kollmus-Heege J, Gerischer L, Herdick ML, Hoffmann S, Lehnerer S, Scheibe F, Stascheit F, Stein M, Buchan AM, Meisel A, Aigner A and Mergenthaler P (2024) Identifying patients at risk for myasthenic crisis with hemogram and inflammation-related laboratory parameters – a pilot study.
Front. Neurol. 15:1297997.
doi: 10.3389/fneur.2024.1297997

Identifying patients at risk for myasthenic crisis with hemogram and inflammation-related laboratory parameters – a pilot study

Anne Mehnert[1], Sivan Bershan[2], Jil Kollmus-Heege[3], Lea Gerischer[1,4], Meret Luise Herdick[1,4], Sarah Hoffmann[1,4], Sophie Lehnerer[1,4,5], Franziska Scheibe[1], Frauke Stascheit[1,4], Maike Stein[1,4], Alastair M. Buchan[2,6], Andreas Meisel[1,2,4], Annette Aigner[2,3†] and Philipp Mergenthaler[1,2,6*†]

[1]Charité - Universitätsmedizin Berlin, Department of Neurology with Experimental Neurology, Berlin, Germany, [2]Charité - Universitätsmedizin Berlin, Center for Stroke Research Berlin, Berlin, Germany, [3]Charité - Universitätsmedizin Berlin, Institute of Biometry and Clinical Epidemiology, Berlin, Germany, [4]Charité - Universitätsmedizin Berlin, Neuroscience Clinical Research Center, Berlin, Germany, [5]Berlin Institute of Health at Charité, Digital Health Center, Berlin, Germany, [6]Radcliffe Department of Medicine, University of Oxford, Oxford, United Kingdom

Background: Myasthenia gravis (MG) is a rare autoimmune disease characterized by fatigable weakness of the voluntary muscles and can exacerbate to life-threatening myasthenic crisis (MC), requiring intensive care treatment. Routine laboratory parameters are a cost-effective and widely available method for estimating the clinical outcomes of several diseases, but so far, such parameters have not been established to detect disease progression in MG.

Methods: We conducted a retrospective analysis of selected laboratory parameters related to inflammation and hemogram for MG patients with MC compared to MG patients without MC. To identify potential risk factors for MC, we applied time-varying Cox regression for time to MC and, as a sensitivity analysis, generalized estimating equations logistic regression for the occurrence of MC at the next patient visit.

Results: 15 of the 58 examined MG patients suffered at least one MC. There was no notable difference in the occurrence of MC by antibody status or sex. Both regression models showed that higher counts of basophils (per 0.01 unit increase: HR = 1.32, 95% CI = 1.02–1.70), neutrophils (per 1 unit increase: HR = 1.40, 95% CI = 1.14–1.72), potentially leukocytes (per 1 unit increase: HR = 1.15, 95% CI = 0.99–1.34), and platelets (per 100 units increase: HR = 1.54, 95% CI = 0.99–2.38) may indicate increased risk for a myasthenic crisis.

Conclusion: This pilot study provides proof of the concept that increased counts of basophils, neutrophils, leukocytes, and platelets may be associated with a higher risk of developing MC in patients with MG.

KEYWORDS

myasthenia gravis, myasthenia, myasthenic crisis, hemogram, laboratory parameters, inflammation, risk prediction

Introduction

Myasthenia gravis (MG) is a rare autoimmune disease caused by an antibody-mediated disturbance of signal transduction at the neuromuscular endplate. The main symptoms are fatigable weakness of the voluntary muscles, worsening with exertion, and fatigue (1). In 70–80% of all patients, MG is caused by pathogenic autoantibodies directed against the acetylcholine receptor (AChR) at the neuromuscular junction (2–4). The loss of functional AChR leads to a reduced amplitude of the endplate potential and, thus, impeded neurotransmission at the neuromuscular endplate (5, 6). MG manifests at the extraocular muscles, leading to ptosis or double vision, and by generalized or bulbar weakness affecting limb muscles or oropharyngeal muscles at manifestation or as the disease progresses.

Critical exacerbation of these symptoms can lead to life-threatening myasthenic crisis (MC), which often requires intensive care treatment with non-invasive or even invasive ventilation and invasive therapy (7). An MC often occurs within the first few years of the disease and can be the first manifestation of MG (8). The lifetime prevalence of MC is 15–20% for patients with MG (9, 10). MC-associated mortality is commonly reported between 5 and 12% (10–13), but mortality up to 22% has also been reported (14, 15). Furthermore, it is established that antibody status or clinical treatment protocols are associated with outcomes after MC (10, 13, 16, 17).

Although it is known that certain drugs, inadequate treatment, surgery, infection, sepsis, and pregnancy can trigger MC (9, 18, 19), the prediction of severe exacerbation of MG or ultimately MC based on laboratory parameters is currently not possible. To this end, so far only a few studies have investigated the relationship between hemogram or inflammation-related laboratory parameters and the disease progression of MG (20–22).

Thus, we hypothesized that certain laboratory parameters could be used to evaluate disease activity in MG even before clinically obvious exacerbations and to identify patients at risk of progressing to MC. We studied highly granular laboratory parameters related to inflammation and hemogram in patients suffering from MC prior to the event, compared to MG without MC to investigate if changes in these parameters could be indicative of the development of MC. This retrospective case–control study with a small number of subjects serves as a pilot study, whose concept and results could later be validated in a larger cohort.

Materials and methods

Standard protocol approvals, registrations, and patient consent

This study was approved by the ethics committee at Charité – Universitätsmedizin Berlin (no. EA4/068/22). Data were collected retrospectively. Due to the retrospective nature, individual patient consent was not obtained in accordance with ethical approval and state and national laws. This manuscript has been posted as a preprint on medRxiv prior to submission to this journal (23).

Study design and patient selection

For this study, we evaluated clinical data from 58 MG patients treated at the integrated Myasthenia Center of the Department of Neurology at Charité – Universitätsmedizin Berlin. It is certified by the German Myasthenia Gravis Society and employs standardized workflows for patient management. The diagnosis of MG was established based on antibody studies, repetitive nerve stimulation, or clinical assessment. MC was defined as the exacerbation of myasthenic symptoms with bulbar or general weakness requiring mechanical ventilation. First, we selected 15 patients who were treated for MC at least once and for whom sufficiently complete medical data were available from all MC patients at our center between 2006 and 2016. MC patients were intended to be matched in a 1:3 ratio with MG patients treated at our center without recorded MC until 2018. Although data for MC patients were available after 2016, they were not included in the analysis to avoid hindsight bias. Matching was based on the criteria sex, age ± 5 years, antibody status (AChR antibodies or negative for AChR, MuSK, LRP4), thymectomy (yes/no), and thymus pathology (thymoma, thymus hyperplasia, and unremarkable). Due to insufficient matching partners with applicable matching criteria, one MC patient could only be matched with one control patient. The final cohort consisted of 58 subjects (15 MC, 43 non-MC patients).

In this study, we focused on the analysis of the following laboratory parameters: hemoglobin, hematocrit, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell count, white blood cell differential count (basophils, eosinophils, monocytes, lymphocytes, neutrophils, and granulocytes), platelet count, and C-reactive protein (CRP). Selected laboratory data were obtained through the Berlin Institute of Health at Charité Health Data Platform (HDP), which hosts up-to-date retrospective data on the hospital management system. For this pilot study, we retrieved all available data for the curated list of laboratory parameters of the selected patients over the entire observation period from 2006 to 2018. For the analysis, we only considered data obtained prior to the occurrence of an MC.

Statistical analysis

We descriptively display all patient characteristics used for matching, separately for cases and controls. Categorical variables are presented as absolute and relative frequencies. To summarize the laboratory parameters, we display the first measurement per patient (baseline) as well as the median value per patient measured before the beginning of the first MC with the median and interquartile range (IQR). Kaplan–Meier curves display the time to first MC stratified by sex (male or female) and antibody status (AChR positive or negative).

We used an Anderson–Gill model, a time-varying Cox proportional-hazards regression, with time to MC as the outcome. To account for the dependency in the data, we used robust standard errors. Time was modeled since the first observation, and patients without any further crises were censored at the time of the database excerpt. This model assumes that the risk of experiencing an MC remains the same, irrespective of whether previous events occurred or not. This means that after an MC has occurred, a subject is treated the same way as a subject who has not experienced an MC. As sensitivity analysis, we also performed a generalized estimating equations (GEE)

10.3389/fneur.2024.1297997

TABLE 1 Cohort demographics and clinical characteristics.

	Myasthenic crisis ($n = 15$)	No myasthenic crisis ($n = 43$)
Sex		
Male, n (%)	7 (46.7)	21 (48.8)
Female, n (%)	8 (53.3)	22 (51.2)
Age		
Age at diagnosis, years, mean (SD)	56 (16.5)	51 (17.2)
Early onset MG, n (%)	6 (40.0%)	16 (37.2%)
Late onset MG, n (%)	9 (60.0%)	27 (62.8%)
Number of MC per patient		
0	-	43
1	11 (73.3%)	-
2	2 (13.3%)	-
3	2 (13.3%)	-
AChR antibodies		
Positive	10 (66.7%)*	28 (65.1%)
Negative for AChR, MuSK, LRP4	5 (33.3%)	15 (34.9%)
Thymectomy		
No, n (%)	5 (33.3%)	15 (34.9%)
Yes, n (%)	10 (66.7%)	28 (65.1%)
Thymus pathology		
Thymoma, n (%)	5 (50.0%)	14 (50.0%)
Hyperplasia, n (%)	2 (20.0%)	5 (18.0%)
Unremarkable, n (%)	3 (30.0%)	8 (29.0%)
Unknown, n (%)	–	1 (4.0%)

*One MC patient was found positive for AChR and MuSK. As there were no appropriate AChR/MuSK-double positive controls, she was considered in the AChR+ group.

logistic regression model, which explains the binary outcome of a potential current crisis with the laboratory parameter measured at the prior visit.

Because of the initial matching, we do not adjust for age and sex in any of the models. Due to the limited number of observed events, all models ran for each laboratory parameter separately (univariable models). Using complete-case analyses, these models are therefore based on a different number of observations, due to the clinical practice of not measuring all laboratory parameters at every time point. Based on these models, we derived hazard ratio (HR) and odds ratio (OR) estimates along with 95% confidence intervals (CI). All analyses were performed using R [R Project for Statistical Computing (24)], as well as additional R packages for data handling and analysis (25–27).

Results

Demographics and clinical characteristics

This pilot study included 58 patients (30 female, 28 male), of whom 15 (26%) suffered from one or more MC (cases) and 43 never had an MC (controls) within the observation period. In the case group, 11 patients suffered one MC, 2 patients suffered two MCs, and 2 patients suffered three MCs. In total, there were 21 MC events (Table 1). Both baseline (i.e., first-ever recorded) and median values for all available measurements prior to MC were similar for all laboratory parameters in both groups. CRP differed in cases and controls (Table 2). The frequency of measurements per person and laboratory parameter varied. For the controls, the median number of observations was 8 (IQR: 3–26, min = 1, max = 714), and for cases 42 (IQR: 18–67.5, min = 1, max = 101). The median number of measurements for the complete blood count with differential was 8 (IQR: 2–19.5, min: 1, max: 56) and 15 (IQR: 4–33, min: 1, max: 158) without differential. For CRP, it was 6 (IQR: 2–15.5, min: 1, max: 93). The median time between two measurements was 3 days (IQR: 2–35, min: 1, max: 3135). Stratified by the outcome, for the 1944 observations where no MC occurred in the subsequent visit, the median time to next visit (i.e., time to next measurement) was 3 days (IQR: 2–34, min: 1, max: 3135) and 39 days (IQR: 19.2–83.8, min: 1, max: 2196) for the 20 observations where an MC occurred. In the MC group, bulbar symptoms preceded the recorded MC in 17 of 21 events (81.0%). As there is no direct comparison to this measure for the control group, we rely on MGFA classification (i.e., the worst ever recorded MGFA) to categorize patients into groups presenting with mainly bulbar or generalized symptoms. Control patients presented with bulbar symptoms in 29 of 43 cases (67.4%) and generalized symptoms in 10 of 43 cases (23.3%). In four cases (9.3%), it was not possible to unequivocally determine the MGFA category.

TABLE 2 Hemogram and inflammation-related laboratory parameter measurements of patients.

	Mysthenic crisis ($n = 15$)	No myasthenic crisis ($n = 43$)
Basophils/nl (baseline)		
Median (IQR)	0.03 (0.02, 0.05)	0.03 (0.02, 0.05)
Missing	1 (6.7%)	1 (2.3%)
Basophils/nl (median)		
Median (IQR)	0.03 (0.02, 0.05)	0.04 (0.02, 0.05)
Missing	1 (6.7%)	1 (2.3%)
C-reactive protein in mg/l (baseline)		
Median (IQR)	7.10 (4.05, 14.93)	3.50 (1.37, 8.07)
Missing	–	19 (44.2%)
C-reactive protein in mg/l (median)		
Median (IQR)	12.00 (4.20, 34.42)	4.35 (1.68, 8.02)
Missing	–	19 (44.2%)
Eosinophils/nl (baseline)		
Median (IQR)	0.06 (0.03, 0.10)	0.08 (0.03, 0.14)
Missing	3 (20.0%)	3 (7.0%)
Eosinophils/nl (median)		
Median (IQR)	0.07 (0.06, 0.12)	0.08 (0.06, 0.14)
Missing	3 (20.0%)	3 (7.0%)
Hematocrit in l/l (baseline)		
Median (IQR)	0.43 (0.40, 0.46)	0.42 (0.40, 0.44)
Hematocrit in l/l (median)		
Median (IQR)	0.41 (0.37, 0.43)	0.42 (0.38, 0.43)
Hemoglobin in g/dl (baseline)		
Median (IQR)	14.20 (13.55, 15.65)	14.10 (13.40, 14.65)
Hemoglobin in g/dl (median)		
Median (IQR)	13.40 (12.15, 14.55)	13.80 (12.85, 14.60)
Leukocytes/nl (baseline)		
Median (IQR)	9.20 (7.28, 13.19)	8.20 (6.06, 10.28)
Leukocytes/nl (median)		
Median (IQR)	8.11 (6.99, 10.25)	7.60 (6.65, 8.96)
Lymphocytes/nl (baseline)		
Median (IQR)	1.57 (1.08, 1.64)	1.52 (1.05, 1.88)
Missing	1 (6.7%)	1 (2.3%)
Lymphocytes/nl (median)		
Median (IQR)	1.30 (0.88, 1.80)	1.33 (1.04, 1.71)
Missing	1 (6.7%)	1 (2.3%)
Mean corpuscular hemoglobin in pg (baseline)		
Median (IQR)	30.80 (29.05, 32.00)	30.70 (29.85, 31.75)
Mean corpuscular hemoglobin in pg (median)		
Median (IQR)	30.70 (29.45, 31.60)	30.60 (29.35, 31.80)
Mean corpuscular hemoglobin concentration in g/dl (baseline)		
Median (IQR)	33.30 (33.05, 34.35)	33.90 (33.30, 34.50)
Mean corpuscular hemoglobin concentration in g/dl (median)		
Median (IQR)	32.80 (32.60, 33.55)	33.60 (32.75, 34.33)

(Continued)

TABLE 2 (Continued)

	Mysthenic crisis (n = 15)	No myasthenic crisis (n = 43)
Monocytes absolute/nl (baseline)		
Median (IQR)	0.59 (0.25, 0.90)	0.51 (0.42, 0.66)
Missing	1 (6.7%)	1 (2.3%)
Monocytes absolute/nl (median)		
Median (IQR)	0.63 (0.47, 0.81)	0.56 (0.49, 0.69)
Missing	1 (6.7%)	1 (2.3%)
Neutrophils/nl (baseline)		
Median (IQR)	6.98 (4.88, 10.75)	5.43 (3.81, 8.01)
Missing	1 (6.7%)	1 (2.3%)
Neutrophils/nl (median)		
Median (IQR)	6.40 (5.41, 7.89)	5.35 (4.25, 6.31)
Missing	1 (6.7%)	1 (2.3%)
Platelets/nl (baseline)		
Median (IQR)	268.00 (220.50, 324.00)	246.00 (206.50, 283.50)
Platelets/nl (median)		
Median (IQR)	260.00 (214.50, 297.00)	249.50 (210.25, 279.50)
Immature granulocytes/nl (baseline)		
Median (IQR)	0.04 (0.02, 0.06)	0.03 (0.01, 0.04)
Missing	1 (6.7%)	1 (2.3%)
Immature granulocytes/nl (median)		
Median (IQR)	0.04 (0.03, 0.07)	0.03 (0.02, 0.05)
Missing	1 (6.7%)	1 (2.3%)

For each laboratory parameter, the baseline (first measurement recorded per patient) and the median of all measurements recorded within the observation period before the beginning of the first MC are shown. Missing data are indicated where observed.

Time to first myasthenic crisis stratified by sex and antibody status

We calculated Kaplan–Meier curves for time to the first MC since the first recorded laboratory parameter, stratified by AChR antibody status and sex. Overall, there was neither a significant difference in the occurrence of MC depending on antibody status (Figure 1A) nor between women and men (Figure 1B).

Laboratory parameters associated with myasthenic crisis

Both statistical models we applied make use of the previous measurement to explain the occurrence of an event (MC or no MC). Laboratory parameter measurements from 1964 observations were used to explain 20 events with subsequent MC (one MC did not have sufficiently complete data to be included) and 1944 observations without subsequent MC.

Univariable Anderson–Gill models showed that basophils, neutrophils, and potentially leukocytes and platelets indicate increase hazards for a myasthenic crisis (Figure 2). Without adjustment for other parameters, an increase of basophils by 0.01 units increased the risk of an MC 1.32-fold (95% CI: 1.02–1.70) and a 1 unit increase in neutrophils 1.4-fold (95% CI: 1.14–1.72). Furthermore, every unit increase in leukocytes increased the hazard for MC 1.15-fold (95% CI: 0.99–1.34), and an increase of 100 units in platelets 1.54-fold (95% CI: 0.99–2.38).

The GEE logistic regression models conducted as sensitivity analyses with the occurrence of an MC in the subsequent patient visit as the outcome also identified basophils, neutrophils, and platelets as potentially relevant laboratory parameters (Figure 3). The odds for MC in the subsequent visit were 1.27-fold (95% CI: 1.08–1.49) per 0.01 unit increase in basophils and 1.15-fold (95% CI: 1.02–1.30) per 1 unit increase in neutrophils. A 100-unit increase in platelets increased the odds for an event 1.29-fold (95% CI: 0.85–1.95), although this association was calculated with low precision. Additionally, higher values in hematocrit (per 0.01 units) and hemoglobin (per 1 unit) resulted in higher odds for a subsequent MC (OR = 1.11, 95% CI: 1.01–1.22, OR = 1.19, 95% CI: 1.01–1.39, respectively).

Both statistical models consider data only before the occurrence of an MC but differently account for time. The Anderson–Gill model is a time-varying Cox regression model and, as such, explores the relationship between the time to the occurrence of an event and the explanatory variables. The dependent variable here is the hazard function at a given time t. Therefore, the model is dependent on time, as the hazard of an MC occuring changes with time. The GEE logistic

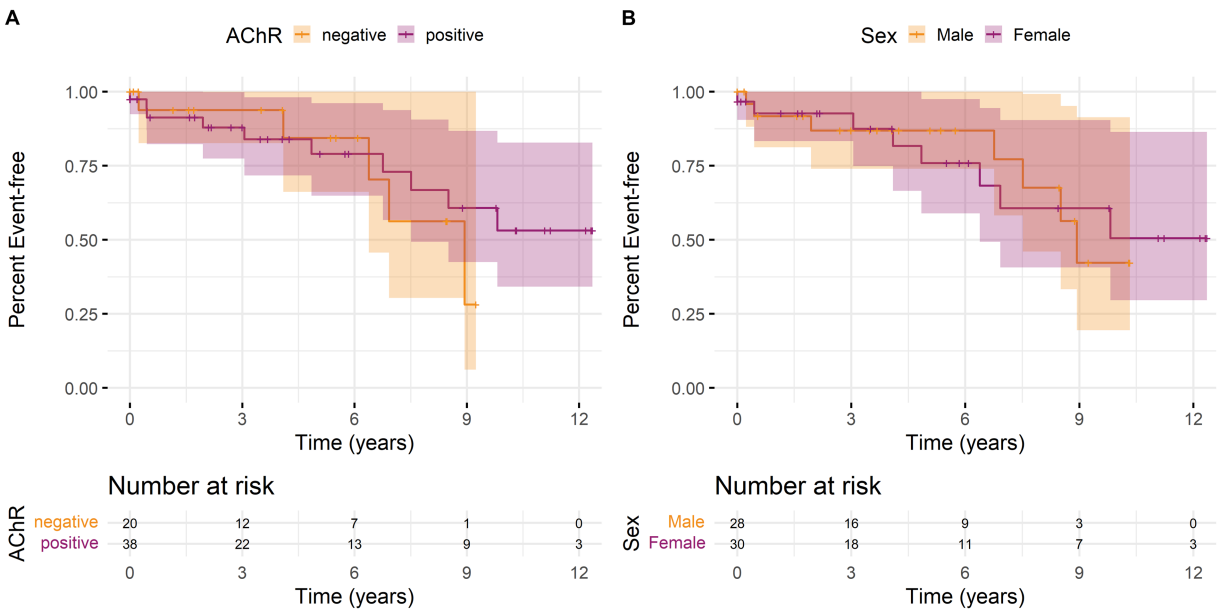


FIGURE 1
Kaplan–Meier curves with 95% confidence intervals (shaded areas) for time to first myasthenic crisis since the first recorded laboratory parameter stratified by **(A)** antibody status or **(B)** sex over the observation period. An event is defined as the first MC of a patient. The individual lines show the stratified survival curves, and the shaded areas present 95% confidence interval. **(A)** Orange lines represent the data for AChR antibody-negative patients and purple lines for AChR antibody-positive patients. **(B)** Orange lines represent the data for male and purple for female patients. Vertical bars indicate the censorship of an observation at that point. Additionally, the number of individuals at risk for a first MC at regular time points is shown below the curves.

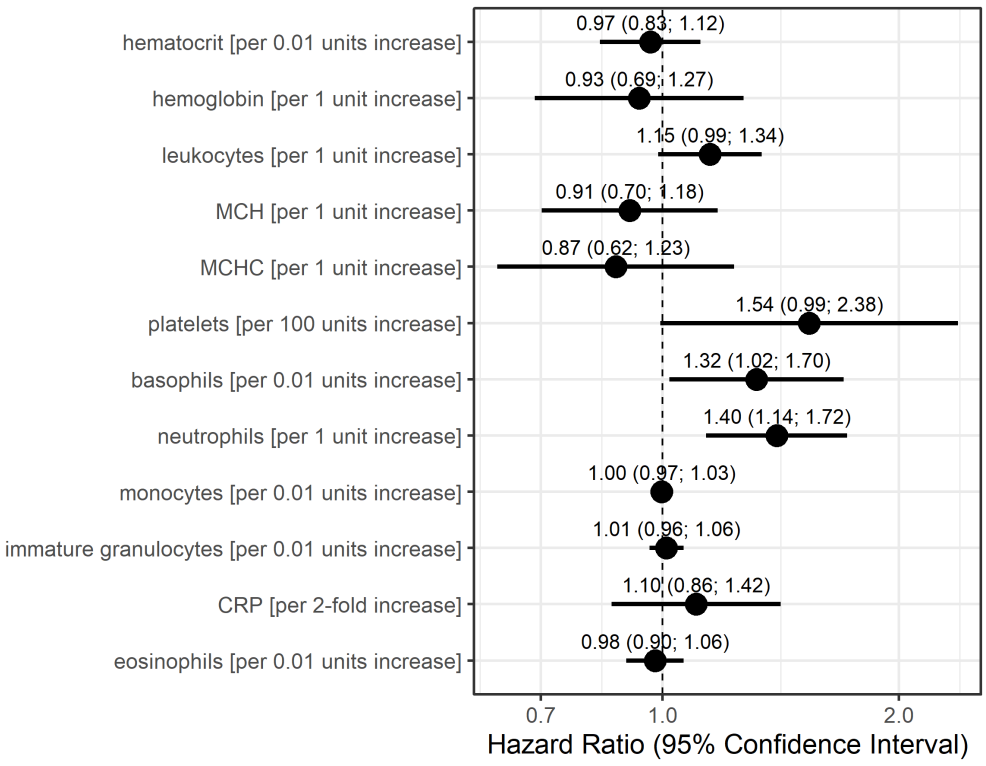


FIGURE 2
Hazard ratio estimates with 95% confidence intervals based on Anderson-Gill models for each laboratory parameter for the occurrence of myasthenic crisis. Dots indicate hazard ratio estimates for each laboratory parameter, and horizontal bars indicate 95% confidence intervals (in parentheses). Units for each laboratory parameter are shown in Table 2.

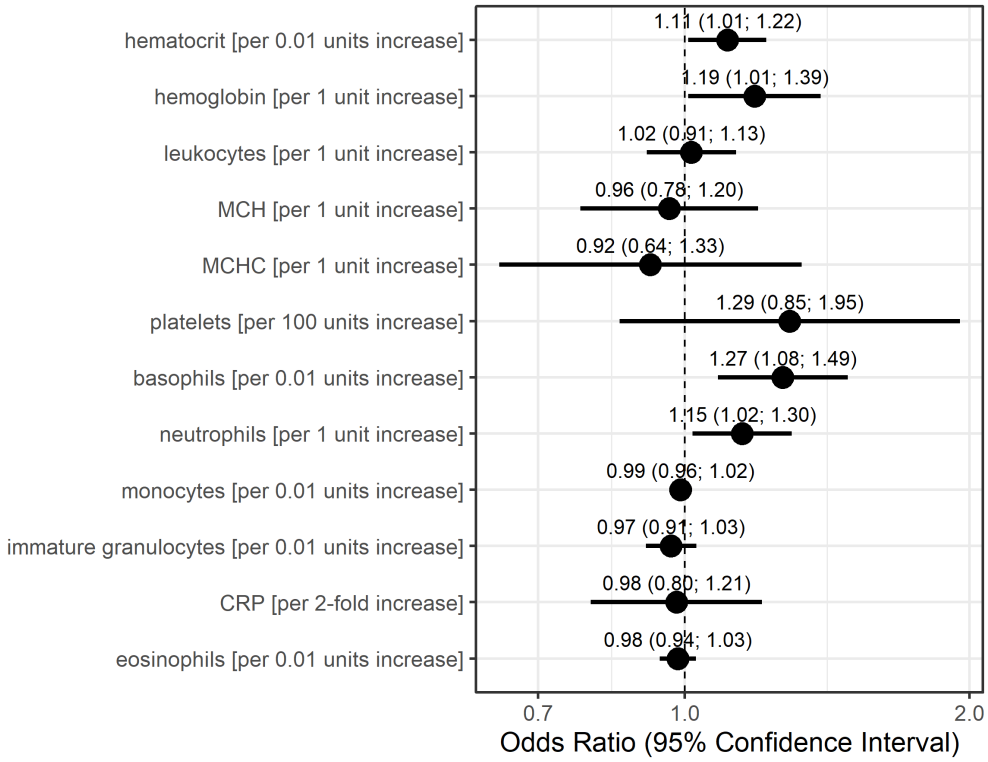


FIGURE 3
Odds ratio estimates and 95% confidence intervals based on generalized estimating equations and logistic regression for each individual laboratory parameter for the occurrence of myasthenic crisis at the subsequent visit. Dots indicate odds ratio estimates for each laboratory parameter, and horizontal bars indicate 95% confidence intervals (in parentheses). Estimates were derived from the generalized estimating equations (GEE) logistic regression. Units for each laboratory parameter are shown in Table 2.

regression estimates the odds for an event (here the occurrence of an MC at the next visit) based on the explanatory variables in the model. Time is only taken into account by the sequence of measurements and the occurrence of an MC at the next visit. It is interesting to note that in 10 of 21 events (47.6%), patient records showed signs of (bacterial) infection prior to the respective MC. It is also noteworthy that infections prior to MC seemed to be more prevalent in male patients (5 of 8 events, 62.5%) than in female patients (5 of 13 events, 38.5%). However, CRP was not identified with increased risk for MC by either statistical model. Based on the results of these models, we conclude that increased values of basophils, neutrophils, and, with lower confidence, also leukocytes and platelets are associated with an increased risk of developing an MC.

Discussion

In this pilot study, we investigated whether routine laboratory data could be used to anticipate the occurrence of MC during the disease course in MG patients. Based on two statistical models with distinct assumptions, we found that from our pre-selected set of laboratory parameters, higher basophils, neutrophils, leukocytes, and platelets measured before an event (i.e., MC) were associated with a higher risk of developing an MC. Although baseline and median CRP as the most obvious laboratory markers of infection (procalcitonin was not determined in any of the cases reported here) before MC were elevated in the MC group compared to the control group, it was not identified as a risk to develop an MC by either statistical model.

Several risk factors for MC have previously been identified from retrospective analyses: infections (15, 19, 28), drugs (28), corticosteroid treatment (29, 30), older age (31), thymoma (15), bulbar symptoms (15, 32), high disease severity (15, 31), male sex (15, 30), and the presence of additional autoimmune diseases (31, 32). Another risk factor for MC is surgery (15, 33), including thymectomy. Although some attempts to establish risk scores for the occurrence of postoperative myasthenic crisis have been made, the identified risk factors (bulbar symptoms, disease severity, decreased vital capacity, and thymoma) generally align with established common risk factors for MC (33–35). Due to the high mortality rate of MC of 5–12% (10, 13, 16, 18), which can be stratified by AChR (10) or MuSK (16) antibodies, and triple-seronegative patients (13), there is a significant need to establish risk scores or identify parameters that can be used to predict the occurrence of MC, and thus aid early intervention.

Our study provides initial indications that routine laboratory parameters assessed before the onset of MC could be used as risk predictors for MC occurrence and facilitate early interventions (e.g., treatment with immunoglobulins or plasma exchange), possibly preventing MC and mitigating the associated morbidity and mortality. To this end, some studies have investigated the prediction of in-hospital mortality in MC based on selected laboratory parameters (22, 36). A recent study derived a predictive score for in-hospital mortality of MC using the Myasthenia Gravis Foundation of America

(MGFA) score at the onset of the MC, septic shock, and cardiac arrest (36). In addition, this study suggested that low serum albumin, low hemoglobin, and a high leukocyte count might be associated with a higher mortality in MC (36). The latter may corroborate our findings that increased leukocyte counts may be associated with an increased risk for an ensuing MC. Further studies described a possible association between infections (37) and signs of inflammation (leukocytosis) (22) and an increased risk of developing MC. Furthermore, a hemogram could provide clues to the course of the disease, as hematological changes have been identified as prognostic factors of mortality for several critical illnesses (22), e.g., endocarditis (38), acute kidney injury (39), and acute myocardial infarction (40, 41). Extreme leukocytosis and anemia have been described as important risk factors for increased mortality in MC (22). Similarly, elevated neutrophil-to-lymphocyte ratios have been reported to be a potential risk factor for indicating the disease severity of MG in children (20) and adults (21). It is interesting that, among others, we identified basophilia as a potential indicator for risk of MC. Classically, basophilia is seen in hypersensitivity reactions of the immediate type (type 1) (42). Basophils are thought to play a role in host defense against parasites (43). Consequently, associations of basophilia with chronic inflammation and autoimmunity have been described (42, 43). As such, our data might open novel opportunities to study biomarkers of disease activity in MG. Together with real-world routine clinical data, inexpensive laboratory studies could allow risk classification for MC that goes beyond known risk factors for MC, such as infection, as exemplified by a recent study that used explainable machine learning to classify the risk for MC based on these parameters (44).

There are several limitations to our study. The dataset, with 58 patients in total, is small. However, the dataset includes 21 MC events and several sequential laboratory measurements per patient since the laboratory parameters were measured frequently. This leads to an uneven distribution of measurements between cases and controls. It is possible that the elevated CRP in the MC group was not identified as a risk factor for MC because of the small size of our cohort. Approximately half of the MCs recorded here showed preceding infections, which is a known risk factor for MC. It is also possible that subgroups (e.g., males) could be more prone to developing MC after infection. However, our study was not designed to address such questions. Furthermore, there is the potential for selection bias due to the retrospective and monocentric design and hand-selection of cases and controls for this pilot study. This study did not consider further clinical data, such as information on infection or co-medication, which are known risk factors for the clinical worsening of MG. Similarly, steroids or steroid-sparing immunosuppression are standard medications in MG patients known to affect blood counts but were not considered confounders. We used complete-case analyses based on a different number of observations, as a result of clinical practice not to measure all laboratory parameters at every time point. This leads to a different number of measurements per parameter per patient, and thus they could only be considered as univariate parameters in the models.

In conclusion, this study indicates that increased basophils, neutrophils, leukocytes, and platelets may be associated with an increased risk for the occurrence of MC in MG patients. The results of this pilot study suggest that it is possible to identify predictors for MC risk based on routine laboratory data. Together with other medical data (44), routine blood biomarkers could serve to develop a risk prediction score to tailor individualized treatment decisions at the point of care. However, larger prospective studies beyond the proof of concept stage are necessary to verify our results.

Data availability statement

The datasets presented in this article are not readily available because ethical approval currently does not permit sharing of raw data. Approval will be sought by the corresponding author upon reasonable request with scientific rationale and sound methodology. Requests for data sharing will be managed in accordance with data access and sharing policies of Charité – Universitätsmedizin Berlin. Requests to access the datasets should be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by the Ethics Committee of Charité – Universitätsmedizin Berlin. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin because data were collected retrospectively. Due to the retrospective nature, individual patient consent was not obtained, in accordance with the ethics approval, and state and national laws.

Author contributions

AMeh: Data curation, Formal analysis, Investigation, Writing – original draft. SB: Formal analysis, Investigation, Writing – review & editing. JK-H: Investigation, Writing – review & editing. LG: Writing – review & editing. MH: Writing – review & editing. SH: Writing – review & editing. SL: Writing – review & editing. FSc: Writing – review & editing. FSt: Writing – review & editing. MS: Writing – review & editing. AB: Formal analysis, Writing – review & editing. AMei: Formal analysis, Investigation, Writing – review & editing. AA: Formal analysis, Investigation, Methodology, Writing – review & editing. PM: Formal analysis, Writing – review & editing, Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation.

Funding

The author(s) declare financial support was received for the publication of this article. This study did not receive dedicated funding. PM is Einstein Junior Fellow, and AB is Einstein Visiting Fellow, both funded by the Einstein Foundation Berlin. PM acknowledges funding support by the Einstein Foundation Berlin (EJF-2020-602; EVF-BUA-2022-694), PM and AB acknowledge joint funding by the Einstein Foundation Berlin (EVF-2021–619), and the Leducq Foundation for Cardiovascular and Neurovascular Research (Consortium International pour la Recherche Circadienne sur l'AVC).

The authors acknowledge financial support for open access publication from the Open Access Publication Fund of Charité - Universitätsmedizin Berlin. The funding organizations did not play any role in the design of the study, preparation, review, approval of the manuscript, or decision to submit the manuscript for publication.

Acknowledgments

The authors thank C. Heibutzki, D. Remstedt, J. Brestrich, and N. Baro for study and patient management; S. Märschenz, S. Lischewski, and M. Heinold for administrative support; and M. Mandrela, P. Brunecker, and the team of the Health Data Platform at the Berlin Institute of Health at Charité for data access support. This manuscript has been posted as a preprint on medRxiv prior to submission to this journal (https://www.medrxiv.org/content/10.1101/2023.09.19.23295421v1).

Conflict of interest

SB is co-owner of exago.ml, a geoanalytics-focused machine learning company. SH has received speaker's honoraria from Alexion, argenx, UCB and Roche and honoraria for attendance at advisory boards from Alexion, argenx and Roche, and is member of the medical advisory board of the German Myasthenia Society. SL has received speaker's honoraria from Alexion, argenx, Hormosan and UCB, and honoraria for attendance of advisory boards from Alexion, argenx, Biogen, HUMA, UCB and Roche. FSt received speaker's honoraria and honoria for attendance of advisory boards from Alexion, argenx and UCB Pharma. MS received speaker's honoraria for attendance at patient events from argenx and Alexion. AM has received speaker's honoraria, consulting fees or (institutional) financial research support from Alexion Pharmaceuticals Inc., Argenx, Grifols SA, Hormosan Pharma GmbH, Janssen, Octapharma, and UCB Pharma, and is chairman of the medical advisory board of the German Myasthenia Society. PM has been on the board of HealthNextGen.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Punga AR, Maddison P, Heckmann JM, Guptill JT, Evoli A. Epidemiology, diagnostics, and biomarkers of autoimmune neuromuscular junction disorders. *Lancet Neurol.* (2022) 21:176–88. doi: 10.1016/S1474-4422(21)00297-0

2. Gilhus NE. Myasthenia gravis. *N Engl J Med.* (2016) 375:2570–81. doi: 10.1056/NEJMra1602678

3. Gilhus NE, Skeie GO, Romi F, Lazaridis K, Zisimopoulou P, Tzartos S. Myasthenia gravis – autoantibody characteristics and their implications for therapy. *Nat Rev Neurol.* (2016) 12:259–68. doi: 10.1038/nrneurol.2016.44

4. Huijbers MG, Marx A, Plomp JJ, Le Panse R, Phillips WD. Advances in the understanding of disease mechanisms of autoimmune neuromuscular junction disorders. *Lancet Neurol.* (2022) 21:163–75. doi: 10.1016/S1474-4422(21)00357-4

5. Meriggioli MN, Sanders DB. Autoimmune myasthenia gravis: emerging clinical and biological heterogeneity. *Lancet Neurol.* (2009) 8:475–90. doi: 10.1016/S1474-4422(09)70063-8

6. Howard JF. Myasthenia gravis: the role of complement at the neuromuscular junction. *Ann N Y Acad Sci.* (2017) 1412:113–28. doi: 10.1111/nyas.13522

7. Sieb JP. Myasthenia gravis: an update for the clinician. *Clin Exp Immunol.* (2014) 175:408–18. doi: 10.1111/cei.12217

8. Roper J, Fleming ME, Long B, Koyfman A. Myasthenia gravis and crisis: evaluation and management in the emergency department. *J Emerg Med.* (2017) 53:843–53. doi: 10.1016/j.jemermed.2017.06.009

9. Gamez J, Salvadó M, Carmona F, de Nadal M, Romero L, Ruiz D, et al. Intravenous immunoglobulin to prevent myasthenic crisis after thymectomy and other procedures can be omitted in patients with well-controlled myasthenia gravis. *Ther Adv Neurol Disord.* (2019) 12:1756286419864497. doi: 10.1177/1756286419864497

10. Neumann B, Angstwurm K, Mergenthaler P, Kohler S, Schönenberger S, Bösel J, et al. Myasthenic crisis demanding mechanical ventilation: a multicenter analysis of 250 cases. *Neurology.* (2020) 94:e299–313. doi: 10.1212/WNL.0000000000008688

11. Liu Z, Yao S, Zhou Q, Deng Z, Zou J, Feng H, et al. Predictors of extubation outcomes following myasthenic crisis. *J Int Med Res.* (2016) 44:1524–33. doi: 10.1177/0300060516669893

12. Alshekhlee A, Miles JD, Katirji B, Preston DC, Kaminski HJ. Incidence and mortality rates of myasthenia gravis and myasthenic crisis in us hospitals. *Neurology.* (2009) 72:1548–54. doi: 10.1212/WNL.0b013e3181a41211

13. Mergenthaler P, Stetefeld HR, Dohmen C, Kohler S, Schönenberger S, Bösel J, et al. Seronegative myasthenic crisis: a multicenter analysis. *J Neurol.* (2022) 269:3904–11. doi: 10.1007/s00415-022-11023-z

14. O'Riordan JI, Miller DH, Mottershead JP, Hirsch NP, Howard RS. The management and outcome of patients with myasthenia gravis treated acutely in a neurological intensive care unit. *Eur J Neurol.* (1998) 5:137–42. doi: 10.1046/j.1468-1331.1998.520137.x

15. Kalita J, Kohat AK, Misra UK. Predictors of outcome of myasthenic crisis. *Neurol Sci.* (2014) 35:1109–14. doi: 10.1007/s10072-014-1659-y

16. König N, Stetefeld HR, Dohmen C, Mergenthaler P, Kohler S, Schönenberger S, et al. Musk-antibodies are associated with worse outcome in myasthenic crisis requiring mechanical ventilation. *J Neurol.* (2021) 268:4824–33. doi: 10.1007/s00415-021-10603-9

17. Angstwurm K, Vidal A, Stetefeld H, Dohmen C, Mergenthaler P, Kohler S, et al. Early tracheostomy is associated with shorter ventilation time and duration of Icu stay in patients with myasthenic crisis-a multicenter analysis. *J Intensive Care Med.* (2022) 37:32–40. doi: 10.1177/0885066620967646

18. Liu F, Wang Q, Chen X. Myasthenic crisis treated in a Chinese neurological intensive care unit: clinical features, mortality, outcomes, and predictors of survival. *BMC Neurol.* (2019) 19:172. doi: 10.1186/s12883-019-1384-5

19. Nelke C, Stascheit F, Eckert C, Pawlitzki M, Schroeter CB, Huntemann N, et al. Independent risk factors for myasthenic crisis and disease exacerbation in a retrospective cohort of myasthenia gravis patients. *J Neuroinflammation.* (2022) 19:89. doi: 10.1186/s12974-022-02448-4

20. Jiang Z, Ning Z, Yang L, Chen B, Tang J, Zhang J, et al. The correlation of neutrophil-to-lymphocyte ratio with the presence and short-time curative effect of myasthenia gravis in children: a retrospectively study. *Int J Neurosci.* (2021) 131:894–901. doi: 10.1080/00207454.2020.1759592

21. Yang DH, Qian MZ, Wei MM, Li J, Yu MM, Lu XM, et al. The correlation of neutrophil-to-lymphocyte ratio with the presence and activity of myasthenia gravis. *Oncotarget.* (2017) 8:76099–107. doi: 10.18632/oncotarget.18546

22. Hsu CW, Chen NC, Huang WC, Lin HC, Tsai WC, Huang CC, et al. Hemogram parameters can predict in-hospital mortality of patients with myasthenic crisis. *BMC Neurol.* (2021) 21:388. doi: 10.1186/s12883-021-02412-4

23. Mehnert A, Bershan S, Kollmus-Heege J, Gerischer L, Herdick ML, Hoffmann S, et al. Identifying patients at risk for myasthenic crisis with hemogram and inflammation-related laboratory parameters – a pilot study. *medRxiv.* (2023). doi: 10.1101/2023.09.19.23295421

24. R Core Team. *R: A language and environment for statistical computing.* Vienna, Austria: R Foundation for Statistical Computing (2021).

25. Therneau TM. A package for survival analysis in R. (2021). Available at: https://CRAN.R-project.org/package=survival.

26. Therneau TM, Grambsch PM. The Cox model In: Dietz, K, Gail, M, Krickberg, K, Samet, J and Tsiatis, A. Eds. *Modeling survival data: extending the Cox model.* New York, NY: Springer (2000). 39–77.

27. Højsgaard S, Halekoh U, Yan J. The R package Geepack for generalized estimating equations. *J Stat Softw.* (2005) 15:1–11. doi: 10.18637/jss.v015.i02

28. Gummi RR, Kukulka NA, Deroche CB, Govindarajan R. Factors associated with acute exacerbations of myasthenia gravis. *Muscle Nerve*. (2019) 60:693–9. doi: 10.1002/mus.26689

29. Lotan I, Hellmann MA, Wilf-Yarkoni A, Steiner I. Exacerbation of myasthenia gravis following corticosteroid treatment: what is the evidence? A systematic review. *J Neurol*. (2021) 268:4573–86. doi: 10.1007/s00415-020-10264-0

30. Abuzinadah AR, Alanazy MH, Butt NS, Barohn RJ, Dimachkie MM. Exacerbation rate in generalized myasthenia gravis and its predictors. *Eur Neurol*. (2021) 84:43–8. doi: 10.1159/000512077

31. de Meel RH, Lipka AF, van Zwet EW, Niks EH, Verschuuren JJ. Prognostic factors for exacerbations and emergency treatments in myasthenia gravis. *J Neuroimmunol*. (2015) 282:123–5. doi: 10.1016/j.jneuroim.2015.03.018

32. Wang L, Zhang Y, He M. Clinical predictors for the prognosis of myasthenia gravis. *BMC Neurol*. (2017) 17:77. doi: 10.1186/s12883-017-0857-7

33. Kato T, Kawaguchi K, Fukui T, Nakamura S, Hakiri S, Nakatochi M, et al. Risk factors for the exacerbation of myasthenic symptoms after surgical therapy for myasthenia gravis and thymoma. *Semin Thorac Cardiovasc Surg*. (2020) 32:378–85. doi: 10.1053/j.semtcvs.2019.09.002

34. Akaishi T, Motomura M, Shiraishi H, Yoshimura S, Abe M, Ishii T, et al. Preoperative risks of post-operative myasthenic crisis (Pomc): a meta-analysis. *J Neurol Sci*. (2019) 407:116530. doi: 10.1016/j.jns.2019.116530

35. Kanai T, Uzawa A, Sato Y, Suzuki S, Kawaguchi N, Himuro K, et al. A clinical predictive score for postoperative myasthenic crisis. *Ann Neurol*. (2017) 82:841–9. doi: 10.1002/ana.25087

36. Lv Z, Zhong H, Huan X, Song J, Yan C, Zhou L, et al. Predictive score for in-hospital mortality of myasthenic crisis: a retrospective Chinese cohort study. *Eur Neurol*. (2019) 81:287–93. doi: 10.1159/000503961

37. Gilhus NE, Romi F, Hong Y, Skeie GO. Myasthenia gravis and infectious disease. *J Neurol*. (2018) 265:1251–8. doi: 10.1007/s00415-018-8751-9

38. Sy RW, Chawantanpipat C, Richmond DR, Kritharides L. Thrombocytopenia and mortality in infective endocarditis. *J Am Coll Cardiol*. (2008) 51:1824–5. doi: 10.1016/j.jacc.2008.01.034

39. Hu SL, Said FR, Epstein D, Lokeshwari M. The impact of anemia on renal recovery and survival in acute kidney injury. *Clin Nephrol*. (2013) 79:221–8. doi: 10.5414/CN107471

40. Menon V, Lessard D, Yarzebski J, Furman MI, Gore JM, Goldberg RJ. Leukocytosis and adverse hospital outcomes after acute myocardial infarction. *Am J Cardiol*. (2003) 92:368–72. doi: 10.1016/s0002-9149(03)00651-9

41. Shu DH, Ransom TP, O'Connell CM, Cox JL, Kaiser SM, Gee SA, et al. Anemia is an independent risk for mortality after acute myocardial infarction in patients with and without diabetes. *Cardiovasc Diabetol*. (2006) 5:8. doi: 10.1186/1475-2840-5-8

42. Miyake K, Karasuyama H. Emerging roles of basophils in allergic inflammation. *Allergol Int*. (2017) 66:382–91. doi: 10.1016/j.alit.2017.04.007

43. Stone KD, Prussin C, Metcalfe DD. Ige, mast cells, basophils, and eosinophils. *J Allergy Clin Immunol*. (2010) 125:S73–80. doi: 10.1016/j.jaci.2009.11.017

44. Bershan S, Meisel A, Mergenthaler P. Classifying the risk for myasthenic crisis using data-driven explainable machine learning with informative feature design and variance control – a pilot study. *medRxiv*. (2023). doi: 10.1101/2023.08.19.23294175

frontiers | Frontiers in Neurology

TYPE Original Research
PUBLISHED 11 March 2024
DOI 10.3389/fneur.2024.1277420

OPEN ACCESS

EDITED BY
German Moris,
SESPA, Spain

REVIEWED BY
Michelangelo Maestri,
University of Pisa, Italy
Marc De Baets,
Maastricht University, Netherlands

*CORRESPONDENCE
João Moura
moura.neuro@chporto.min-saude.pt

RECEIVED 14 August 2023
ACCEPTED 26 February 2024
PUBLISHED 11 March 2024

CITATION
Moura J, Fernandes J, Lima MJ, Sousa AP, Samões R, Martins Silva A and Santos E (2024) Treatment strategies and treatment-related adverse events in MG according to the age of onset.
Front. Neurol. 15:1277420.
doi: 10.3389/fneur.2024.1277420

Treatment strategies and treatment-related adverse events in MG according to the age of onset

João Moura[1,2]*, Joana Fernandes[1], Maria João Lima[3], Ana Paula Sousa[4], Raquel Samões[1,2,5], Ana Martins Silva[1,2,5] and Ernestina Santos[1,2,5]

[1]Department of Neurology, Centro Hospitalar Universitário de Santo António, Porto, Portugal, [2]Unit of Multidisciplinary Research in Biomedicine (UMIB), Instituto de Ciências Biomédicas Abel Salazar (ICBAS), University of Porto, Porto, Portugal, [3]Department of Neurology, Unidade Local de Saúde de Matosinhos, Porto, Portugal, [4]Department of Neurophysiology, Hospital de Santo António, Centro Hospitalar Universitário Do Porto, Porto, Portugal, [5]Laboratory for Integrative and Translational Research in Population Health (ITR), Porto, Portugal

Introduction: Early-onset (EOMG) and late-onset (LOMG) are distinct groups of MG patients. It is unclear if treatment strategies and treatment-related adverse events may differ according to the age of MG onset.

Methods: This single-center retrospective study includes all MG patients followed at a tertiary center since 2007. We reviewed the electronic clinical records.

Results: In total, 212 patients were identified, 142 (67.0%) females, with a median disease duration of 10years. The median age of symptom onset was 42.0 (26.0–64.5) years, with 130 (61.3%) EOMG cases and 82 (38.7%) LOMG. EOMG were more frequently female, had longer disease duration and often more generalized MG (p<0.001). Comorbidities were significantly more frequent in LOMG (67.1%) compared to EOMG (53.1%) (p=0.002). Steroid-related adverse effects motivating the switch to steroid-sparing agents (82.0%) were different between groups, with hypertension, hypercholesterolemia, diabetes mellitus and malignancies being more common in LOMG. At the same time, osteoporosis and dyspepsia were more frequent in EOMG (p<0.001). The most common first-line choice was azathioprine (45.8%), and rituximab was used in 4 patients (1.9%).

Conclusion: Our study shows that treatment modalities are similar between EOMG and LOMG, while steroid-related adverse events appear to be distinct.

KEYWORDS

myasthenia gravis, late-onset, comorbidities, steroid-sparing, immunosuppression

Introduction

Myasthenia gravis (MG) is a chronic autoimmune neuromuscular disease characterized by ocular and generalized muscle weakness. This disorder has a heterogeneous pathogenesis and variable phenotype associated with distinct disease subtypes (1, 2). When considering the age of onset, patients <50 years are considered early-onset MG (EOMG), while patients with

disease onset above 50 years belong to the late-onset MG group (LOMG).

Classically, treatment response is considered satisfactory in MG. The approval of novel immunosuppressive drugs to treat MG cases offers the opportunity to tailor the treatment to each patient. Baseline comorbidities and treatment-related side effects are essential aspects to consider in this process (3). Some studies suggest that EOMG and LOMG may differ in these characteristics (4, 5). However, it is still unclear if treatment-related adverse events are associated with the age of MG onset.

This study aims to describe a MG cohort and evaluate comorbidities, treatment strategies and treatment-related adverse events between EOMG and LOMG.

Methods

We retrospectively analyzed the medical records of MG patients from an institutional database that contains all MG cases followed in the Centro Hospitalar Universitario de Santo António Neuroimmunology Outpatient Clinic. This database is updated annually since 2007. MG was diagnosed by practicing neurologists specialized in neuroimmunology (ES, AMS, APS, and RS) based on a combination of clinical features, neurophysiological studies, antibody testing and response to pyridostigmine (2). Patients aged <50 years were classified as EOMG, while patients ≥50 years were considered LOMG (1).

We collected information concerning sex, age of onset and age at diagnosis. We extracted data on the prevalence of different comorbidities using what was defined in previous studies. Comorbidities were classified as treatment-related if they appeared after the initiation of a specific treatment and if the neurologist considered the comorbidity an effect of treatment, according to the medical records. The baseline Myasthenia Gravis Foundation of America (MGFA) score and the occurrence of myasthenic crisis or hospital admission due to MG during the disease course were retrospectively collected. Data on the use of anticholinesterases (and maximum dose), steroids (and maximum dose), and other immunosuppressors used during the disease course was additionally detailed.

Qualitative variables were studied using absolute and relative frequencies. The median and interquartile range (p25–p75) (IQR) were calculated for quantitative variables. An X^2 was used to compare categorical variables, while a Mann–Whitney U test was used for continuous variables. Statistical analysis was performed in SPSS Statistics version 29. A p-value < 0.05 was considered statistically significant.

This study was approved by Centro Hospitalar Universitário de Santo António Ethical Committee. Informed consent was waived due to the retrospective nature of the study.

Results

In total, 212 patients were identified, 103 (79.2%) female. The median disease duration was ten years, and the median follow-up time was 8 years. The median age of symptom onset was 42.0 (26.0–64.5) years, with 130 (61.3%) EOMG cases and 82 (38.7%) LOMG. Table 1 summarizes the characteristics of the cohort. Overall, EOMG were more frequently female and had longer disease duration. MG phenotypes were significantly different between groups, with EOMG patients having significantly more generalized MG (80.0% vs. 56.1%) and LOMG patients having more ocular MG (43.9% vs. 20.0%) ($p < 0.001$). Figure 1 shows the distribution of baseline MGFA scores concerning the age of onset.

At least one comorbidity was present in 124 patients (58.5%), with a median of 1.0 (0.0–2.0) comorbidity per patient. Comorbidities were significantly more frequent in LOMG (67.1%) compared to EOMG (53.1%) ($p = 0.002$). In particular, arterial hypertension, dyslipidemia, diabetes mellitus and venous thromboembolism were significantly more associated with LOMG, as shown in Table 1. Patients with and without thymomas had a similar number of comorbidities ($p = 0.582$).

Fifty patients (23.6%) had at least one myasthenic crisis requiring acute treatment throughout the disease course. There was no difference concerning age of onset (26.1% EOMG vs. 19.5% LOMG, $p = 0.320$). In total, 41 (19.3%) patients required hospital admission due to MG exacerbations, 20 EOMG (16.1%) and 21 LOMG (25.6%) ($p = 0.335$). Regarding the treatment regimens, pyridostigmine was offered to 93.9% of patients, with significantly more EOMG patients receiving treatment ($p = 0.024$). The median highest dose of anticholinesterase was 300.0 (240.0–360.0), which was higher in EOMG compared to LOMG (300.0 versus 240.0, $p < 0.001$). Acute treatment modalities comprised human immunoglobulin (30.7%) and plasmapheresis (6.1%). Steroids were used in 74.5% of patients, with 15.6% requiring prednisolone as part of maintenance treatment. Treatment-related side effects were the most common reason for choosing a steroid-sparing agent (82.0%). From these, hypertension, hypercholesterolemia, diabetes mellitus and malignancies were more common in LOMG, while osteoporosis and dyspepsia were more frequent in EOMG ($p < 0.001$) (Figure 2).

Most patients were switched to steroid-sparing agents, with the most common first-line choice being azathioprine (45.8%). Rituximab was used as a first-line option in 4 patients (1.9%): 2 anti-MuSK-associated MG cases, 1 with comorbid autoimmune disease and 1 EOMG case with an aggressive course. There was no significant difference between EOMG and LOMG regarding first-line options. Second-line immunotherapy was required in 26.9%, mainly for improving disease control (85.8%). Treatment-related adverse events motivating a therapy switch to second-line agents (3.8%) included hepatitis (2.4%), repeated infections (0.9%) and pancytopenia (0.5%). The most common second and third-line agents were methotrexate (12.3%) and rituximab (6.1%), respectively. Tocilizumab (0.5%) and efgartigimod (0.5%) were only used as third-line alternatives in EOMG cases. Thymectomy was performed in 81 (38.2%) patients, more frequently in EOMG (81.5%) than LOMG (18.5%). The median time to thymectomy was 1.0 (1.0–3.0) years. Thymoma was identified in 30 (14.2%), most commonly of type 2B (53.3%), while hyperplasia was present in the remaining patients. Although more thymomas being identified in EOMG (56.7% vs. 43.3%), this difference was not statistically significant.

Discussion

This study underscores the importance of considering the age of MG onset and comorbidities in treatment selection.

10.3389/fneur.2024.1277420

TABLE 1 Characterization of the MG cohort according to age of onset.

Variable	EOMG 130 (61.3%)	LOMG 82 (38.7%)	*p*
Female, *n* (%)	103 (79.2)	39 (47.6)	<0.001
Disease duration, median (IQR)	28 (15.0–39.0)	7.0 (4.0–13.0)	<0.001
Disease type, n (%)			
Ocular	26 (20.0)	36 (43.9)	<0.001
Generalized	104 (80.0)	46 (56.1)	
Antibodies, n (%)			
Anti-AchR	84 (64.6)	51 (62.2)	0.878
Anti-MuSK	9 (6.9)	2 (2.4)	0.497
Anti-titin	7 (5.4)	19 (23.2)	<0.001
Thymoma, *n* (%)	17 (13.1)	13 (15.9)	<0.001
Deceased	2 (1.5)	16 (19.5)	0.002
Number of comorbidities, median, IQR	1 (0.0–1.0)	1.0 (0.0–3.0)	0.002
Comorbidities, n (%)			
HTA	10 (7.7)	25 (30.5)	<0.001
Hypercholestrolemia	9 (6.9)	22 (26.8)	<0.001
Diabetes	14 (10.8)	20 (24.4)	0.012
Other cancers	12 (9.2)	12 (14.6)	0.268
Cataracts	8 (6.2)	4 (4.9)	0.770
Osteoporosis	7 (5.4)	2 (2.4)	0.487
Oportunistic infections	5 (3.9)	6 (7.3)	0.343
Dyspepsia	4 (3.1)	2 (2.4)	1.000
PVT/PE	0	4 (4.9)	0.021
Thyroid disease	7 (5.4)	6 (7.3)	0.570
Other autoimmune disease	26 (20.0)	6 (7.3)	0.031
Ashtma	5 (3.9)	4 (4.9)	0.737
Psoryasis	3 (2.3)	0	0.285
Neuromyotonia	4 (3.1)	0	0.160
Coronary disease	8 (6.2)	5 (6.1)	1.000
Atrial Fib	3 (2.3)	4 (4.9)	0.434
Prostate hyperplasia	0	2 (2.4)	0.148
Depression	4 (3.1)	3 (3.7)	1.000
Sleep apnea	5 (3.8)	1 (1.2)	0.409
Stroke/TIA	3 (2.3)	2 (2.4)	1.000
CKD	4 (3.1)	5 (6.1)	0.313
PVD	1 (0.8)	1 (1.2)	1.000
COPD	0	1 (1.2)	0.387
HF	2 (1.5)	2 (2.4)	0.641
Hypoacusis	0	2 (2.4)	0.148
Chronic liver disease	1 (0.8)	1 (1.2)	1.000
Headache	1 (0.8)	0	1.000
Bronchiectasis	1 (0.8)	0	1.000
Dementia	1 (0.8)	3 (3.7)	0.301
Gout	0	2 (2.4)	0.148
Epilepsy	0	1 (1.2)	0.387

(Continued)

TABLE 1 (Continued)

Variable	EOMG 130 (61.3%)	LOMG 82 (38.7%)	*p*
PD	1 (0.8)	3 (3.7)	0.301
Alcoholism	1 (0.8)	1 (1.2)	1.000
Psychosis	0	1 (1.2)	0.387
Treatment			
Piridostigmine ever, *n* (%)	127 (97.7)	72 (87.8)	0.024
Piridostigmine dose, median (IQR)	300.0 (240.0–360.0)	240.0 (180.0–300.0)	<0.001
Steroids ever, *n* (%)	103 (79.2)	55 (67.1)	0.219
Steroids dose, median (IQR)	40.0 (20.0–60.0)	30.0 (20.0–52.5)	0.118
Immunoglobulin during acute phase, *n* (%)	43 (33.1)	22 (26.9)	0.328
Plasmapheresis during acute phase, *n* (%)	8 (6.2)	5 (6.1)	0.599
Thymectomy, *n* (%)	66 (50.8)	15 (18.3)	<0.001
Steroid sparing, first line, *n* (%)			0.074
AZA	69 (53.1)	28 (34.1)	
MMF	5 (3.8)	3 (3.7)	
RTX	3 (2.3)	1 (1.2)	
MTX	4 (3.1)	4 (4.9)	
CYC	0	1 (1.2)	
Steroid sparing, second line, *n* (%)			0.068
MTX	18 (13.8)	8 (9.8)	
MMF	16 (12.3)	5 (6.1)	
RTX	5 (3.8)	0	
AZA	3 (2.3)	1 (1.2)	
CYC	1 (0.8)	0	
Steroid sparing, third line, *n* (%)			0.042
RTX	9 (6.9)	4 (4.9)	
MMF	6 (4.6)	0	
MTX	3 (2.3)	0	
CYC	1 (0.8)	0	
Efgartigimod	1 (0.8)	0	
Tacrolimus	1 (0.8)	0	

AZA, azathioprine; MMF, mycophenolate mofetil; MTX, methotrexate; RTX, rituximab; CYC, cyclophosphamide; IQR, Interquartile range; PVT, Peripheral venous thrombosis; PE, pulmonary embolism; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; HF, heart failure; PD, Parkinson's Disease.

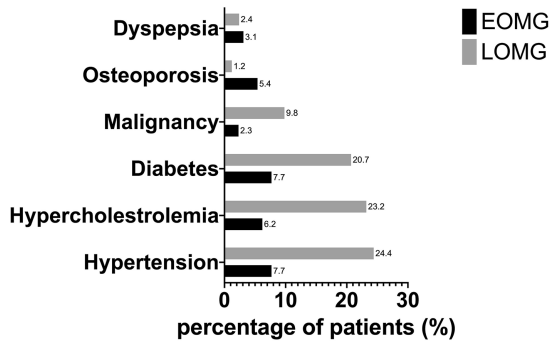


FIGURE 1
Distribution of MGFA scores according to the age of onset.

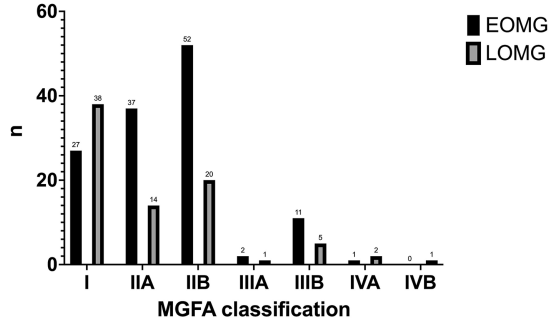


FIGURE 2
Adverse effects associated with steroids in EOMG and LOMG.

Overall, our MG cohort follows what is described in the literature for EOMG concerning female predominance and tendency toward generalized disease (1, 6). Thymomas were more frequently found in LOMG cases ($p < 0.001$), which is consistent with the literature (7). However, patients proposed for thymectomy were already the ones with an increased probability of having a thymoma based on imaging studies, which could introduce bias.

In our cohort, most MG patients had at least one comorbidity (approximately 59%), these being more frequent in LOMG cases. LOMG had significantly more comorbidities, particularly comorbidities associated with increased cardiovascular risk (arterial hypertension, dyslipidemia, and diabetes mellitus), consistent with previous reports (4). However, we could not exclude that these resulted from older age and not disease type. A previous study compared age-and sex-matched groups of EOMG and LOMG and found no significant differences, consistent with comorbidities probably resulting from the cumulative effect of increased aged than with MG type (5). Some conditions like dyslipidemia and hypertension may be particularly bothersome in MG since some treatment options (statins and beta-blockers) might worsen MG symptoms (8).

Steroid-related adverse effects motivating treatment change were frequent, consistent with the general recommendations for corticotherapy. However, the profile of side effects differs between LOMG and EOMG. Steroid-treated LOMG more frequently developed malignancies and the same comorbidities that were already more frequent in this subgroup (arterial hypertension, hypercholesterolemia, diabetes mellitus). This effect might be explained by age, as previously addressed. In the EOMG group, osteoporosis and dyspepsia were more frequent. Steroid-induced osteoporosis mainly affects individuals 20–45 years, consistent with our findings (9, 10). This probably results from the cumulative effect of corticotherapy for several years, since the mean dose was not significantly different between groups.

Immunosuppression is generally associated with favorable outcomes in MG, irrespective of the age of onset (11). In most cases from our study, steroid sparing agents were introduced first, and the dose of steroids progressively tapered while a steroid-sparing agent was introduced. The choice of steroid-sparing agents followed what is recommended in the literature, with the most frequent options being azathioprine, mycophenolate mofetil and methotrexate (1, 8, 12). MMF has been suggested as an alternative first-line steroid-sparing agent, due to its favorable profile (13). The results from our practice, with AZA as the commonest option, are in line with recently published guidelines (14). Anti-CD20 antibody therapy (rituximab) was generally reserved to more severe or refractory cases. These findings are consistent with other studies showing that the therapeutic management does not seem to differ between EOMG and LOMG, despite the latter having more comorbidities (11, 15). Rituximab has also been shown to be a safe and effective treatment in late-onset cases of aggressive generalized MG (16). Regarding other monoclonal antibodies, eculizumab and tocilizumab were used in 1 EOMG patient each, which is statistically non-significant. Both treatments have been shown to be safe and effective in patients with later age of onset (17, 18). Overall, there was a tendency toward late-onset forms requiring fewer drugs and thus being less frequently treatment-refractory, confirming findings from a recent study (6).

This study has several limitations that must be addressed. First, its retrospective design based on clinical records with a relatively small sample size. Second, we opted to use the age of 50 as a cut-off to define EOMG and LOMG, but there are other cut-off values proposed in the literature (19). Moreover, we have not considered the subgroup of very-late onset forms that is increasingly recognized (20). We have not specifically addressed the efficacy of different treatment modalities based on the MGFA score, as the purpose of this study was to specifically study treatment modalities and respective side effects in EOMG and LOMG. In the future, doing a longitudinal study with this purpose would be interesting.

MG is a complex neuroimmunological disorder whose treatment implicates taking into account patient comorbidities and strategies to avoid treatment-related adverse events. The growing subgroup of LOMG poses further challenges to neurologists handling this disorder. Our study describes the similarities in treatment modalities between EOMG and LOMG and the differences in steroid-related adverse events between each group.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Comissão de ética do Centro Hospitalar Universitário de Santo António. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation because the retrospective study was based on information from clinical records.

Author contributions

JM: Conceptualization, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. JF: Conceptualization, Formal analysis, Methodology, Writing – original draft. ML: Conceptualization, Formal analysis, Methodology, Writing – original draft. AS: Writing – review & editing. RS: Writing – review & editing. AM: Writing – review & editing. ES: Conceptualization, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Gilhus EN, Verschuuren JJ. Myasthenia gravis: subgroup classification and therapeutic strategies. *Lancet Neurol*. (2015) 14:1023–36. doi: 10.1016/S1474-4422(15)00145-3

2. Vincent A, Palace J, Hilton-Jones D. Myasthenia gravis. *Lancet*. (2001) 357:2122–8. doi: 10.1016/S0140-6736(00)05186-2

3. Narayanaswami P, Sanders BD, Wolfe G, Benatar M, Cea G, Evoli A, et al. International consensus guidance for management of myasthenia gravis. *Neurology*. (2021) 96:114–22. doi: 10.1212/WNL.0000000000011124

4. Misra KU, Kalita J, Singh KV, Kumar S. A study of comorbidities in myasthenia gravis. *Acta Neurol Belg*. (2020) 120:59–64. doi: 10.1007/s13760-019-01102-w

5. Klimiec-Moskal E, Quirke M, Leite IM. Comorbidities in older patients with myasthenia gravis — Comparison between early-and late-onset disease. *Acta Neurol Scand*. (2022) 145:371–4. doi: 10.1111/ane.13549

6. Cortés-Vicente E, Álvarez-Velasco R, Segovia S, Paradas C, Casasnovas C, Guerrero-Sola A, et al. Clinical and therapeutic features of myasthenia gravis in adults based on age at onset. *Neurology*. (2020) 94:e1171–80. doi: 10.1212/WNL.0000000000008903

7. Mao Z-F, Mo X-A, Qin C, Lai Y-R, Hackett LM. Incidence of thymoma in myasthenia gravis: a systematic review. *J Clin Neurol*. (2012) 8:161–9. doi: 10.3988/jcn.2012.8.3.161

8. Sanders BD, Wolfe IG, Benatar M, Evoli A, Gilhus NE, Illa I, et al. International consensus guidance for management of myasthenia gravis. *Neurology*. (2016) 87:419–25. doi: 10.1212/WNL.0000000000002790

9. Khosla S, Lufkin EG, Hodgson SF, Fitzpatrick LA, Melton LJ. Epidemiology and clinical features of osteoporosis in young individuals. *Bone*. (1994) 15:551–5. doi: 10.1016/8756-3282(94)90280-1

10. Mazziotti G, Angeli A, Bilezikian PJ, Canalis E, Giustina A. Glucocorticoid-induced osteoporosis: an update. *Trends Endocrinol Metab*. (2006) 17:144–9. doi: 10.1016/j.tem.2006.03.009

11. Yildiz Celik S, Durmus H, Yilmaz V, Saruhan Direskeneli G, Gulsen Parman Y, Serdaroglu Oflazer P, et al. Late-onset generalized myasthenia gravis: clinical features, treatment, and outcome. *Acta Neurol Belg*. (2020) 120:133–40. doi: 10.1007/s13760-019-01252-x

12. Lascano MA, Lalive HP. Update in immunosuppressive therapy of myasthenia gravis. *Autoimmun Rev*. (2021) 20:102712. doi: 10.1016/j.autrev.2020.102712

13. Hanisch F, Wendt M, Zierz S. Mycophenolate mofetil as second line immunosuppressant in myasthenia gravis - a long-term prospective open-label study. *Eur J Med Res*. (2009) 14:364–6. doi: 10.1186/2047-783X-14-8-364

14. Wiendl H, Abicht A, Chan A, Della Marina A, Hagenacker T, Hekmat K, et al. Guideline for the management of myasthenic syndromes. *Ther Adv Neurol Disord*. (2023) 16:17562864231213240. doi: 10.1177/17562864231213240

15. Pasqualin F, Guidoni VS, Ermani M, Pegoraro E, Bonifati MD. Outcome measures and treatment effectiveness in late onset myasthenia gravis. *Neurol Res Pract*. (2020) 2:2. doi: 10.1186/s42466-020-00091-z

16. Monte ME, Giménez GF, Morán DAJ, Andres LMJ. Rituximab para el tratamiento de la miastenia grave generalizada: experiencia en la práctica clínica. *Rev Neurol*. (2021) 73:416–20. doi: 10.33588/rn.7312.2021166

17. Howard FJ, Bril V, Vu T, Karam C, Peric S, Margania T, et al. Safety, efficacy, and tolerability of efgartigimod in patients with generalised myasthenia gravis (ADAPT): a multicentre, randomised, placebo-controlled, phase 3 trial. *Lancet Neurol*. (2021) 20:526–36. doi: 10.1016/S1474-4422(21)00159-9

18. Jia D, Zhang F, Li H, Shen Y, Jin Z, Shi FD, et al. Responsiveness to tocilizumab in anti-acetylcholine receptor-positive generalized myasthenia gravis. *Aging Dis*. (2023):0. doi: 10.14336/AD.2023.0528

19. Hellmann AM, Mosberg-Galili R, Steiner I. Myasthenia gravis in the elderly. *J Neurol Sci*. (2013) 325:1–5. doi: 10.1016/j.jns.2012.10.028

20. Tang Y-L, Ruan Z, Su Y, Guo RJ, Gao T, Liu Y, et al. Clinical characteristics and prognosis of very late-onset myasthenia gravis in China. *Neuromuscul Disord*. (2023) 33:358–66. doi: 10.1016/j.nmd.2023.02.013

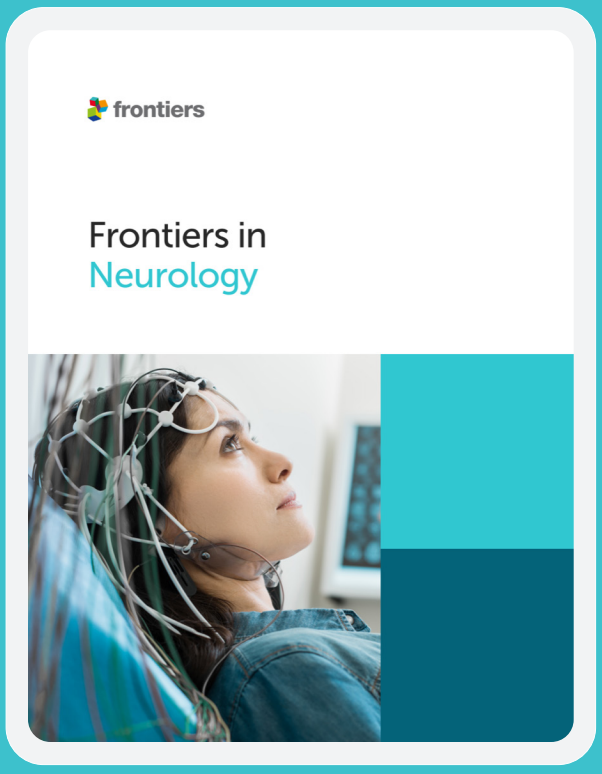
Frontiers in Neurology

Explores neurological illness to improve patient care

The third most-cited clinical neurology journal explores the diagnosis, causes, treatment, and public health aspects of neurological illnesses. Its ultimate aim is to inform improvements in patient care.

Discover the latest Research Topics

See more →



Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact