NEWS AND VIEWS IN THE MANAGEMENT OF MYASTHENIA GRAVIS

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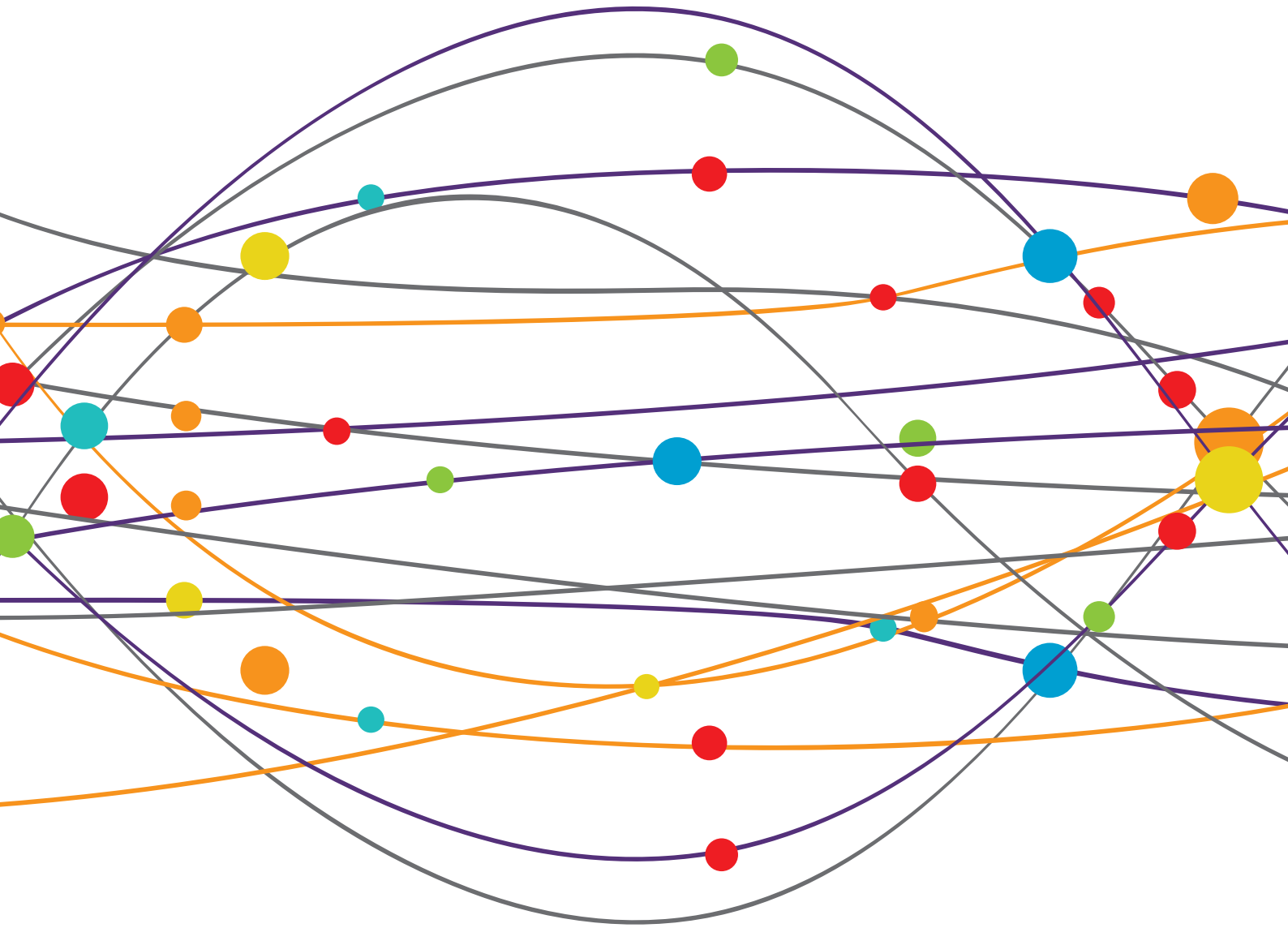

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NEWS AND VIEWS IN THE MANAGEMENT OF MYASTHENIA GRAVIS

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Table of Contents

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EDITORIAL
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Editorial: News and Views in the Management of Myasthenia Gravis

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Keywords: myasthenia gravis, acetylcholine receptor antibodies, muscle-specific tyrosine kinase antibodies, immunosuppressive therapy, quality of life measures

Editorial on the Research Topic

News and Views in the Management of Myasthenia Gravis

Myasthenia gravis (MG) is a rare disease of the neuromuscular transmission and one of the best characterized autoimmune diseases. The aim of this Research Topic was to provide an overview of current issues in the management of this disease.

In MG, pathogenic antibodies (Abs) bind to key components of the motor end-plate and cause morphological and functional alterations of the postsynaptic membrane leading to loss of acetylcholine receptors (AChRs) and impairment of neuromuscular transmission. The clinical hallmark is fatigable weakness of striated muscles with broad phenotypic variability. The AChR is the main antigen in MG, followed by the muscle-specific tyrosine kinase (MuSK) and the low-density lipoprotein-related protein 4 (LRP4). Specific Abs identify disease subtypes with distinctive pathogenic aspects, clinical features, and response to therapy. In addition, Abs to synaptic proteins (agrin and collagen Q) and muscle proteins like titin, the ryanodine receptor, Kv1.4 potassium channel, and cortactin can be found in MG patients. Patient subgrouping according to the Ab profile is considered a prerequisite to optimizing treatment (1). Two contributions are focused on the role of Abs in the immuno-pathogenesis and management of MG. Frykman et al. review the Ab effects at the neuromuscular junction and propose a useful algorithm for MG serological diagnosis. Lazaridis and Tzartos discuss recent advances in Ab testing and prospects for future innovative antigen-specific therapies.

Like other autoimmune diseases, the etiology of MG is multifactorial, including self-tolerance disruption, genetic predisposition, and environmental factors. An imbalance between antigen-specific CD4+ T helper cells and regulatory cells is thought to be crucial in promoting B cell activation and high-affinity Ab production (2). Wu et al. revise the evidence for the involvement of different subsets of regulatory cells in MG pathogenesis and discuss the difficulty in translating these findings into the heterogeneous MG population. Vitamin D has modulatory effects on both innate and adaptive immune responses (3). The study by Han et al., investigating the association of vitamin D receptor polymorphisms with MG in the Chinese Han population, reports an increased frequency of the rs731236 variant in adult AChR-negative patients. With reference to the increased frequency of autoimmune diseases in MG patients compared to healthy subjects, Li et al. focus on the rare association with primary Sjögren's syndrome.

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The MG diagnosis may be challenging in individuals with isolated ocular symptoms (ocular MG-OMG) given the low positivity rates of serological testing and repetitive nerve stimulation. In their review, Wirth et al. summarize the evidence on sensitivity and specificity of repetitive ocular vestibular evoked myogenic potentials (roVEMPs), a promising new technique that can detect muscle fatigability through direct recording from extrinsic ocular muscles.

MG management takes into consideration weakness extension and severity, associated Abs, age at onset, and thymus pathology. Current treatment is based on the use, generally in combination, of cholinesterase inhibitors, corticosteroids, other immunosuppressants, and, in selected subgroups, thymectomy. Plasma exchange and high-dose intravenous Ig (IVIg) are used in deterioration phases or as periodic treatment in patients with refractory disease. Farrugia and Goodfellow provide a comprehensive overview of MG management in adult patients, including data from the authors' own experience. Three contributions investigate selected therapeutic options. Imai et al. compare the efficacy of different prednisolone regimens in the long-term course of MG. Fan et al. assess the therapeutic effect and safety profile of tacrolimus monotherapy in patients with ocular and generalized disease. Putko et al. report the results of a *post-hoc* analysis, based on an open-label prospective trial of subcutaneous Ig (SCIg), to evaluate the correlation of SCIg dosage and serum IgG levels with clinical response.

Several articles in this collection focus on subgroups of patients in whom MG management poses specific problems or is complicated by the rarity of the disease and lack of randomized controlled trials (RCTs). N.E. Gilhus reviews the potential risks for mother and child during pregnancy, delivery, and the postpartum/postnatal period. Heckmann and Marais describe the characteristics of MG in human immunodeficiency virus (HIV) infected persons and discuss safety concerns related to immunosuppressive therapy. Evoli and Iorio provide an overview of OMG epidemiology, rate of progression to generalized MG, clinical aspects, and treatment issues. Two contributions focus on juvenile MG (age at onset $\leq$18 years). O'Connell et al. discuss the current evidence on disease management, propose a treatment algorithm, and highlight controversial issues in diagnosis and treatment. The study by Popperud et al. investigates the long-term effects of early-life thymectomy on the immune system by measuring T cell subsets at different intervals after surgery. Two articles address MG with Abs to MuSK (MuSK-MG). Rodolico et al. review the disease epidemiology, clinical phenotypes, diagnostic challenges, and response to therapy. Zhang et al. examine MuSK-MG severity and prognosis in Northern China through a comparison with AChR-MG and AChR/MuSK negative (double seronegative—DSN) MG.

Although most patients respond satisfactorily to conventional therapy, drug-free remission is rare, chronic immunosuppression is generally required, and 10–15% of patients have refractory disease (4). Targeted immunotherapies, including B cell depletion, inhibition of complement activation, and increased IgG clearance through interference with the Fc neonatal receptor, are promising alternatives to conventional immunosuppression. Two contributions focus on therapeutic advances in MG. Menon et al. review the rationale for the use of novel agents in MG and the status of related RCTs. Mantegazza and Antozzi examine the unmet needs in MG treatment, discuss the potential advantages of the early use of biologic drugs, and the prospects for new therapeutic approaches. Among non-pharmacological interventions, there has been increasing awareness of the beneficial effects of an active lifestyle. O'Connor et al. discuss the difficulty in quantifying fatigue perception in MG patients and review the current evidence on physical activity and tailored exercise training in patients with stable disease.

Outcome measures, including disease-specific scales aimed at quantifying muscle weakness and self-perceived quality of life (QoL) are crucial to assess the response to treatment in clinical practice (5) and patients' satisfaction with disease control (6). Thomsen and Andersen revise the use of ordinal scales in recent RCTs, and highlight some drawbacks such as the limited correlation between muscle weakness and disability, a considerable floor effect in milder cases, and lack of data about the performance of the scales in different patient populations. Applying the short-form 36-item questionnaire for health survey to a large patient cohort, Szczudlik et al. show that together with symptom severity, age and employment status are among the main determinants of reduced QoL in MG. The steroid- and, in general, the immunosuppressive therapy-sparing effect has increasingly been used as a treatment end-point. This approach has a strong rationale, as immunosuppression tapering is an indirect measure of disease control and the burden of treatment-related side effects has a negative impact on QoL. Nowak et al. report the changes in patients' exposure to conventional immunosuppression during the open-label extension of the REGAIN trial, which investigated the efficacy and safety of eculizumab in refractory AChR-MG (7). Around 50% of patients could withdraw one immunosuppressant and most could taper other agents with sustained disease control.

Phenotypic variability, immunopathological heterogeneity, and symptom fluctuations all contribute to the complexity of MG management. In addition to established protocols for disease confirmation and treatment, new diagnostic techniques and more selective immunotherapies have become available of late. Clinicians must be aware of their advantages and limitations in order to optimize treatment. This Research Topic addresses a broad range of clinical issues and should contribute to reach this goal.

AUTHOR CONTRIBUTIONS

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

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REVIEW
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Myasthenia Gravis Can Have Consequences for Pregnancy and the Developing Child

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Myasthenia gravis (MG) with onset below 50 years, thymic hyperplasia and acetylcholine receptor (AChR) antibodies is more common in females than in males. For a relatively large group of MG patients, pregnancy represents therefore an important question. The muscle weakness, the circulating autoantibodies, the hyperplastic thymus, the MG drug treatment, and any autoimmune comorbidity may all influence both mother and child health during pregnancy and also during breastfeeding in the postpartum period. Mother's MG remains stable in most patients during pregnancy. Pyridostigmine, prednisolone, and azathioprine are regarded as safe during pregnancy. Mycophenolate, methotrexate and cyclophosphamide are teratogenic and should not be used by women with the potential to become pregnant. Rituximab should not be given during the last few months before conception and not during pregnancy. Intravenous immunoglobulin and plasma exchange can be used for exacerbations or when need for intensified therapy. Pregnancies in MG women are usually without complications. Their fertility is near normal. Vaginal delivery is recommended. MG patients have an increased rate of Cesarean section, partly due to their muscle weakness and to avoid exhaustion, partly as a precaution that is often unnecessary. Around 10% of the newborn develop neonatal myasthenia during the first few days after birth. This is transient and usually mild with some sucking and swallowing difficulties. In rare cases, transplacental transfer of AChR antibodies leads to permanent muscle weakness in the child, and arthrogryposis with joint contractures. Repeated spontaneous abortions have been described due to AChR antibodies. MG women should always give birth at hospitals with experience in newborn intensive care. MG does not represent a reason for not having children, and the patients should be supported in their wish of becoming pregnant.

Keywords: myasthenia gravis, autoimmunity, autoantibodies, pregnancy, neonatal myasthenia, arthrogryposis, breastfeeding, teratogenicity

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INTRODUCTION

Myasthenia gravis (MG) is an autoimmune disorder where well-defined muscle antibodies bind to the post-synaptic membrane at the neuromuscular junction (1). These antibodies induce the muscular weakness typical for MG. In most patients, the antibodies bind to acetylcholine receptors (AChR), but alternative targets are muscle-specific tyrosine kinase (MuSK) and lipoprotein-related

peptide 4 (LRP4) (2). Antibody binding leads to destruction and reduced receptor function through cross-linking of membrane molecules, complement activation, and blockade of ligand-binding epitopes. AChR antibody binding to the cell membrane leads in addition to a cascade of intracellular events that influences muscle cell function. AChR and MuSK antibodies are highly specific for MG, as they do not occur without muscle weakness and in the healthy population. MG severity is not linked to autoantibody concentration, illustrating the variation and complexity in the antigen-antibody interaction as well as the individual variation in the consequences of this interaction.

Most females in reproductive age with MG have an enlarged and hyperplastic thymus with widespread germinal follicles (3). A thymoma is present in 10% of all MG patients but is less common among young females with the disease. The thymus pathology induces the production of AChR antibodies in lymphoid tissue widespread in the body. The trigger for thymic hyperplasia is not known, but virus infection has been suggested, in genetically predisposed individuals and perhaps at an especially vulnerable time point. Thymectomy early in the disease improves MG in females in reproductive age (4). Thymic hyperplasia does not occur in other autoimmune disorders, and the therapeutic effect of thymectomy is specific for MG with AChR antibodies.

Untreated MG is a severe disease with 50% mortality after ten years. With modern treatment, no patients should die from their MG. Therapy combines symptomatic and immunosuppressive drugs, thymectomy, and supportive therapy such as physical training, vigorous treatment of infections, respiratory support in the rare occasions it becomes necessary, and optimal treatment of comorbidities. Most patients do well and have modest, minimal or no muscle weakness. However, 10–20 % have a disease that is relatively resistant to standard therapies.

MG prevalence in the general population is 150–250 individuals per million, and with an annual incidence of 8–10 individuals per million (5). As both prevalence and incidence increase with increasing age, these figures are somewhat lower among females in reproductive age. MG prevalence in European females below age 50 years is thought to be 120 per million, and annual incidence 5–10 per million (5). MG with onset below age 50 years and AChR antibodies is 2–3 times more common in females than in males, and women have an incidence peak at age 30 years. In China and other Far East countries, juvenile MG is much more common than in Western populations, and MG with debut in childhood represents a third incidence peak (6). MuSK MG is more common in older age groups. However, in a multinational study from mainly Western countries, 70% had MuSK MG debut before age 40 years, and the females had a mean debut age of 31 years (7). MuSK MG is twice as common in females as in males. The relative number of MG patients in different age groups depends for a large part on population demographics. In younger populations in Africa, South-America and Africa, pregnancy and childbirth is relevant for a much larger proportion of MG patients than in Europe.

Mother's age when giving birth has increased markedly during the last decades, especially in Western countries. In Norway, the mean age was in 2018 31 years, up from 29 years ten years ago. Similarly, mean age at first childbirth has increased from 23.5 years in 1975 to 25.5 years in 1990, and to 29.5 years in 2018 (www.ssb.no/fodte). This increased age at childbirth means that a higher proportion of MG females will experience childbirth after manifest disease.

For females in reproductive age with MG, one of their major concerns is potential consequences for fertility, pregnancy, giving birth, and lactation (8). Any risks for the child as well as for themselves are of the highest importance. Furthermore, they would like to know about any genetic MG predisposition for their children. Precise information about these factors to the patients and to all caretakers during the pregnancy and in the perinatal period should have a supportive and encouraging effect, and also improve the outcome. MG females often have exaggerated worries and postpone or avoid pregnancy unnecessarily.

MOTHER'S MG

The much higher MG frequency in females than in males during the whole reproductive period strongly indicates that sex hormones play a role in MG pathogenesis. Experimental studies support a role of estrogens and progesterone (9). Thus, both pregnancy, puerperium and lactation would be expected to have the capacity to influence the course of MG. There are several case reports of MG debut during pregnancy, both for AChR- and MuSK antibody-mediated disease. Relative risk of MG onset before, during, and shortly after pregnancy has been calculated in a population-based cohort study combining data from Norway and The Netherlands. 246 women with MG onset at age 15–45 years were included (10). The authors found that the relative risk for onset during pregnancy was not increased. In contrast, this risk increased markedly, with a factor of around five, during the first 6 months postpartum. During the next 6 months, the relative risk normalized. The risk was highest after the first childbirth. Similar results have been reported for other autoimmune disorders such as thyroiditis and rheumatoid arthritis (11). Both hormonal, immunological, and stress mechanisms have been put forward as explanations for MG debut shortly after childbirth.

Established and stable MG can be influenced by pregnancy. Pregnancy is associated with changes in immune and endocrine signaling that can influence autoimmune diseases in general (12). In a series of 69 MG pregnancies, 30% had an exacerbation, 45% had no change, and 25% improved (13). In several similar case series, each with relatively few patients, a deterioration occurred in 35–45% of MG pregnancies (14–18). The rates for exacerbation tended to be higher than for improvement, whereas a substantial proportion remained unchanged. The exacerbations were generally mild to moderate, and myasthenic crisis during pregnancy is rare. Exacerbations occurred more commonly during the first 6 months postpartum than in the pregnancy (17). There were no specific characteristics for the MG patients with exacerbations during pregnancy. Neither previous thymectomy, AChR antibody concentration, nor years since MG debut seemed to be determinants. MG with more severe symptoms before pregnancy usually remained more severe also during this period. More surprisingly, the outcome regarding mother's MG during

previous pregnancies did not predict the development next time. This supports the conclusion that pregnancy by itself represents no major risk factor for MG and that non-pregnancy factors are more important both for short-term and long-term MG outcome. In the postpartum period, however, there is an increased risk for both debut of MG and MG deterioration. Among 27 pregnancies either before or during MG with MuSK antibodies, the pregnancy and puerperium did not precipitate or influence mother's muscle weakness (19).

Symptomatic treatment with the acetylcholine esterase inhibitor pyridostigmine is regarded safe and should be continued during pregnancy (20, 21). The drug does not cross placenta in significant amounts. Optimal pyridostigmine treatment is important for most MG women's general health during pregnancy. Some of the reported MG exacerbations during pregnancy is probably due to dose reduction or withdrawal of effective treatments due to fear for harmful effects for the child. Intravenous injections of acetylcholine esterase inhibitor should be avoided during pregnancy as this can lead to increased uterine contractions.

Mycophenolate mofetil, methotrexate and cyclophosphamide are teratogenic immunosuppressive drugs that should not be given to pregnant women (20–22). These drugs should therefore be avoided for all women in reproductive age, at least if there is any chance for pregnancy. Both prednisone/prednisolone and azathioprine are regarded as safe during pregnancy. These are the most common first-line immunosuppressive drug therapies for MG. Rituximab is increasingly used for moderate and severe MG. This is a monoclonal antibody that crosses the placenta. The drug will bind to B-lymphocytes in the developing child and should therefore be avoided the last months before as well as during pregnancy. Newborns of mothers treated with rituximab have transient B-cell depletion (23). This will normalize after 6 months, but it is not known if such children will experience any long-term immunoregulatory complications. Teratogenicity is not a risk for rituximab. Intravenous immunoglobulin and plasma exchange represent safe treatments during pregnancy. Due to convenience and general safety, intravenous immunoglobulin is often the preferred treatment for an MG exacerbation during pregnancy, and for a stable severe or moderately severe MG condition as well. Thymectomy as MG treatment should not be undertaken during pregnancy.

PREGNANCY IN MG

MG is not expected to influence fertility. There is an overlap with other autoimmune disorders, and with some that may be associated with female infertility. This has been reported for thyroid disease with reduced thyroid function, SLE, and anti-phospholipid syndrome (24). Autoantibodies *per se* do not seem to be associated with infertility. The commonly used drugs in MG should not reduce fertility. Females with MG tend to have fewer children than healthy women, but this can be explained by other reasons than reduced fertility (8).

Pregnancy is for the large majority of MG females uncomplicated, and MG women should be supported when they wish to have children. However, in a cross-sectional study from Germany, one half of the MG females reported that they had abstained from having a child or further children due to their disease (8). The most common cause was fear of adverse drug effects on the child. The knowledge level was generally low among the MG women. Most pregnancy complications occur with a similar frequency with and without MG, including preeclampsia and eclampsia. However, preterm rupture of amniotic membranes shows an increased frequency, and especially in those with MG deterioration during the pregnancy (15, 25, 26).

Spontaneous abortion may occur with a slightly increased frequency in MG. The exact frequency of miscarriages is difficult to know due to small case series reported, and in addition the possibility of selection bias in the reports. Seven miscarriages among 36 pregnancies were found in a French study (18), 10 among 64 in a similar Italian study (14), 4 among 27 in a Turkish cohort (15), and 5 among 35 in Brazil (16). This indicates a rate of around 15%. This is similar to the miscarriage rate in the general population of 10–20% among women who know they are pregnant. A recent study reported a 24% pregnancy loss rate in females with a spectrum of medical disorders on azathioprine and a 50% risk on mycophenolate mofetil (27).

Folic acid supplement is recommended for MG women in the same way as for other women. The standard recommendation is 400 mg daily before and during pregnancy to reduce the risk of birth defects (28).

GIVING BIRTH IN MG

MG women should be advised to give birth by vaginal delivery, similar to women without MG. However, all case series reports show an increased frequency of Cesarean section. In a national and registry-based Norwegian cohort, 17% of MG females had Cesarean section compared to 8.6% in the total population (25). Both elective and emergency sections were increased. Interestingly, the Cesarean section rate was 15% also in females that had no MG diagnosis when giving birth but had developed overt MG at a later delivery (29). In other MG patient series, the Cesarean section rate is much higher, but with similarly increased rates for the general population. In Taiwan, 45% of MG women had Cesarean section, compared to 37.4% of the general population (30). More than 50 countries in the world have Cesarean section rates above 27% for the total population (31). The British guidelines state that Cesarean section in MG should be performed only for obstetric indications (20). These include prolonged labor with an exhausted mother. Interventions with vacuum or forceps are slightly more common in MG, 9% in MG vs. 6% in the general population in the Norwegian cohort (25).

MG women should continue with their standard drug treatment during the last part of pregnancy and during labor. Epidural analgesia is preferable to general anesthesia whenever possible (20), and is performed in the large majority of those with Cesarean section (16). Most anesthetic drugs are, however,

safe in MG. Giving birth at a hospital with experience in neonatal intensive care and with access to a multidisciplinary team involvement by obstetrician, anesthetist, neonatologist, and neurologist is strongly recommended. A protocol with epidural labor analgesia and early use of vacuum extraction for maternal MG has been suggested (32).

NEONATAL MYASTHENIA

Around 10% of the babies of mothers with MG have a transient muscle weakness. This is due to antibodies against AChR or MuSK that are transported from the mother's circulation, across placenta, and to the fetus (21, 33). In the baby, these antibodies may bind to their respective antigens and induce muscle weakness. If present, the weakness will nearly always appear during the first 24 h after birth. As mother's IgG antibodies are broken down in the baby and gradually disappear, the muscle weakness improves, and normal function is achieved (14). The weakness usually lasts for up to 4 weeks but is most pronounced during the first week.

Typical symptoms are some general hypotonia and poor sucking due to reduced muscle strength. Dysphagia and a weak cry are other possible manifestations. Insufficient respiration, aspiration and pneumonia are rare complications, but make neonatal ward observation necessary for these babies.

In a Norwegian nationwide cohort without selection bias, 5 out of 125 MG babies had definite neonatal myasthenia and another 10 were transferred to a neonatal ward (26). Various case series have reported transient neonatal myasthenia in 4/31, 6/27, 2/30, 1/36, and 5/55 mothers with MG (14–18). This sums up to a frequency of around 10%. The different results can probably best be explained by variation in diagnostic sensitivity for neonatal myasthenia.

Neonatal myasthenia can occur in babies of MG mothers with both AChR and MuSK antibodies, but also in patients without detectable muscle antibodies (34). A large proportion of MG patients where no antibodies can be detected by routine assays, still have such antibodies but with low affinity or in low concentration (35). There is no direct correlation between severity of mother's MG and risk for neonatal myasthenia, nor is there a correlation to antibody concentration in the mother. Transport of IgG across placenta shows individual variation and depends also on properties of the antibodies such as IgG subclass. The serum IgG concentrations in mother and child at delivery are similar, illustrating the efficient transplacental transport during the end of the pregnancy. Epitope specificity of the AChR or MuSK antibody is an important determinant for myasthenic disease, and the configuration and antigenicity of AChR differ between mother and her newborn child (26). Neonatal myasthenia in a previous child increases the risk for the condition in the next ones (36). Previous thymectomy seems to reduce the risk for neonatal myasthenia (37).

Most cases of neonatal myasthenia are so mild that no treatment is needed. Very low doses of the acetylcholine esterase drugs pyridostigmine and neostigmine will improve muscle strength (20). Supportive treatment, for example help with breastfeeding, is important. Treatment with intravenous immunoglobulin or plasma exchange is only very rarely needed.

PERSISTENT SEQUELA IN THE CHILD

The great majority of children of MG mothers are healthy and with no persistent muscle weakness or motor disabilities. This is true also for those with transient neonatal myasthenia. IgG transport across placenta does not appear until pregnancy week 13, after the organ-forming period. In pregnancy week 17–22, the IgG concentration in the child is still only 5–10% of that in the mother (38, 39).

Arthrogryposis with skeletal abnormalities and joint contractures is a rare condition but has increased frequency in children of MG mothers (40). Five out of 127 such children (3.9%) in the Norwegian national cohort had such malformations (25). No cases of congenital malformations have been reported in other case series with 26 and 30 children (16, 17). MG in mother does not seem to be a major causative risk factor for arthrogryposis (41). As for neonatal myasthenia, a previous child with arthrogryposis represents a definite risk factor in the next pregnancy (26). Such women should be treated with intravenous immunoglobulin or plasma exchange in all later pregnancies. The cause of arthrogryposis is restricted fetal movements *in utero*. When mother has MG the movement restriction is due to mother's IgG antibodies binding to fetal type AChR with gamma subunits. Arthrogryposis can occur in babies of mothers also with only mild MG. Fetal movements should be monitored as accurately as possible (40) in all women with MG, as there is effective treatment to inhibit arthrogryposis to develop in MG mothers.

In rare, single cases, a permanent muscle weakness has been reported in children of MG mothers (42, 43). This weakness can be generalized or isolated, for example as a facial paresis. This is not a fluctuating condition due to persistent antibodies, but rather a permanent change in the postsynaptic membrane induced by mother's AChR antibodies during fetal life. Such a fetal AChR inactivation syndrome has been reported in eight children from four families (42).

BREASTFEEDING

Breastfeeding should be encouraged for MG mothers (20, 21). This is true both for those with AChR and MuSK antibodies. Maternal lgG levels in milk comprise only 2% of that in serum. In humans, breast milk does not represent a source for immunity transfer from mother to child. Breastfeeding is recommended also for babies with neonatal myasthenia. Being breastfed has many advantages, including a reduced risk for autoimmune disease later in life (44).

Breastfeeding is not known to influence mother's MG. There is an increased risk for worsening of MG in the puerperium, similar to other autoimmune disorders. Boldingh et al. found that debut of MG in the postpartum period was more common in The

Netherlands than in Norway, and they speculated that the much higher frequency of prolonged breastfeeding in Norway might have a protective role (10).

Breastfeeding is advised against in MG mothers with ongoing treatment with cyclophosphamide, mycophenolate mofetil or methotrexate (20). The reason is the teratogenic potential of these drugs. Cyclophosphamide is excreted into breast milk. Maternal treatment with pyridostigmine, prednisolone/prednisone, or azathioprine represents no contraindication for breastfeeding. Transfer of these drugs and their metabolites into breast milk is minimal. Breastfeeding is most probably safe also for treatment with rituximab, cyclosporine, and tacrolimus. The concentration of rituximab in breast milk is 200 times less than in serum (45). Intravenous immunoglobulin or plasma exchange can be used for MG exacerbations in the postpartum period irrespective of breastfeeding or not. Breastfeeding should be encouraged for women on treatment with monoclonal antibodies, and at the same time register outcome (46).

COMORBIDITIES

MG women have an increased frequency of all other autoimmune disorders (47). Such disorders need to be taken into consideration for women before and during pregnancy, both their clinical manifestations and their treatment. In a minority of young women, the MG is caused by a thymoma. Most MG-related thymomas should not influence pregnancy, but in rare cases either thymoma treatment or non-MG thymoma-associated autoimmune disease may be of significance. Infections should always be treated actively in MG patients, and with specific considerations regarding choice of anti-infectious drugs (48).

CONCLUSION

MG women with a child wish should be supported and encouraged. Pregnancy and childbirth have similar complication rates as for the non-MG population. Optimal drug treatment for MG should be continued. Vaginal delivery is recommended, and indications for Cesarean section are obstetrical and the same as for non-MG women. Breastfeeding is safe and should be supported. However, there are a few important warnings. Mycophenolate mofetil, methotrexate and cyclophosphamide should not be given to any females that may become pregnant as these drugs have a teratogenic potential. Rituximab should be stopped some months before a pregnancy. MG women should always give birth at a hospital with intensive care services for the newborn, as 10% of the babies have transient neonatal myasthenia. All babies by MG mothers should be observed in hospital for at least 48 h. Correct information to all females in reproductive age is important. Obstetrical and neurological follow-up during pregnancy makes a difference. Many MG women have exaggerated worries and practice unnecessary limitations or restrictions regarding pregnancy.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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REVIEW
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Novel Treatments in Myasthenia Gravis

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Myasthenia gravis (MG) is the prototypical autoimmune disorder caused by specific autoantibodies at the neuromuscular junction. Broad-based immunotherapies, such as corticosteroids, azathioprine, mycophenolate, tacrolimus, and cyclosporine, have been effective in controlling symptoms of myasthenia. While being effective in a majority of MG patients many of these immunosuppressive agents are associated with long-term side effects, often intolerable for patients, and take several months to be effective. With advances in translational research and drug development capabilities, more directed therapeutic agents that can alter the future of MG treatment have been developed. This review focuses on the aberrant immunological processes in MG, the novel agents that target them along with the clinical evidence for efficacy and safety. These agents include terminal complement C5 inhibitors, Fc receptor inhibitors, B cell depleting agents (anti CD 19 and 20 and B cell activating factor [BAFF)]inhibitors), proteosome inhibitors, T cells and cytokine based therapies (chimeric antigen receptor T [CART-T] cell therapy), autologous stem cell transplantation, and subcutaneous immunoglobulin (SCIG). Most of these new agents have advantages over conventional immunosuppressive treatment (IST) for MG therapy in terms of faster onset of action, favourable side effect profile and the potential for a sustained and long-term remission.

Keywords: myasthenia gravis, treatment, immunotherapy, complement, Fc receptor

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INTRODUCTION

Myasthenia gravis (MG) is the prototypical autoimmune disorder caused by specific autoantibodies at the neuromuscular junction. Broad-based immunotherapies, such as corticosteroids, azathioprine, mycophenolate, tacrolimus, and cyclosporine, have been effective in controlling symptoms of myasthenia (1). Corticosteroids are effective in a majority of MG patients; however, these are associated with many long-term side effects, often intolerable for patients. Traditional steroid-sparing agents have shown mixed efficacy in trials, and usually take several months to be effective. Recently more directed, novel immunotherapies have been developed. These include terminal complement C5 inhibitors and Fc receptor inhibitors (2). These treatments work at different points of the immune pathology and are likely to be complementary in action. FC receptor inhibitors reduce the level of circulating pathogenic autoantibody, whereas terminal complement C5 inhibitors block the formation of the membrane attack complex at the last step of immune injury. This review discusses novel agents that act on other nodal points in MG pathogenesis, autologous stem cell, and chimeric antigen receptor T (CART-T) cell therapy in MG. These new treatments may help reduce the use of steroids, and their relatively fast onset of action makes them attractive options to traditional steroid-sparing agents. These treatments usher in a new era of more focused MG management that promises to improve the lives of people with MG.

OVERVIEW OF MYASTHENIA GRAVIS PATHOGENESIS

MG is an antibody mediated disease in which the immunopathogenesis is T cell driven and there exists a complex interplay between CD4+ T cells and B cells. The normal immune system weeds out autoreactive T cells early and these are destroyed in the thymus by the process called central tolerance. Autoreactive T cells that escape this process or arise *de novo*, are kept in check in the peripheral circulation by a subset of CD4+ cells called Treg cells that bring about apoptosis, anergy or suppression of autoreactive cells (3). These T reg cells which are outsourced from the thymus gland are crucial in maintaining immune tolerance and are found to be functionally deficient in MG. The immunological process in MG begins when immune tolerance is broken by a hitherto unidentified trigger, probably infectious agents, with "molecular mimicry" between the infectious antigen and the acetylcholine receptor(AChR) protein (4). The antigen presenting cells submit the AChR to the CD4+ cells leading to upregulation of proinflammatory cytokines such as interleukins and tumor necrosis factors (5). Also the defect in Treg cells results in upregulation of the proinflammatory CD4+ T cell effector subtypes Th1, Th2, Th17, and these stimulate B cells which proliferate to plasma blasts, plasma cells, and memory B cells (2, 6, 7). The antibodies secreted by the plasma cells in AChR antibody positive MG are mainly of IgG1 and IgG3 subclass (8, 9). These antibodies bring about the pathogenic immune cascade, binding by their fragment binding (Fab) site to the AChR and by the fragment crystallization (Fc) portion to the respective Fc receptors (FcR) expressed in all immunocytes (10). The various FcR subfamilies for IgG are either activating receptors (FcγRI, FcγRIIa/c, FcγRIII) or inhibitory receptors (FcγRIIb). Agents that modulate the function of these receptors are being recognized as novel therapeutic agents in many autoimmune diseases. While germinal centers in the thymus gland are the primary site of anti-AChR producing B cells, later, secondary lymphoid organs in the periphery can take over this function (11). Also, the integrity of the NMJ and effective AChR clustering depend on the effective interaction of other post-synaptic proteins such as muscle specific kinase (MuSK), low density lipoprotein receptor-related protein 4 (LRP4), agrin and rapsyn, amongst others. The binding of agrin to LRP4 results in dimerization of MuSK which is vital for effective AChR clustering in the post-synaptic membrane (5, 12). Antibodies against MuSK and LRP4 have been found to be pathogenic in MG. The pathogenic process is different in muscle specific kinase (MUSK) MG in that the thymus is not involved in the pathology and the MuSK antibody, belonging to IgG4 subclass, does not activate the complement system. This binding of anti-MuSK antibodies masks the site for normal MuSK-LRP4 interaction, thus preventing acetylcholine receptor clustering necessary for normal neuromuscular function (5). The anti-LRP4 antibodies belong to the IgG1 subclass, and, in addition to disrupting LRP4-agrin interactions, also activate the complement pathway leading to damage of the NMJ (12). Knowledge of the various processes involved in the immunopathogenesis of MG has led to identification of potential targets that can selectively inhibit the immune cascade leading to MG.

CONVENTIONAL TREATMENT OF MYASTHENIA GRAVIS

The existing standard of care in the management of myasthenia gravis includes 'broad-spectrum' immunosuppressive treatment (IST) with medications such as corticosteroids, azathioprine, mycophenolate, methotrexate, cyclosporine, tacrolimus, and immunomodulatory treatments such as plasma exchange (PLEX) and intravenous immunoglobulin (IVIG) (1). The mechanisms of action of immunosuppressive agents include activating or suppressing target genes and thereby causing a multitude of changes including suppression of antigen production and reducing circulating T cells (corticosteroids), interfering with T and B cell proliferation by cell cycle arrest (azathioprine, methotrexate, and mycophenolate), inhibition of T cell activation (cyclosporine, tacrolimus), inhibition of antigen presenting cell interaction with T cells and Fc receptor blockade among other actions (IVIG) (13–15). While these treatments are time-tested and remain the commonly used agents for MG, the disadvantages are many such as increased susceptibility to life threatening infections, a wide range of deleterious systemic side effects, delayed onset of action and minimal but definite increased long term risk of malignancy and drug toxicity. With advances in immunology, molecular biology and drug development, newer agents that have more selective immunological targets, spare the rest of the immune system, with lesser toxicity, and more rapid onset of action with possibly sustained remission and cure, are being developed at a rapid pace. **Figure 1** outlines the immune system and potential targets for novel therapies.

NOVEL IMMUNOTHERAPIES FOR MG

Complement Inhibitors in MG

Once the binding of IgG to the AChR epitopes has occurred, it sets in motion the cascades of the classical and common complement pathways. The final steps in the cascade result in formation of C5 convertase, which splits C5 into C5a and C5b. C5b combines with C6-C9 factors to form the membrane attack complex (MAC) which incorporates into the cell membrane resulting in cell damage and lysis (16, 17). This is evidenced by the presence of IgG, C3, and MAC deposits at the neuromuscular junction in affected postsynaptic membrane and also by the low circulating complement levels due to the consumption of these factors, both in affected humans and in animal models (18). The low complement titres correlate with clinical severity and with higher levels of AChR antibodies (19). Moreover, complement knockout mice have significantly lower incidence and severity of experimental autoimmune myasthenia gravis (EAMG) (20). These observations led to studies of several complement inhibitors such as cobra venom factor, soluble complement receptor 1, anti C5 and anti C6 antibodies. All showed improvement in AChR content at the post synaptic

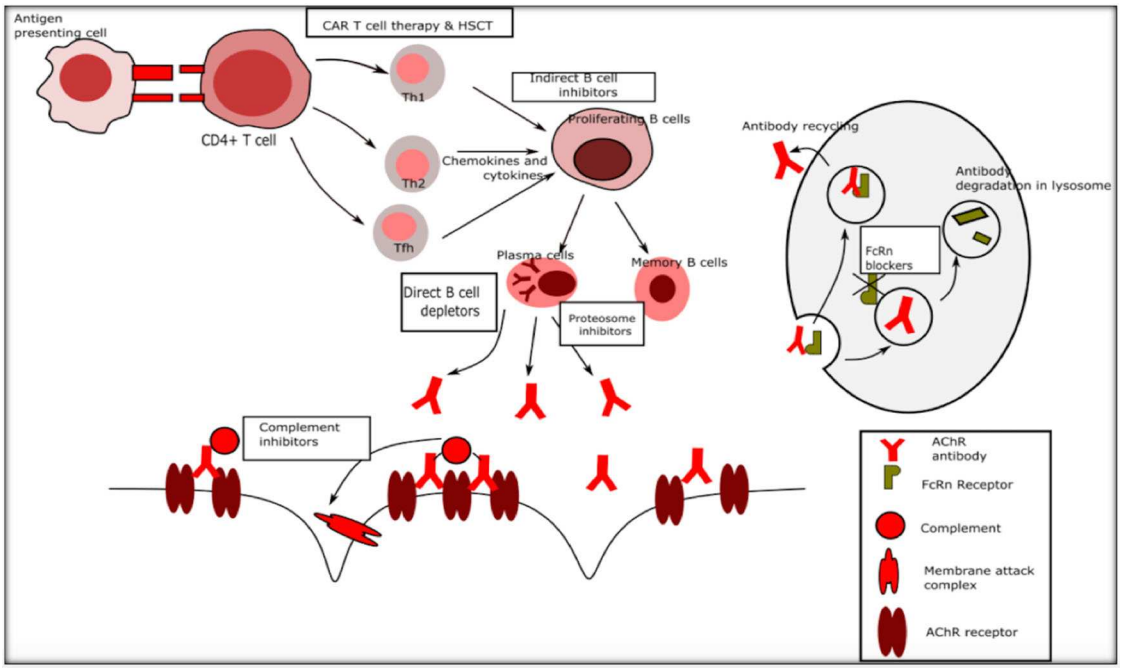

FIGURE 1 | Immune targets for novel therapies in myasthenia gravis. Th1 Type 1 T helper cells, Th2 Type 2 T helper cells.

junction, reduced MAC deposition despite elevated levels of IgG, and a parallel improvement in muscle weakness in animal models (18, 21). Based on this evidence, anti C5 antibodies that inhibit the common complement pathway were developed, but had the disadvantage of increased risk of opportunistic infections leading to further development of molecules such as small interfering RNAs that selectively inhibit the classical complement pathway, the latter being in preclinical development (22). The role of the complement system in seronegative MG (SNMG), and therefore the utility of complement inhibitor therapy in this group of patients, is questionable. However, recent pathological evidence based on samples from external intercostal muscle biopsies from patients with SNMG showed complement deposition at the NMJ, suggesting importance of complement in this subgroup of patients with MG (23). The efficacy of complement inhibitors in SNMG remains to be demonstrated in appropriate clinical trials.

Eculizumab

Eculizumab is a recombinant humanized monoclonal antibody that binds to C5 complement and prevents its cleavage to active C5a and C5b factors and is the first available drug that targets the complement system, specifically C5 (24). It was initially approved by the Food and Drug Administration (FDA) for the treatment of paroxysmal nocturnal hemoglobinuria in 2007 and has since then been approved for use in atypical haemolytic uraemia, generalised MG and neuromyelitis optica (**Table 1**) (40, 41).

A large phase 3 trial (REGAIN) showed major benefits in patients with refractory generalized MG although the response rate was not 100% and most patients required ongoing chronic therapy with other ISTs. The REGAIN study enrolled 125 patients with refractory generalised MG randomized to either intravenous eculizumab or placebo as an add on medication to existing IST treatments, excepting PLEX and IVIG, for 26 weeks,. The dosage schedule was induction with 900 mg on days 0, 7, 14, and 21; 1200 mg at week 4; and then maintenance dosing of 1,200 mg every second week for 26 weeks. The primary efficacy endpoint was the change from baseline to week 26 in total score of MG-Activities of Daily Living (MG-ADL) measured using worst-rank ANCOVA. The trial did not meet the primary outcome efficacy parameter, but multiple secondary end-point measures such as change in MG-ADL, Quantitative MG (QMG) score and MG-Quality of Life (MG-QOL15 scores) showed significant improvement from baseline values in the eculizumab group compared with placebo (42). Eculizumab inhibits complement at the last stage of the immune cycle as noted above, but does not change abnormal antibody production and other potential immune mechanisms underlying MG. The ongoing requirement for other ISTs as observed in the open-label extension study is likely due to the presence of these other immune mechanisms, such as blocking or cross-linking effects of the abnormal antibodies that are unaffected by eculizumab. A major potential risk of eculizumab is that of meningococcal meningitis leading to the need for appropriate immunization prior to the initiation of eculizumab therapy. Hence Neisseria meningitidis vaccination is advised at least 2 weeks before starting treatment and revaccination after 2–5 years (25). If vaccination is not possible before beginning treatment, then prophylactic antibiotics are advised until 2 weeks after vaccination.

The results of the REGAIN trial led to approval for the use of eculizumab in refractory generalized, AChR antibody

TABLE 1 | Novel immune therapies for treatment of myasthenia gravis.

Agent	Action	FDA approval/ Ongoing trials	MG serology and type	Route	Dosage and interval	Main safety concerns	Remarks
Eculizumab	C5 inhibitor	FDA approved	AChR positive gMG	IV	900 mg weekly x 4 weeks, followed by 1,200 mg every alternate weeks	Neisseria meningitis, infections	Vaccinate at least 2 weeks prior (25)
Zilucoplan	C5 inhibitor	Ongoing phase III trial(RAISE) in MG, (26) FDA orphan drug approval	AChR positive gMG	SC	0.3 mg/kg daily	Concerns of meningitis	
Ravulizumab	High affinity C5 inhibitor	Ongoing phase III trial in MG (27)	Unspecified gMG	IV	Weight based 2400–3000 mg every 15 days	Headache	FDA approved for PNH,
Efgartigimod	FcRn blocker	Ongoing phase III trial (28)	AChR positive gMG	IV	10 mg/kg weekly	Headache, reduced monocyte count	
Nipocalimab	High affinity FcRn blocker	Ongoing phase II (29)	Unspecified gMG	IV	Every 2 weeks (multiple doses, under phase II study)		Potentially safe in pregnancy
Rozanolixizumab	High affinity FcRn blocker	Ongoing phase II (30)	AChr or MuSK positive gMG	SC	7 mg/kg once a week	Headache forcing withdrawal,	No increased infection in trials
RVT 1401	FcRn blocker	Ongoing phase II (31)	AChR positive	SC or IV	340/680 mg weekly for 4 weeks followed by 340 mg every 2 weeks	No severe adverse effects	
Rituximab	Anti CD20 antibody	Phase II trial, data unpublished	AChR or MuSK positive gMG	IV	375 mg/m^2 body surface area per week for 4 weeks, repeated after 6 months	Infusion reactions, rare long term risk of PML	Second line option especially in refractor MUSK positive MG
Belimumab	BAFF inhibitor	Phase II trial, no significant benefit to standard of care (32)	AChR or MuSK positive	IV	10 mg/kg at 2–4 weeks interval	Influenza, gastric side effects	No ongoing trials
Bortezomib	Proteosome inhibitor	Phase II trial terminated due to recruitment issues (33)	AChR positive, anecdotal reports in MuSK positive	SC	2 cycles, each consisting of 2 doses of 1.3 mg/m^2 body surface area, at 10 day intervals	Sensory motor polyneuropathy	May require acyclovir and trimethoprim-sulfamethoxazol prophylaxis
CAR T cell therapy	Autologous T cell directed against BCMA	Ongoing phase I and phase II trials (34)	Not specified gMG	IV		Cytokine release syndrome	FDA approved for refractor B cell leukemia and lymphoma
Hematopoetic stem cell transplantation	Ablation of auto-reactive T and Memory B cells	Ongoing Phase II (35)	Ideally in seropositive MG	IV		Complications related to conditioning regime	
SCIG	Broad spectrum immunomodulation	Phase II trials, As efficacious as IVIG, better patient satisfaction (36–38)	AChR or MuSK positive	SC	IVIG equivalent dose weekly divided dose	Injection site reactions	For maintenance treatment
Monarsen	Antisense oligonucleotide against ACHE-R isoform	Phase II trial (2008), Modest improvement (39)	AChR positive	Oral	500 mg/kg	None	No ongoing trials

(AChRAb) positive MG by the FDA, Health Canada, the European Medicines Agency (EMA) and the Pharmaceuticals and Medical Devices Agency (PMDA) in the US, Canada, Europe and Japan respectively. In the open label extension of REGAIN where 117 patients received 1,200 mg every 2 weeks for a median of 22.7 months, there was 1 case of meningococcal meningitis which resolved with antibiotics. Infections occurred in about 19% of patients including infections

with pseudomonas, cytomegalovirus and aspergillus as well as septic shock. A significant reduction in exacerbation rates, MG related hospitalization and rate of rescue therapy was seen in the double blind study and most patients reported global clinical improvement. More than half of the patients achieved minimal manifestation status or pharmacological remission (43). Additional analyses of the data from the REGAIN and the open-label extension studies have confirmed the benefits of eculizumab treatment as more refractory MG patients on eculizumab have minimal symptom expression compared with those on placebo (44). The success of eculizumab has led to the development of other complement inhibitors.

Zilucoplan

Working in a similar fashion to eculizumab, zilucoplan is a synthetic macrolide peptide complement inhibitor that prevents cleavage of C5 complement protein into active C5a and C5b fragments, thus preventing downstream formation of MAC (45). A recent phase II randomized placebo-controlled trial compared two doses of subcutaneous zilucoplan in patients with moderate to severe generalised MG (defined as QMG score $\geq$ 12), and positive AChR antibodies. The higher dose group (0.3 mg/kg daily) achieved significantly lower mean QMG and MG ADL scores (primary end points) and also lower MG composite (MGC) and better MGQOL (secondary end points) at 12 weeks compared to baseline and no patient required rescue therapy. There were no serious side effects reported and minor side effects included injection site reactions (46). A phase III study is currently under way to study the safety, efficacy and tolerability of zilucoplan in AChRab positive patients with moderate to severe generalised MG (26).

Ravulizumab

Ravulizumab, another humanized monoclonal antibody, is a novel C5 complement inhibitor which differs from eculizumab by aminoacid substitutions in the Fc region of eculizumab that provide a high affinity for C5 and immediate and sustained reduction in C5 (47). This modification confers a longer half life of the antibody due to recycling through the FcRn pathway. As a result, patients can be given ravulizumab every 8 weeks, an advantage over eculizumab which is administered biweekly. Phase 3 trials have shown the outcome of ravulizamb to be non-inferior to eculizumab in PNH and this medication has been approved by the FDA for the treatment of PNH (48, 49). A phase III randomized placebo-controlled multicentre study to evaluate the safety and efficacy of ravulizumab administered once every 15 days in generalized MG is underway. The serological status was not specified (27).

Fc Receptor Inhibitors

Among the Fc receptors, neonatal FcR (FcRn) play a pivotal role in maintaining IgG homeostasis and are recognized as a treatment target in myasthenia. While initially recognized as the mediators of passive transfer of immunity from mother to fetus, their role in protecting IgG from lysosomal degradation and prolonging the half-life of immunoglobulins, has been recognized subsequently (50). By blocking the FcRn receptor, the recycling of IgG is reduced because IgG is being degraded in lysosomes. Since production of IgG does not compensate for this decrease, FcRn receptor blockade causes a rapid fall in all IgG subclasses (50, 51). In rat models of MG, treatment with anti-FcRn-antibody showed significant reduction in severity of symptoms and lowering of total and anti-AChR IgG levels providing pre-clinical proof of concept (52).

Efgartigimod

Efgartigimod is a mutated human IgG1 Fc portion with increased affinity for FcRn at both physiological and acidic pH, whereas regular IgG-FcRn binding occurs strictly in acidic pH (53). In healthy volunteers, a single dose of 50 mg/kg reduced the total IgG by about 50% and multiple doses further reduced IgG levels by a total of 75%, with return to near baseline levels after ∼8 weeks (54). The study subjects had only minor adverse effects such as headache and chills at higher doses. In a phase II study of 24 patients with generalized, AChRab positive MG, on stable doses of standard treatment, randomized to IV efgartigimod (maximum dose of 1200 mg per infusion) or placebo for 3 weeks showed safety and tolerability of efgartigimod. The most common adverse events were headache and reduced monocyte counts which were minor. A rapid reduction in all IgG subclass levels was observed in the first week after treatment, and further decreases to a total 70% reduction from baseline levels with subsequent doses. There was a gradual but incomplete return to baseline levels (20% reduction) at 8 weeks after treatment, with parallel changes in AChRab levels. Interestingly, there were improvements in all the MG scales used in the study, and these mirrored the fall in IgG levels, but persisted even after the IgG levels had increased close to baseline levels (55). A phase III study is currently underway to evaluate the efficacy and safety of efgartigimod 10 mg/kg per week for 26 weeks in patients with generalised AChRab positive MG (28).

Nipocalimab

Nipocalimab (M281) is a fully humanized monoclonal IgG1 anti-FcRn antibody which binds with extremely high affinity to FcRn both at endosomal and extracellular pH blocking the binding of IgG to FcRn. It occupies the FcRn receptor throughout the cell cycle and has high specificity, minimizing off-target effects, and is unlikely to cross the placenta (53). A phase I placebo controlled study in 50 subjects examined both single (at 0.3, 3, 10, 30, and 60 mg/kg) and multiple ascending doses (four weekly doses of 15 or 30 mg/kg). Nipocalimab achieved rapid FcRn receptor occupancy and up to 80% reduction in IgG levels with 30 or 60 mg/kg doses and 50% reduction persisting for 18 and 27 days respectively for 30 or 60 mg/kg doses. There were no severe or serious adverse effects or increased risk of infections (56). A phase II trial is underway in AChRab or MuSK positive generalised MG exploring the safety and efficacy of nipocalimumab (29). An added advantage is its probable safety profile in pregnant women, and a clinical trial is now underway in pregnant women at high risk for severe haemolytic disease of the fetus and newborn (57). Because women of reproductive age constitute a large proportion of early-onset MG cases (58), interventions compatible with pregnancy are much needed.

Rozanolixizumab

Rozanolixizumab is a humanized high affinity anti FcRn monoclonal IgG4 antibody. A four week study in cynomologus monkeys showed marked decrease (75–90%) in IgG concentrations with 50 and 150 mg/kg doses every 3 days for 4 weeks, with maximum effect by day 10. There were no safety concerns or increased infections (59). In a phase I randomized placebo controlled study to evaluate safety, healthy subjects were randomized to single infusion of intravenous or subcutaneous doses of 1, 4, or 7 mg/kg of razonolixizumab. The most common adverse events were headache (38.9%), vomiting (25%), nausea (19.4%), and pyrexia (19.4%), all occurring more frequently with intravenous administration compared to subcutaneous treatment. The reduction in IgG concentration peaked at 7–10 days and gradually returned to baseline by day 57 (59). In a subsequent phase II trial, 43 patients with AChR or MuSK positive generalised MG were randomized to 3 weekly subcutaneous infusions of placebo or rozanolixizumab, and then 4 weeks later, were re-randomized to 3 weekly doses of either 4 or 7 mg/kg. Standard of care MG treatments were stable during the study. The study showed clinical benefits across several endpoints, including QMG, MGC and MG-ADL scores as well as marked reduction of total IgG and AChRab levels. There was a greater frequency of headache (57.1%) compared to placebo (13.6%) and three patients withdrew from the study due to headache (53, 60). A 240 patient, phase 3, parallel design, randomized, double-blind, placebo-controlled, multi-centre clinical study of rozanolixizumab is ongoing currently (30).

RVT 1401

RVT 1401 is a fully humanized monoclonal FcRn antibody for subcutaneous or intravenous injection. Limited information from an unpublished phase 1 trial on healthy volunteers report that a single, subcutaneous, 765 mg dose of RVT 1401 reduced IgG by 47% with further reduction after continued weekly injections. All adverse events were mild to moderate in severity, with no subjects requiring premature discontinuation due to AEs (61). A phase II trial comparing weekly subcutaneous 680 and 340 mg RVT 1401 doses to placebo in patients with AChR antibody positive MG is in progress. The study also has an open label extension arm with 340 mg every 2 weeks for 6 weeks (31).

These studies of complement inhibitors and FcRn inhibitors are not without certain limitations. None of these trials have included seronegative MG patients though this group of patients may resemble antibody positive patients in response to immune therapies (62, 63). Longer treatment durations are necessary to confirm the long term efficacy and potential adverse effect of these agents. Since the patients in these trials were continued on stable doses of standard agents, the utility of these newer agents in crisis and the timing of their introduction into the care regimen remain uncertain. FcRn inhibition also has the potential to alter serum levels of therapeutic monoclonal antibodies and the pharmacokinetic interactions among these agents remain unexplored (64).

B Cell Depleting Agents

As MG is primarily mediated by humoral mechanism, B cells play a central role in MG pathology.

Role of B Cells in MG

Under the influence of the Tfh subset of CD4 T cells and with regulatory Tfr CD4 T cells being defective, B cells differentiate into memory B cells, plasmablasts and plasma cells in the thymic germinal centers (65). The plasma cells are the terminal differentiated effector B cells and along with plasmablasts secrete the pathogenic antibodies. The various plasma cell populations differ in their phenotypes in the expression of cell surface molecules, for example, CD20 which are less expressed in fully mature plasma cells and memory cells (66). In addition to their main pathogenic role in autoantibody generation, B cells also serve as efficient antigen-presenting cells to T cells and, by this means, trigger there activation and proinflammatory upregulation (67). The germinal centres of the thymus provide an ideal environment for differentiation and proliferation for autoreactive B cells (11). There are a number of molecular and cellular factors that influence this proliferation among which BAFF deserves special mention. Both normal and more importantly autoreactive B cells are very much dependent on BAFF for their survival and maturation (68). The beneficial effect of thymectomy is explained by the removal of the thymus associated germinal centers (69). However, after thymectomy the antibody levels do not disappear completely disappear from the serum. This may due to persisting memory B cells and long lived plasma cells which can be localized in secondary lymphoid organs and can replenish short lived plasma cells that secrete antibodies. The concept that long-lived plasma cells are not affected by IST drugs such as corticosteroids or cyclophosphamide, or by B cell depletion, has identified them as a novel target cell requiring specific therapeutic approaches (66). There are various steps at which B cells can be targeted either directly or indirectly.

DIRECT B CELL DEPLETORS

Rituximab (RTX)

RTX has gained popularity in recent times and has been employed for MG in many centers across the world. This is despite that most of the data for RTX in myasthenia comes from single centre experiences and case series and its use remains an off-label treatment for myasthenia.

RTX was developed in the 2000s for cancer and other autoimmune disorders and is a murine-human chimeric anti-CD20 glycoprotein monoclonal antibody. CD20 is a transmembrane protein expressed by B cells, but not by long-lived plasma cells and plasmablasts. It can induce killing of CD20+ cells via multiple mechanisms. The direct effects of RTX include complement-mediated cytotoxicity and antibody-dependent cell-mediated cytotoxicity, and the indirect effects include structural changes, apoptosis, and sensitization of cancer cells to chemotherapy. RTX also increases Treg cells which favourably influences MG immunology. A systematic review of the efficacy and safety of RTX in MG (99 patients AChRab

positive, 57 patients MuSK positive) showed that MG Foundation of America (MGFA) minimal manifestation status, or better, was achieved in 44% of patients, and combined pharmacological and complete medical remission was observed in 27%. MuSK positive patients had better response than AChRAb positive patients, with 72% of MuSK patients achieving minimal manifestation status or remission, compared to 30% of AChR positive patients. Relapses were also less frequent in MuSK MG. Other predictors of a positive response were younger age of onset and milder disease. Reduction in antibody titers was not correlated with clinical response to RTX in AChR or MuSK patients (70). A multi-centre, retrospective study of RTX in MuSK positive MG, also showed that individuals who received RTX had better outcomes than those on standard treatments (71). A recent, retrospective nationwide study from Austria showed that, at a median follow-up of 20 months, MG patients (70% AChRab positive, 25% MuSK positive) treated with rituximab achieved remission in about 43% and minimal manifestations in 25% (70). Remission was more frequent in MuSK positive patients than in AChRab positive patients (71 vs. 36%) (72). Another recent retrospective review from Stockholm showed that rituximab shortened the time to remission and the need for additional immunosuppressive therapies in patients with new-onset MG treated within 12 months after diagnosis, compared to the longer time for remission in those with refractory disease (73). The time to remission was shorter with rituximab compared to those who received other immunosuppressive therapy. A recent randomized controlled trial, compared RTX to placebo as add-on treatment in patients with AChRAb positive MG (the BEAT-MG study) (74). Patients were required to be on prednisone $\geq$ 15 mg/day with or without additional ISTs, and they received RTX or placebo every 6 months for 2 cycles, with final follow up at 52 weeks. At the end of the study, RTX did not have a corticosteroid-sparing effect compared to placebo; additionally there were no significant differences in outcomes of disease severity. However, the baseline scores on different outcome measures were relatively low, so it is possible that the population selected was too mildly affected to show significant change (75). While the full BEAT-MG results are currently unpublished, there may be other reasons for the negative results such as the potential development of human antichimeric antibody (HACA) against RTX. Also, since long lived plasma cells lacking CD20 are not targeted by RTX, any clinical benefits may be transient and would require chronic infusions—beyond the 2 cycles in the study— to maintain the effects (76).

Despite the lack of robust evidence, RTX is the second-line drug for treatment of MG in some areas of the world (2). The evidence for efficacy in MuSK MG is more robust, although randomized controlled trials are lacking. Since patients with MuSK MG tend to have refractory disease RTX has been proposed as first line of treatment in this population (2, 71). Although RTX may be safe for long-term use in MG, there is a risk, although low, of progressive multifocal leukoencephalopathy with this treatment, so its use needs to be cautious (77).

Next Generation Anti-CD-19 and Anti-CD-20 Biologicals

Next generation anti-CD20 and anti-CD19 biologicals have been considered as possible treatments for MG. Most of the second generation anti-CD20 agents such as ocrelizumab, ofatumumab, obinutuzumab, veltuzumab, and ofatumumab have the advantage of being fully-humanized and thus may be better tolerated and more efficacious in haematological malignancies and autoimmune disorders (78, 79). Ofatumumab showed sustained remission in a patient with refractory MG who had previously responded to RTX but developed hypersensitivity reactions to repeated RTX infusion (80). Given the lack of phase III studies, there is insufficient data to recommend these newer agents for use in MG at present.

Anti CD19 agents offer several advantages over anti CD20 agents. CD19 is a B cell marker that is expressed much earlier than CD20 and, as a result, may be a better target and might possibly act synergistically with anti CD20 agents. The most promising anti CD19 agents are blinatumomab, SAR3419 and MEDI-551 which are currently in phase II studies in haematological malignancies (79).

INDIRECT B CELL INHIBITORS

Belimumab

Belimumab (Benlysta, Rockville, MD), is a human immunoglobulin (Ig) G1λ monoclonal antibody against B-lymphocyte stimulator (BLyS) also called BAFF. Elevated BAFF levels have been identified in patients with MG, highlighting it as a potential treatment target (81).

BAFF belongs to the tumor necrosis factor (TNF) superfamily and is a costimulator for B-cell survival and function. The binding of BAFF to B cell receptor promotes the survival of the autoantibody-producing B cells by preventing their apoptosis. Transgenic mice overexpressing BAFF have excessive numbers of mature B cells and autoantibodies as well as an overall increased autoimmune response while BAFF deficient animals have marked reduction in B cells and hypogammaglobulinemia (82, 83). Belimumab has been approved by the FDA for the treatment of lupus (84). However, in a phase II randomized, placebo controlled trial in AChRab positive generalised MG, patients on belimumab did not have a significant difference in QMG scores or MG-ADL at week 24 compared to patients who were on placebo (32). While this might be due to a lack of effect of belimumab in MG, other potential reasons for the negative results include: a population of stable patients with mild disease, leading to floor effect of the MG scales, and exclusion of MuSK positive patients.

Proteosome Inhibitors

The immune system contains long-lived memory plasma cells which are terminally differentiated B cells that have lost cell surface markers and are as a result resistant to most agents such as RTX. These plasma cells reside in niches and form sentinels of adaptive immunity (85). Such plasma cells have not been targeted and may be responsible for treatment resistance in autoimmune diseases. Given the high rate of immunoglobulin

synthesis, these plasma cells are sites of high protein turn over. Many of these cellular proteins need effective degradation and removal for cellular homeostasis. Proteosomes are hollow, cylindrical protein structures which are an integral part of the ubiquitin-proteosome pathway that plays a major role in clearing intracellular proteins (86). Inhibition of proteosomes causes accumulation of misfolded proteins and apoptosis of highly active plasma cells and this therapy is employed in treatment of multiple myeloma (87). In EAMG animals, bortezomib efficiently reduced the rise of AChRab titers, prevented ultrastructural damage of the postsynaptic membrane, improved neuromuscular transmission, and decreased myasthenic symptoms (88). An open label trial to investigate the use of bortezomib in treatment of resistant autoimmune diseases including MG, SLE and RA was terminated early due to recruitment issues (33, 89). Bortezomib was tried in a patient with resistant MuSK positive MG with moderate improvement, but the patient had received RTX nineteen days before the initiation of bortezomib, a major confounder (90). Although bortezomib may be promising in MG, further studies are needed. A limiting factor is the potential for development of sensory neuropathy observed in 30–40% of those treated with bortezomib, and this neuropathy is disabling and permanent in some patients (87). More selective inhibition of the proteosome subunit, called the immuneproteosome which may have less neurotoxicity, is in preclinical development. ONX 0914, an immuneproteosome inhibitor, reduced the severity of EAMG through varied mechanisms including reduction of autoantibody affinity, and reduction of Tfh cells and antigen presenting cells, but additional studies are required prior to clinical use (91).

T Cells and Cytokine Based Treatment in Myasthenia

With Treg cell dysfunction and Th1, Th2, and Tfh over action being major factors in MG pathogenesis, agents that target T cells, promoting regulation or inhibition, may be attractive options for MG treatment. Given that Th1, Th2, and Tfh cells act through various cytokines to induce B cell proliferation and differentiation into plasma cells, drugs designed to inhibit cytokines are also attractive treatment options (21). Animal models with inborn deficiencies of cytokines and those treated with cytokine inhibitors of IL1, IL6, and TNF, were resistant to EAMG (22, 92, 93). Several monoclonal antibodies have been developed to target Th cells or cytokine pathways. These include secukinumab (inhibits IL 17A), rontalizumab (inhibits INFalpha pathway), and tocilizumab (inhibits IL6 pathway) (6). Many of these agents have been approved for treatment of psoriasis and psoriatic arthritis (6). Tocilizumab has been reported to be beneficial in patients with refractory MG, one of whom failed to benefit with RTX (94). At present, none of these agents are being studied in MG clinical trials.

CHIMERIC ANTIGEN RECEPTOR—T (CAR-T) CELL THERAPY

The concept of adaptive T cell immunity had been evolving in cancer therapy, The concept is to treat patients with advanced cancer using their own T cells which have been harvested, manipulated *ex-vivo*, expanded and then re-infused. The presumed, increased effectiveness of a patient's own T cells against the malignancy is thought to occur by redirecting the native T cells against selected antigens expressed only by the tumor cells. The CAR-T cells are genetically engineered and expanded autologous T cells that are infused into the patient and recognize tumor cell antigens, and thus bring about tumor cell destruction (95). The major adverse effect of this therapy is the cytokine releasing syndrome (CRS) which can range from mild constitutional symptoms to severe CRS leading to multi-organ dysfunction (96). CAR-T cell therapy has received FDA approval for the treatment of refractory B cell acute lymphocytic leukemia, B cell lymphoma, and non-Hodgkin lymphoma, but is likely to have wider application in hemato-oncology (97). Applying these principles to treatment of autoimmune disorders, chimeric autoantibody receptor T (CAAR-T) cells have been developed to target autoreactive B cells secreting autoantibodies. Pre-clinical studies have found efficacy in various animal models of autoimmune disorders including autoimmune encephalomyelitis, lupus and pemphigus (98). Thus CAR T cell therapy offers a novel and attractive treatment opportunity in MG. Currently phase I and phase II trials are underway using CD8 positive CAR T therapy directed against plasma cells that express B-cell maturation antigen (BCMA) (34).

HEMATOPOETIC STEM CELL TRANSPLANT (HSCT)

The data for the use of HSCT in various refractory immune mediated neurological disorders have been accumulating over the past two decades, most notably for multiple sclerosis (99, 100). Autologous stem cell transplantation has the advantage over allogenic transplantation in having lesser risk for graft vs. host disease. The basic mechanism of action of HSCT is ablation of all existing autoreactive T cells and B cells, including memory cells and long-living plasma cells, during the conditioning phase using cytotoxic therapies or radiation, depending on the conditioning regime (101). The subsequent autologous hematopoetic transplantation helps in recovery from the post-conditioning aplasia and enhances immunotolerance by increasing regulatory T cells, reducing autoantibodies and rejuvenating thymic function (102, 103).

A retrospective case series of seven patients with severe refractory MG treated with HSCT showed that all patients were in complete stable remission at the median follow-up time of 40 months. At 8 months after HSCT, all patients had discontinued ISTs (104). An intensive conditioning regimen was employed in all patients but acute complications were transient and none of the patients required ICU care. A subsequent systematic review of HSCT therapy showed that 2.2% of all articles were in MG, 29.4% in graft versus host disease and 19.8% in multiple sclerosis (105). With better and safer induction regimens, HSCT may be a reasonable treatment option in severe refractory MG in the future. However, factors to consider in assessing these reports are

whether these patients had received adequate trials with other immunosuppressants prior to transplant, and whether using only high dose cyclophosphamide induction, without transplant, would have induced sustained remission (106). A phase II trial to evaluate the safety and efficacy of high dose chemotherapy in autoimmune neurological disorders, including MG, is in progress (35).

SUBCUTANEOUS IMMUNOGLOBULIN (SCIG)

IVIG is a useful treatment option when a rapid response is required in worsening or poorly controlled MG. While there is class I evidence for the short term use of IVIG in acute worsening or myasthenic crisis, data for maintenance therapy is less robust, and is restricted to class III evidence (107–109). Immunoglobulins(Ig) have broad-spectrum immunomodulatory actions and exert their influence by a number of B-cell, T-cell, complement and Fc receptor modifying actions (110). However, some of these same actions and other factors such as increased blood viscosity, rapid exposure to high foreign protein load and rapid intravenous volume expansion lead to the frequent adverse effects of IVIG ranging from 2.5 to 87.5% with repeated infusions (111). Subcutaneously administered immunoglobulin (SCIG) has advantages over IVIG since the slow and sustained intravascular absorption avoids the abrupt vascular volume load, and subcutaneous administration eliminates the need for intravascular access. Many patients report improved quality of life (QOL) with greater freedom, control, and independence in their treatment with immunoglobulin (112). With these attractive advantages, SCIG was used initially in primary immunodeficiency disorders and was as effective as IVIG in preventing infections with a lower incidence of serious adverse events (113). The utility of SCIG as maintenance therapy for MG was examined in a retrospective case series of 9 patients. At a mean follow-up period of about 7 months, all had stable or improved MGFA status, significant improvement in MG-ADL, MG-QOL and the visual analogue scale (VAS) for patient satisfaction (36). The efficacy, safety and tolerability of SCIG in 22 seropositive MG patients was assessed in a multicentre North American open label trial (37). After a 10 week screening period with periodic IVIG treatments, stable patients were transitioned to weekly SCIG for 12 weeks. The study showed improved scores in the QMG, manual muscle testing (MMT), and MGC with high patient satisfaction and no serious adverse effects. The treatment success rate at 12 weeks was 85% (37, 38). Thus SCIG offers a novel, efficacious and patient-friendly alternative to IVIG in maintenance therapy for MG, although it has not been tested for acute management of MG. Additionally, its corticosteroid-sparing effects have not been established.

OTHER NONIMMUNE TREATMENTS

Antisense Oligonucleotide Treatment Against Acetylcholinesterase

While acetylcholinesterase inhibitors (ACHEI) were the first agents to be tried in MG and provided symptomatic improvement, the focus of attention has shifted mainly to treating the primary aberrant immunological processes of MG. However, the use of an antisense oligonucleotide which hybridizes with ACHE mRNA may be a therapeutic option. Splicing of the ACHE gene normally produces different ACHE isoforms, the predominant one being the ACHE-S isoform in physiological condition. Acute exposure to anticholinesterases shifts the splicing of the AChE pre-mRNA to the normally rare, AChE-R variant (114). The increase in AChE-R levels enhances ACh hydrolysis and restores the balance between the ACh and AChE levels. The antisense oligonucleotide EN101, or Monarsen, targets exon 2 of the AChE mRNA and results in AChE-R mRNA being more susceptible to destruction which decreases its activity, and hence maintains levels of acetylcholine in the synaptic cleft (115). Monarsen, intravenous and oral, reduces AChE-R levels in EAMG rat muscle and plasma and enhances task performance. Initial phase 2a studies in MG patients showed modest improvement in QMG scores and that the treatment was safe and well tolerated (39). Additional studies of Monarsen are not underway at this time.

CONCLUSION

The availability of more focused immune therapies provides greater treatment options for both patients and treating physicians in the management of MG. A favourable benefit-side effect profile and more rapid onset of action are advantages over current ISTs. However, the long term efficacy and safety of novel treatments are yet to be understood fully. Furthermore, the high and sometimes prohibitive cost of many novel agents prevents access for many patients particularly those in developing countries. Given the wide range of treatment options for MG, cost becomes an important factor, and less expensive agents may be considered preferable in many cases. Health economic studies are necessary to understand the cost-effectiveness of novel treatments compared with traditional alternatives. More data is required to develop greater patient and physician confidence in these agents before wide scale use.

AUTHOR CONTRIBUTIONS

DM performed the literature search, wrote and edited the manuscript. CB wrote and edited the manuscript. VB planned the review, supervised DM in his review, wrote and edited the manuscript.

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REVIEW
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A Practical Approach to Managing Patients With Myasthenia Gravis—Opinions and a Review of the Literature

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When the diagnosis of myasthenia gravis (MG) has been secured, the aim of management should be prompt symptom control and the induction of remission or minimal manifestations. Symptom control, with acetylcholinesterase inhibitors such as pyridostigmine, is commonly employed. This may be sufficient in mild disease. There is no single universally accepted treatment regimen. Corticosteroids are the mainstay of immunosuppressive treatment in patients with more than mild MG to induce remission. Immunosuppressive therapies, such as azathioprine are prescribed in addition to but sometimes instead of corticosteroids when background comorbidities preclude or restrict the use of steroids. Rituximab has a role in refractory MG, while plasmapheresis and immunoglobulin therapy are commonly prescribed to treat MG crisis and in some cases of refractory MG. Data from the MGTX trial showed clear evidence that thymectomy is beneficial in patients with acetylcholine receptor (AChR) antibody positive generalized MG, up to the age of 65 years. Minimally invasive thymectomy surgery including robotic-assisted thymectomy surgery has further revolutionized thymectomy and the management of MG. Ocular MG is not life-threatening but can be significantly disabling when diplopia is persistent. There is evidence to support early treatment with corticosteroids when ocular motility is abnormal and fails to respond to symptomatic treatment. Treatment needs to be individualized in the older age-group depending on specific comorbidities. In the younger age-groups, particularly in women, consideration must be given to the potential teratogenicity of certain therapies. Novel therapies are being developed and trialed, including ones that inhibit complement-induced immunological pathways or interfere with antibody-recycling pathways. Fatigue is common in MG and should be duly identified from fatigable weakness and managed with a combination of physical therapy with or without psychological support. MG patients may also develop dysfunctional breathing and the necessary respiratory physiotherapy techniques need to be implemented to alleviate the patient's symptoms of dyspnoea. In this review, we discuss various facets of myasthenia management in adults with ocular

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and generalized disease, including some practical approaches and our personal opinions based on our experience.

Keywords: ocular myasthenia, generalized myasthenia, refractory myasthenia, thymectomy, myasthenic crisis, fatigue, dysfunctional breathing

INTRODUCTION

Myasthenia gravis (MG) is a rare acquired autoimmune disorder of the neuromuscular junction (NMJ), caused by antibodies that target the post-synaptic membrane (1). These antibodies commonly are to the nicotinic acetylcholine receptor (AChR) but in a smaller proportion of cases, antibodies to muscle specific tyrosine kinase (MuSK) or to lipoprotein receptor-related protein 4 (Lrp-4) can be present instead (1–3). In an even smaller cohort of MG patients, no antibodies are detected on conventional antibody assay testing and we refer to these patients as "seronegative." Patients with MG typically present with fatigable muscle weakness. They commonly present first with ocular manifestations such as asymmetrical fatigable ptosis with or without blurred or double vision. The majority, however, evolve further into generalized muscle weakness involving the facial and bulbar muscles, the neck and axial muscles and the limbs, with the upper limbs often being more severely affected than the lower limbs. In myasthenic crisis, the severe end of the disease spectrum, there is neuromuscular dysphagia rapidly evolving into complete loss of swallow function, and often in association with respiratory muscle weakness and type 2 respiratory failure. This is a clinical emergency that requires management in an intensive care setting. Therapies in the field of MG have significantly advanced over the years. Now, more than ever, the treating physician must carefully contemplate which treatments are best suited for an individual MG patient since the "one size fits all" approach may not be as relevant. There are specific clinical scenarios where one must be extra cautious, for instance the newly diagnosed young female patient, who may be imminently planning a pregnancy, in contrast to a newly diagnosed elderly patient with multiple comorbidities. This review discusses the literature with some emphasis on our practice based over a time-span of over a decade where we have treated an excess of 900 MG patients.

PHARMACOLOGICAL THERAPIES IN GENERALIZED MG

Medical therapies are used in MG patients for either direct alleviation of symptoms, or as immunomodulatory drugs with the aim of dampening the underlying immunopathology causing the disease. The aim of treatment is to induce remission (pharmacological in the majority or complete stable remission which is rarely achieved) or minimal manifestations (MM). The Myasthenia gravis Foundation of America (MGFA) post-intervention status (PIS) (4) defines MM in a patient who has no symptoms or functional limitations from MG but has some weakness on examination of some muscles. There are four different categories of MM depending on whether the patient is receiving treatment and if this includes immunosuppression and/or symptomatic treatment (for example pyridostigmine as will be discussed below). This contrasts to complete stable remission (CSR) where the patient has no symptoms of MG and no weakness (excluding residual weakness of eye closure) and has received no therapy for a minimum period of 1 year, and pharmacological remission (PR) which is the same as CSR but the patient would have received some therapy for MG excluding symptomatic treatment.

SYMPTOMATIC THERAPIES

Pyridostigmine is by far the most commonly used symptomatic therapy. This is an acetylcholinesterase inhibitor which blocks the degradation of acetylcholine at peripheral cholinergic synapses, including the neuromuscular junction (NMJ). Originally, physostigmine and prostigmine (neostigmine) were identified by Mary Broadfoot Walker, a physician in Scotland in the late 1880s, as drugs that temporarily improved muscle strength in patients with MG (5). These drugs work by prolonging the action of any acetylcholine released into the synaptic cleft and compensates for the structural and functional deficits in NMJ transmission that characterizes MG. In early or mild disease pyridostigmine allows significant and rapid improvement in muscle strength (6, 7). However, with longstanding or severe disease this pharmacological compensation may be insufficient and there may be minimal clinical effect. Peak blood levels of pyridostigmine occur 1.5–3 h after oral intake but significant clinical effect occurs within 30 min. Dosing 4–5 times per day leads to very stable blood levels. Renal impairment leads to reduced clearance of pyridostigmine and doses must be adjusted.

Patients are usually prescribed doses of 180–240 mg daily but patients may require up to 480 mg daily. Although generally well-tolerated, side effects from pyridostigmine are very common, are usually dose dependent, and can be debilitating necessitating reduction of dose or slower titration. Most side effects arise from the action of pyridostigmine at non-NMJ muscarinic peripheral synapses and include, gastrointestinal disturbance (abdominal cramps, bloating, diarrhea, frequency, nausea), urinary frequency, hypotension, bradycardia, sweating, salivation, lacrimation, increased bronchial secretions, and

Abbreviations: AChR, Acetylcholine receptor; C5, Complement component 5; CSR, complete stable remission; DM1, myotonic dystrophy type 1; EFT, early fast-acting treatment; FSHD, Facioscapulohumeral muscular dystrophy; IvIG, intravenous immunoglobulin; MG, Myasthenia gravis; MGFA, Myasthenia Gravis Foundation of America; MGTX, thymectomy trial in non-thymomatous MG patients; MM, minimal manifestations; MMF, mycophenolate mofetil; MuSK, Muscle specific tyrosine kinase; NIV, non-invasive ventilation; PE, plasma exchange/plasmapheresis; PIS, post-intervention status; PR, pharmacological remission; RATS, robotic assisted thymectomy surgery; VATS, Video-assisted thoracoscopic surgery.

other symptoms of cholinergic excess. Some elderly patients can be extremely sensitive to the cardiac side effects and have experienced syncope even with low doses of pyridostigmine. Some asthmatic patients may show increased sensitivity and experience increased bronchospasm with pyridostigmine. At high doses side effects can be severe, and lead to the entity of "cholinergic crisis," where neuromuscular weakness worsens along with the above symptoms leading to bulbar or respiratory crisis from drug excess rather than worsening MG (8). Such extreme manifestations are uncommon but it is very frequent for patients to have gastrointestinal symptoms on starting or increasing doses. These tend to lessen within a few days but can persist in some. Propantheline is an antimuscarinic agent that counteracts many of the cholinergic side effects of pyridostigmine without reducing its action at the NMJ. It can be very effective at reducing the side effects of pyridostigmine if given ∼15 min beforehand. Loperamide can alternatively be prescribed but is not as effective at reducing the other muscarinic side effects. When patients fail to respond to pyridostigmine, the physician should be cautious about increasing the dose particularly in dysphagic patients, since pyridostimgine will increase salivary secretions and exacerbate their swallowing difficulties.

Neostigmine is an alternative acetylcholine esterase inhibitor that can be used in MG (9, 10). This should only be given via the subcutaneous route in MG and not intravenously. It is useful in patients with MG who cannot absorb via the oral route (e.g., a MG patient with acute bowel obstruction) but should not be first line if the patient has impaired swallow. Swallowing difficulties are very common in patients with MG and if there are concerns about aspiration with oral intake, including medications, the first strategy should always be to place a nasogastric tube and administer pyridostigmine via this. Only if this cannot be undertaken should subcutaneous neostigmine be used. It has the same side effect profile as pyridostigmine albeit with more marked cardioinhibitory effects and a shorter half-life leading to more frequent dosing. However, neostigmine should always be used with caution since it may cause excessive salivary secretions and as a result may further negatively impact and exacerbate swallowing difficulties.

Experimental models of AChR deficiency show how oral ß-2 adrenergic receptor agonists such as salbutamol enhance function of the NMJ (11). Oral salbutamol can rarely be of clinical utility in mild autoimmune MG disease too especially where the patient has not tolerated pyridostigmine. We have used successfully in a couple of patients. Side effects commonly include tachycardia, tremor and a sense of anxiety and these can be limiting factors. MG patients with MuSK antibodies tolerate albuterol and 3,4-diaminopyridine (12) more than pyridostigmine which, in MuSK-MG, is commonly associated with enhanced side effects especially of cramp and muscle fasciculations. A small clinical trial (phase IIB) studying amifampridine phosphate in MuSK-MG demonstrated this drug to be safe and effective (13). Ephedrine, a sympathomimetic agent, can also be used as an add-on treatment and improves symptoms and weakness (14). Tirasemtiv has been explored in a clinical trial and found to increase the muscle response to calcium and improves muscle strength in MG (15). This remains an experimental drug.

IMMUNOMODULATORY THERAPIES FOR GENERALIZED MG

Corticosteroids

Prednisolone or prednisone constitute the main immunomodulatory therapy in the long-term management of patients with MG (16, 17). The majority will require long-term oral corticosteroid therapy and it is crucial to have the appropriate discussion with newly diagnosed patients, indicating that this will not be a short course of treatment. It is equally important to discuss with patients the long list of potential side effects from steroids, necessitating bone and gastric protection. Patients should also be adequately monitored for the development of diabetes mellitus, hypertension, with careful counseling on potential excessive weight gain and the necessary dietary changes that they may need to pre-emptively and pro-actively address. Other side effects include the formation of cataracts, raised intraocular pressures, mood and sleep disturbances, peripheral oedema and susceptibility to frequent infections or even sepsis. The latter may result in failure of response to conventional MG therapies or even a chronic refractory state and decline in status with multiple hospital admissions.

There can be a paradoxical worsening of MG symptoms on commencing corticosteroids at high doses (18). Therefore, our practice is to start at a low dose and escalate the dose gradually (16). Our initial practice was to use an alternate day regimen of steroids, where side effects are probably reduced when compared to the daily dosing schedule. However, we have encountered many difficulties with the alternate day regimen including patients and physicians in primary and secondary care becoming easily confused, and we have therefore resorted, in the last 3 years or so, to applying the daily steroid regimen. We initiate prednisolone at 5 mg daily and increase every third dose (day) by 5 mg until we achieve stability in MG symptoms and significant improvement, with our ceiling dose usually being 50 mg daily but higher doses have been prescribed in a few select cases.

We treat the majority of patients in the outpatient setting, giving clear instructions to the primary care physician and to the patient, with contact details of the myasthenia team. The nurse specialist phones in on the patient regularly to ensure that the treatment plan is being ensued and to monitor patients' symptoms over the phone. In patients demonstrating significant bulbar weakness, our preference is to admit them immediately to the neurology ward and to initiate treatment accordingly including symptomatic treatment with pyridostigmine and where necessary intravenous immunoglobulin (ivIG).

With the slow steroid dose escalation that we apply, patients improve after 2–4 months of initiation, but some do take much longer to improve significantly. This can be problematic in some, and occurs in circa 20% of patients that we manage. In patients with moderate bulbar muscle involvement or disabling fatigable

limb weakness, we prefer to admit to the acute neurology ward or to the day-case ward (if they are generally stable) to treat them with a course of ivIG during the steroid escalation process in order to help expedite the process of their recovery. Occasionally, patients require more than a single course of ivIG to help stabilize their symptoms or to significantly improve their symptoms while increasing their corticosteroid dose. Some patients may not respond to ivIG. In this case, we employ plasma exchange (PE) if we feel their symptoms are sufficiently disabling. If patients are stable (but symptomatic) then PE can be administered in a day-case unit in an outpatient setting and PE carried out through peripheral venous access.

The slow steroid escalation regimen of treatment that we employ is in contrast to the early fast-acting treatment (EFT) strategies applied by the Japanese group (19, 20). This strategy always involves patients being admitted to hospital for treatment where they would receive 1–2 plasmapheresis sessions followed immediately by high-dose intravenous methylprednisolone (0.5–1 g), with or without intravenous immunoglobulin therapy. Treatment would be repeated if significant improvement did not take place. Patients were then discharged from hospital on the lowest dose possible of oral steroids. In some patients, who did not have severe MG symptoms, high dose methylprednisolone was not required. Achievement of MM was more frequent and occurred earlier in the EFT therapy cohort were compared to those in the non-EFT one (19, 20). While this regimen of treatment is highly attractive, it does require easy access to neurology inpatient beds and the necessary manpower (for instance accessibility to the plasmapheresis team) and would not be practical in our regional neurology center (which has 21 neurology beds serving a population of 2 million).

Steroid Sparing Immunosuppressive Agents

Until recently our practice has been to initiate a steroid sparing agent such as azathioprine, almost simultaneously as initiating corticosteroids and using a dose of 2.5 mg/kg/day (17). This was based on the study by Palace et al. (21) which showed that azathioprine was an effective adjunct treatment to prednisolone and was effective in reducing the long-term maintenance prednisolone dose, in reducing relapses, and in achieving remission in the long-term. However, our practice changed a few years ago (16, 22), when we began to treat newly-diagnosed MG patients with steroids alone first. A steroid-sparing immunosuppressive agent would be later added if the patient relapsed while reducing their steroid dose indicating that they will require more than 10 mg daily of prednisolone to maintain MM and thus justifying the addition of such an agent. We also consider adding in immunosuppression early if the patient has pre-existing comorbidities such as diabetes, significant depression (with steroids potentially exacerbating their mood), osteoporosis, leg ulcerations, that would be compounded by several-month treatment with corticosteroids. Also, in patients who are demonstrating a slow response with corticosteroid treatment then we would an immunosuppressant early in the course of treatment. Furthermore, in some patients, corticosteroid treatment is absolutely or relatively contraindicated because of background comorbidities and in this scenario we immediately prescribe a steroid-sparing immunosuppressant agent without the addition of steroids. Stabilization can be prolonged with this strategy, and we prescribe ivIG in the interim with or without low-dose corticosteroids depending on the clinical picture. Some patients refuse to be started on steroids because of concerns of side effects and in these circumstances adding a steroid-sparing immunosuppressant at diagnosis is a viable option. A retrospective study by Abuzinadah et al. (23), showed that a satisfactory response (which included CSR, PR, and MM) was achieved in about 50% of MG patients with generalized disease when they were maintained on low dose prednisolone, without a steroid-sparing immunosuppressant with follow-up extending up to 6 years.

We advocate checking thiopurine S-methyltransferase (TPMT) levels (24) prior to initiating azathioprine treatment. If levels are in the normal range, we initiate azathioprine at 25 mg daily and increase weekly by 25 mg until target dose is reached, with blood monitoring carried out in primary practice. Generally, the drug is well-tolerated and we rarely encounter idiosyncratic reactions in our population. The drug however takes 8–12 months to become effective and we counsel patients about this. In our opinion the drug is not entirely benign and we have observed many patients develop multiple skin lesions namely actinic keratosis, as a result of long-term azathioprine use and also skin malignancies such as squamous cell carcinoma. If the TPMT levels are deficient but not absent, then we consider using lower doses of azathioprine, monitoring the level of the active metabolite, 6-thioguanine nucleotides (6-TGN), in the blood and titrating the dose accordingly.

Our second steroid-sparing agent of choice is mycophenolate mofetil (MMF) at a dose of 1 g twice daily. In general, we have found it practical to use this drug and it is well-tolerated and (as previously reported in the literature) (25, 26) except for a small number of patients who complain of associated side effects including disabling dizziness and insomnia, and have discontinued this as a result. In patients with very high body mass indices, we have used doses of up to 2.5 g daily. Infrequently we have prescribed mycophenolic acid which can be better tolerated than MMF, in those with side effects from MMF. We find that the efficacy of MMF is noted after circa 6 months of treatment as was also observed in previous studies (27). Based on our clinical observations, and in contrast to the findings from a previous randomized controlled trial (28) oral weekly methotrexate is as effective as MMF and its efficacy becomes apparent at around the same time-point as MMF. It is about 20 times cheaper than MMF. Nausea and vomiting can be limiting side effects experienced by some. In general, folic acid 5 mg daily is prescribed day 4 after methotrexate but when nausea is prominent, daily folic acid (except for the day of methotrexate dosing) can help alleviate this. Ciclosporin (used at a dose of 3.5 mg/kg/day) is probably the most potent immunosuppressive agent with the added advantage that it is not teratogenic (29). From our clinical observations, we have deduced that ciclosporin is, at minimum, effective within 3 months of initiation. However, we have observed that the

majority of patients prescribed this drug run into problems with significant side effects including hypertension, alteration in their glomerular filtration rates, nephrotoxicity, tremor and in female patients also problems with hirsutism. We have prescribed ciclosporin in around 25 MG patients, where they have proven refractory to other steroid-sparing immunosuppressants, and usually belonging to a younger age-group. We avoid prescribing in older patients because of the potential complications and side effects and aim to reserve for younger patient groups. Tacrolimus is of similar efficacy (30) but with a similar side effect profile as ciclosporin. We have not prescribed cyclophosphamide in MG but there is a role for prescribing this drug as a monthly intravenous pulsed treatment in patients with refractory disease and who are unable to reduce their maintenance steroid doses (31), and this is generally tolerated without significant side effects.

Withdrawing Symptomatic Therapies and Achieving Maintenance Therapy

When the MG status starts to stabilize, MG patients no longer experience the significant fluctuation and variability in symptoms, become less fatigable and their strength starts to normalize. We educate patients about this time-point being reached and trying to recognize when they no longer need to reach out for their next pyridostigmine dose which is a good prognostic sign for stabilization. At that stage, while maintaining the same dose of corticosteroids, we advise patients to reduce their pyridostigmine dose by 30 mg per week (or sometimes faster), with the aim to wean this altogether but in some cases patients prefer or require to remain on low doses of up to 120 mg daily. Reduction of their steroid dose then ensues following a similar regimen previously described (16) −5 mg reduction per month down to 20 mg daily, then 2.5 mg reductions per month down to 10 mg daily, then 1 mg reduction per month or slower, aiming to reach 5 mg daily. In some cases, it is possible to wean steroids altogether especially if a steroid sparing immunosuppressive agent has already been added. However, if this is not the case then careful consideration needs to be taken, with detailed discussion with the patient, about withdrawing steroids altogether and a potential risk of future relapse. There is an argument for maintaining on low-dose prednisolone such as 5 mg daily for life where the cumulative life-time risk is likely to be small vs. further reduction or absolute withdrawal of prednisolone that might trigger a significant relapse of MG. In our experience, most patients favor the former option. Also, we are of the opinion that the long-term risk of such low-dose prednisolone (development of diabetes, hypertension, osteoporosis, glaucoma etc.) is significantly less than for example being maintained on 100 mg of azathioprine for life—although there are no long-term studies that quantitate this risk.

REDUCING THE DOSE OF SECOND-LINE AGENTS

When patients have achieved pharmacological remission and have successfully withdrawn corticosteroids, then it would be sensible to consider a gentle reduction in their steroid sparing immunosuppressant dose (17). The difficulties are 2-fold: firstly there is little data on the actual risk of relapse on withdrawal of immunosuppression and secondly there is no consensus or guideline on how rapidly the dose should be reduced. With regards to the first point, the limited studies on this indicate that the risk of relapse on withdrawal of immunosuppression may be rather high. In two respective studies, more than 50% of patients who were in CSR and who were prescribed azathioprine (32) and nearly all patients who had significantly reduced the dose or withdrawn MMF, experienced a relapse in their MG (33) necessitating the reintroduction of immunosuppression. With regards to the second difficulty: we usually take an ultra-conservative approach when reducing the dose of any immunsuppressant. In the case of azathioprine we reduce the dose by 25 mg every 6 months (infrequently weaning altogether) while with MMF we reduce no faster by 500 mg per year, as previously reported (34). We always advocate close monitoring of patients' MG status and symptoms during the reduction process. The rate of CSR is low and we often opt, after discussion with patients and balancing the decision against their age and comorbidities, to maintain them on the lowest dose possible of immunosuppressant in the long-term unless there is a pressing requirement that this is discontinued altogether.

Thymectomy

Thymectomy in generalized AChR antibody positive MG should be considered as early as possible in the management plan and thymectomy should be performed where relevant when the MG status has been stabilized (17). Imaging of the thymus gland, using CT or MR modalities, should be performed in all AChR antibody positive MG patients, also to rule out thymoma and in the younger patients to look for evidence thymic hyperplasia. The role of the thymus gland in driving MG has been known for almost a century (35, 36). The results from the international thymectomy trial (MGTX) have been crucial in underscoring the role of thymectomy in the management of MG (37). In this trial, non-thymoma MG patients up to the age of 65, with generalized disease and with positive AChR antibodies, were recruited. Patients whose MG onset was up to 5 years prior were recruited. The goal of the surgical procedure, in those who received thymectomy, was to remove all thymic tissue including ectopic tissue and surrounding fat. The results showed that patients who had thymectomy (which involved an extended trans-sternal procedure) required lesser doses of corticosteroids both in the short and in the long-term (38), had better functional outcomes, were less likely to be hospitalized due to their MG and were less likely to require additional immunosuppression with azathioprine for instance. The benefit was seen across all age-groups and was sustained on follow-up. This trial has been pivotal in the way we neurologists are now approaching MG management. Thymectomy now is more widely offered to patients with generalized disease associated with AChR antibodies, including patients with late-onset MG and up to the age of 65, as part of the overall treatment of their MG.

Minimally-invasive thymectomy surgery has been further revolutionary in the field. Reports of video-assisted thorascopic surgery (VATS) thymectomy began to emerge in 1993 and 1994,

with a number of centers using alone or in combination with a trans-cervical approach (39–41). Reports of robotic surgery in the field of thoracic surgery began to emerge in the early 2000s (42) with the use of the da Vinci robotic system applied in a 28-year old patient with MG. With both types of minimally invasive procedures, patients are reported to experience less blood loss intra-operatively, complain of less pain post-operatively and have a shorter post-operative hospital stay when compared to open thymectomy. In a systemic review comparing robotic assisted thymectomy surgery (RATS) with VATS and open surgery (43), there are clear advantages of RATS or VATS over open surgery, but no significant advantage of RATS over VATS at least to date. Clinical outcomes have been compared in a retrospective study in MG and found to be comparable between thoracoscopic vs. trans-sternal thymecetomy (44). Data analyses, after propensity score matching, also confirmed that robotic thymectomy in early stage thymoma was safe and feasible with oncological outcomes that were comparable to trans-sternal thymectomy (45) and in thymoma exceeding 5 cm (46, 47). In relation to MG outcomes, it would be very challenging to design a further trial that would compare clinical MG status and outcomes after open thymectomy vs. minimally invasive surgery.

In our experience, patients with non-thymoma MG are now more encouraged to pursue thymectomy, during the course of their MG management, when provided with the results from the MGTX trial. We have also observed that patients are also more comfortable in pursuing minimally invasive thymectomy surgery in contrast to open surgical approaches. For the past 3 years, our thoracic surgeons have been employing RATS, which we perceive as further advantageous specifically from the perspective of post-operative morbidity. For those who are in employment, who drive, who are parents looking after young children, and also for those younger adults who may be pursuing studies at school or university, RATS evokes less anxiety about the post-operative period impacting on their work, studies, social, or family life. Minimally invasive thymectomy procedures also overcome the aesthetic problems that patients faced with open thymectomy mediastinal scars. We, as a center, have also gained confidence in referring older MG patients for thymectomy, acknowledging that there is data to support its benefit also in this age-group (48, 49), and since the MGTX trial, we have been consistently referring patients up to the age of 65.

Although it is perceived that that there is a 2-year window of opportunity for thymectomy from disease onset, there is no evidence to suggest that the MG status is negatively impacted when thymectomy is performed beyond this time-frame. In the MGTX trial there was no evidence to support that patients who had thymectomy within 2 years did better than those who had thymectomy within 5 years of disease onset. This is particularly relevant to patients, who have proven refractory to all conventional immunosuppression, and where thymectomy at a later time-point in their disease could potentially offer additional benefit; we have been exploring this as an option in a small category of patients. In contrast, there are various reports indicating that thymectomy is contraindicated in MuSK-MG, with patients' MG status often worsening after the procedure and, therefore, thymectomy should not be pursued if MuSK antibodies are present (50). The jury is out as to whether thymectomy would benefit MG patients without AChR or MuSK antibodies (traditionally referred to as double seronegative) and if there is a role for thymectomy in MG with LRP-4 antibodies (51). Leite et al. (52) had shown that the thymic abnormalities in double seronegative patients had more thymic abnormalities than the MuSK-MG thymus, but less than seen in the AChR-MG cohort (52). Thus, these patients may benefit from thymectomy too but this is an area that requires further research.

Thymoma, in contrast, is a rare epithelial tumor of the anterior mediastinum and 50% of cases occur in association with MG. Thymoma occurring in association with MG, should always be surgically removed (17). Minimally invasive surgical approaches are feasible in most but may not be possible in the larger tumors. Complete surgical resection is aimed for but radiotherapy may be required for invasive thymomas. Where resection is incomplete and/or surgical margins are positive for thymoma, radiotherapy improves the prognosis by 50–60% (53, 54). Thymomas are also chemosensitive. Platinum-based agents show consistent efficacy (55) and can improve the outcome of Masaoka stage III and IV thymomas or recurrent thymomas. Non-platinum based regimens are also prescribed in some tumors and the role of immunotherapy still remains to be further investigated.

Ocular MG

Isolated ocular myasthenia is rare. While ptosis and diplopia are common presenting symptoms including in patients who will eventually evolve into generalized myasthenia, only 20% of patients will turn out to have pure ocular MG—signifying that these patients will never develop generalized disease) (56, 57). The diagnostic difficulty with this entity is that only 50% have detectable antibodies to the AChR (57). Single fiber EMG studies support the diagnosis of neuromuscular transmission failure in patients without detectable antibodies, including ocular myasthenia (58). The main differential diagnoses include thyroid eye disease, and a progressive external ophthalmoplegia associated with a mitochondrial disorder. The latter group of patients may also have some minor abnormalities on single fiber EMG studies with borderline increased jitter values making the diagnosis even more challenging (58). Other diagnostic cues are therefore crucial, and ultimately a muscle biopsy may be necessary to clinch the diagnosis.

First-Line Pharmacological Therapy in Ocular MG

While ocular MG is not life-threatening, diplopia is a very disabling symptom. It significantly impacts an individual's quality of life—it impacts patients' driving ability, it may impact their employment, their social life, and their hobbies including sports, reading etc. When a patient presents with ocular myasthenia, the first treatment that should be initiated is pyridostigmine in order to achieve symptom control and to determine reversibility. This may be sufficient in patients with mild symptoms and signs, but is unlikely to be adequate in patients with significant ocular motility disturbance. If patients remain symptomatic despite maximal doses of pyridostigmine, then the next step should be prompt treatment with corticosteroids (59–63). Early treatment

of ocular myasthenia improves the chances of reversibility or significant improvement in the long-term (64). There is some evidence to indicate that early treatment of ocular myasthenia delays or prevents the development of generalized disease (64–66). Delaying corticosteroid treatment, in our experience, reduces the chances of recovery of the extraocular muscles. In some patients, in spite of prompt and adequate treatment, they do not respond to therapies and are left with a fixed ophthalmoplegia in the absence of any other signs or symptoms. This may reflect the complex sarcomeric organization (67, 68), gene expression (69), distinct complement expression (68, 70), and unique metabolic demands and vulnerability of mitochondrial oxidation pathways within the extraocular muscles (71) that are susceptible to disease including autoimmune disorders. In patients who are refractory to treatment, and especially when they have no detectable antibodies and/or equivocal SFEMG findings, there is scope for investigating with an MRI scan of the orbits with gadolinium to exclude alternative, namely inflammatory, processes for instance thyroiditis. Commonly in ocular myasthenia patients with refractory disease and fixed ophthalmoplegia, the MRI shows atrophic extraocular muscles with asymmetric involvement and with no enhancement following gadolinium administration.

The ceiling steroid dose in ocular myasthenia is deemed to be lower than that used in generalized myasthenia (16, 57). One usually aims for a maximal steroid dose (prednisolone/prednisone) of around 25 mg daily (or equivalent of 50 mg alternate days) but in some instances higher doses may need to be considered particularly if there is a delay in the correction of the ocular motility disturbance and if diplopia remains a persistent symptom. Recovery of the extraocular muscles in ocular myasthenia may take several months and there may be scope for adding in immunosuppressive agents for the same reasons as in generalized MG (16, 22, 72). The indications for this includes patients whose ocular motility does not respond to corticosteroids alone, or who experience frequent relapses and are unable to reduce the corticosteroid dose below an acceptable level, or the physician feels that additional treatment is required especially if there has been incomplete response to corticosteroids and pyridostigmine. Other patients are unable to tolerate corticosteroids or may have comorbidities such as diabetes, osteoporosis, depression, or glaucoma that preclude the long-term use of steroids.

It is important to monitor patient's response to treatment carefully and working with an orthoptist can be of immense assistance. There also needs to be an objective assessment of ptosis and ocular motility for instance using the Jampolsky scheme (73, 74), and collaborative work with an orthoptist is often very helpful in monitoring response to treatment and progress.

Non-pharmacological Therapies in Ocular MG

In the short term, patients may be fitted with a Fresnel prism to allow some correction of their double vision (75). Reducing the strength of the prism over time is a clear indication of response and improvement. Some patients, however, will continue to rely on their prism in the long-term. Using a patch over one eye in the short-term is another option for some patients to help obliterate the false image while others tolerate using an occlusive contact lens.

Residual ptosis can be a significant problem in some patients either causing obstruction of one's vision or from an aesthetic perspective. In older patients, ptosis may be aggravated further by senile dehiscence or dermatochalasia. In general, if patients' ptosis does not reverse in spite of maximal treatment received over a 2-year period, then the chances of recovery after that period of time are rather slim. In a select group of patients, ptosis repair surgery performed by an oculoplastic surgeon may be indicated (72, 76). The surgeon needs to ensure that the risk of corneal exposure is minimal and repeated procedures are best to be avoided. In contrast using ptosis props is a less invasive way of dealing with the problem but some patients complain that these cause discomfort or corneal dryness since the props limit blinking, and may be simply impractical for some. In some patients, the extraocular motility may remain abnormal in spite of adequate treatment with steroids, and may become fixed. In a highly select group, strabismus surgery may be of benefit but careful discussion with an ophthalmologist who specializes in squint surgery is required for these cases. Botulinum toxin to correct the strabismus should be avoided altogether in MG because of the toxin's systemic effects, which may destabilize MG patients even when their status (other than their ocular features) has been stable for many years (77).

Thymectomy in ocular myasthenia remains controversial but there are various small studies indicating that this is beneficial particularly when considered early in the disease (78–82). The task force for the EFNS/ENS guidelines (62) agreed that thymectomy is not recommended for ocular myasthenia as first-line treatment but should be considered if drug therapy was not successful and may prevent MG generalization (good practice point). Given that ocular myasthenia often evolves into generalized disease (and there are no markers to predict this) and given the increased access to minimally invasive thymectomy surgery, early intervention may be of benefit. For these reasons, we have increasingly been referring ocular MG patients for thymectomy in the last 3 years. Furthermore, it is unknown, if early thymectomy may also prevent these patients developing a fixed ophthalmoplegia in the long-term.

MG IN SPECIFIC PATIENT GROUPS

The Pregnant Patient

In practice, the majority of MG patients, who are treated adequately before pregnancy, do not experience any complications during pregnancy or in the post-partum phase. However, some report an increased risk of MG relapse during pregnancy that varies between 17% (83) to 41% (84). Some patients' MG status improves during pregnancy (85) as one observes with other autoimmune conditions such as multiple sclerosis. In the ideal scenario, the pregnancy is planned to allow optimization of MG status and withdrawal of teratogenic medications where relevant. Pyridostigmine, corticosteroids, and azathioprine are all safe to be used in pregnancy and

should not be discontinued during pregnancy (85, 86). MMF and Methotrexate are teratogenic and should be avoided (85). Ciclosporin and tacrolimus are not teratogenic but their use can be associated with the development of hypertension and gestational diabetes and, therefore, the patient requires close monitoring (85). IvIG and PE are also safe to be used during pregnancy (87). There are some reports suggesting that MG patients are at risk of preterm rupture of membranes (88, 89). We have not encountered this in our practice, however.

Therapy for MG should be optimized where possible before and during pregnancy. The neurologist and obstetrician should be in regular dialogue particularly in the third trimester of pregnancy, when plans should be initiated on how the baby should be best delivered. Medications for MG should continue uninterrupted before and throughout labor. Patients should undergo spontaneous vaginal delivery in most cases and epidural labor analgesia should be considered early in patients who are likely to experience fatigue during labor (90). Nitrous oxide is safe to use (85).

Surgical delivery should be considered in those who MG status is poorly controlled and in those patients where muscle weakness is significant or their MG is considered brittle. Ideally this should be planned adequately in advance with multidisciplinary team discussions throughout but especially in the latter part of the pregnancy. MG patients are usually extremely sensitive to depolarizing muscle relaxants, and should be administered the least possible dose (85). Magnesium sulfate for the treatment of eclampsia should be avoided in MG since this will exacerbate myasthenic weakness (85). Opiates for pain relief especially in the post-partum phase should be used with caution since they too may exacerbate weakness. Breast feeding of the newborn should be encouraged. Neonatal myasthenia, with temporary and usually mild myasthenic weakness, occurs in 10% of neonates due to transplacental transfer of antibodies (86, 91). It usually resolves spontaneously within 3 weeks of the birth of the infant. Rarely the presentation of the neonate can be more complex, especially if the mother's MG was undertreated during pregnancy, and may require the neonate to be managed in an intensive care setting for a short period. Very rarely, infants of MG mothers are born with mild myopathy and—at the severe end of the spectrum—arthrogryposis multiplex congenita (92). The mothers may in fact be asymptomatic or minimally symptomatic with elevated AChR antibodies and some may be asymptomatic with antibodies specific to the fetal AChR γ subunit (92).

The MG Patient in Crisis

MG crisis occurs in circa 20% of MG patients who are newly presenting with MG (93, 94). It occurs more frequently in MG patients who are undertreated, or who have newly presented and whose treatment is being slowly escalated but whose presentation has evolved more rapidly than therapy has originally been scheduled for stabilization. Patients develop severe muscle weakness including weakness of the respiratory muscles, commonly preceded by severe bulbar weakness with dysphagia, with or without palatal weakness and nasal escape. In this situation, patients require a nasogastric tube to be inserted to allow medications to be administered and for feeding. This clinical picture must be promptly recognized and the patient requires to be monitored closely in hospital, usually in a high dependency unit setting, since this clinical picture often evolves further with significant respiratory muscle weakness. Arterial blood gases should be checked to identify when type 2 respiratory failure occurs. At the bedside, assessing the patient's respiratory rate and forced vital capacity, and observing whether the patient is using their accessory muscles are all helpful measurements, predictors or cues. If parameters allow then the patient could be treated with non-invasive ventilation (NIV) first but if parameters fail to improve or the patient continues to tire with NIV or is intolerant of this, then treatment must be quickly escalated and the patient must be intubated and mechanically ventilated in an intensive care setting.

The two primary pharmacological therapies to treat MG crisis are ivIG, at a dose of 0.4 g/kg/day for 5 days or PE—usually 4–6 exchanges (17). They are equally effective in the treatment of MG crisis or a significant MG relapse (95). We commonly prescribe ivIG first, unless there are contraindications, and resort to PE as second-line therapy if the patient fails to respond to ivIG. However, if PE is readily available we would recommend using as first-line in the context of MG crisis since it is more rapid in its effect than ivIG. This has been our experience and also previously shown by Qureshi et al. (96). PE is not without risk however. It is more invasive, more labor-intensive and more expensive than ivIG (97). PE should be performed via peripheral venous access, where feasible, but central catheters may be necessary in some which pose additional risks of an infection source if mishandled or if left *in situ* for too long (98). The same dose of ivIG could be administered over a shorter period for example 2–3 days if tolerated by the patient. We prefer to administer over 5 days, especially in patients who are ivIG naïve at least initially, and we consider administering over 2–3 days in subsequent treatments.

Corticosteroids are added or increased simultaneously with ivIG or PE therapy (16). In our practice, we still initiate corticosteroids at low doses but then we escalate the dose more rapidly over 5–7 days, since the steroid dip is likely to be counteracted by the simultaneous use of ivIG or PE. The role of acetylcholinesterase inhibitors is limited in MG crisis. They may exacerbate bronchial secretions and so one should be mindful of identifying the clinical situation when they are likely to be of benefit even to the MG patient in crisis. Some patients may require further courses of PE or ivIG 4–5 weeks after their initial therapy and may relapse even after their initial significant improvement. This is because the effect of corticosteroids may be apparent after 6–8 weeks while the effect of ivIG or PE usually lasts circa 4 weeks.

Weaning from the ventilator should be considered when the patient demonstrates an improvement in vital capacity and is strong enough to transition to spontaneous mode ventilation, which allows the patient to initiate breathing (99). The patient should be observed for fatigability with switch-over to assisted-ventilation when they fatigue. There is concomitant improvement in bulbar and neck muscle strength when respiratory muscle improvement is observed. If their cough remains weak and the patient is struggling to clear their airways

secretions, then extubation is likely to be precocious and failure is more likely to occur.

Consideration for thymectomy should be considered where relevant and after the patient has been weaned off ventilation and extubated. Also, they should demonstrate stability in their MG status, have been stepped down to a regular ward and are becoming less dependent for their daily activities of daily living. The prognosis of MG crisis is worse in patients with thymoma. In this group of patients, managing their MG crisis can be challenging and response to therapy may be delayed (93). When their MG status has been stabilized, however, thymectomy should follow on promptly when safe to do so.

The Older MG Patient

World-wide epidemiological studies confirm that the incidence of MG is increasing among male and female patients who are older than 65 years (100–102) and the prevalence is also rising due to patients living longer (103, 104). Multiple comorbidities often exist in older patients. They are less likely to tolerate the more potent immunosuppressive agents that benefit the younger MG patients (105–107). In older patients, careful consideration needs to be given of the potential impact of corticosteroid treatment on other systems for example the development of diabetes, hypertension, obesity with cardiac strain and heart failure, significant osteoporosis with vulnerability to various fractures. They become more vulnerable to infection including recurrent infections and sometimes resulting in life-threatening sepsis especially when more potent immunosuppressive agents such as MMF or Methotrexate are prescribed. Some older patients suffer recurrent infections when managed with maximal immunosuppression for their MG which in turn results in hospitalization, further deconditioning and a significant delay in recovery from their MG. From our experience, we have noted that in the older perhaps frailer patients it may be safer in the longer term to slightly undertreat their MG rather than aim to induce remission, since prescribing conventional doses of immunosuppression in this age-group often leads to fatal consequences. MG patients are also more vulnerable to developing osteoporosis (108) and the prescribing neurologist needs to be aware of this and monitor closely patients' bone densities since osteoporotic fractures result in significant morbidity, chronic pain and reduced mobility, which may already be compromised in an older patient.

The Refractory MG Patient and Novel Therapies

About 20% of MG patients are refractory to all conventional treatments. Monoclonal antibody treatments that bind the B lymphocyte membrane protein CD20, such as Rituximab have been increasingly prescribed in this group of patients with successful outcomes. The rationale behind preparations such as Rituximab is that they destroy and deplete pathogenic B cells and decrease AChR antibody production. Rituximab influences the whole spectrum of B cell function including antigen presentation, cytokine production, and T cell stimulation and hence has a role in T cell mediated autoimmune diseases too (109). Studies have demonstrated that clinical improvement even with one cycle of Rituximab is sustained (110, 111) allowing subsequent reduction in steroid doses and in some inducing remission (112). Patients with MuSK-MG respond extremely well to Rituximab and the drug often induces remission without the requirement for subsequent infusions (112, 113). Rituximab has a role in patients presenting aggressively and explosively at onset and who are refractory to all conventional therapies. Brauner et al. (114) demonstrated that clinical outcomes were better in patients who were treated early rather than later with Rituximab. There is scope for considering Rituximab in patients who are in crisis and who are not responding to high dose corticosteroids or ivIG or PE, and when patients demonstrate resistance in weaning off ventilation during the treatment pathway of MG crisis. Caution must be exerted in this scenario, acknowledging that Rituximab will not be effective immediately and may pose an added risk to the patient for developing infection. Rituximab is contraindicated during pregnancy (87).

In our experience, where we have treated a small cohort of 17 MG patients with MuSK-MG, AChR-MG, and MG with no detectable antibodies, the majority of patients improved significantly but remain dependent on immunosuppression (unpublished data). Our single MuSK-MG patient, within this small cohort, responded best to Rituximab although this did not induce complete remission of her disease. In contrast, about a third of MG patients did not respond to Rituximab and their MG status was not altered by this therapy. In general, we have found that the drug is well-tolerated with minimal side effects. However, in two patients we have observed delayed neutropenia developing many months after Rituximab treatment, including one patient whose presentation was complicated by two neutropenic sepsis episodes several months after their Rituximab treatment. This has been observed in other patient groups treated with Rituximab (115–117).

In a large systemic review of 169 MG patients who received Rituximab, remission (PR or CSR) and MM was achieved in 72% of MuSK-MG patients in contrast to 30% of AChR-MG patients, with post-treatment relapses being markedly reduced in the MuSK-MG cohort (118). It is still unclear why MuSK-MG patients respond so well to this drug. It would be crucial for biomarkers to be developed that will allow physicians to predict a patient's response to Rituximab. There is also a similar crucial need for robust trial data for this drug, since the efficacy of Rituximab in AChR-MG is still debatable and the studies that are available may be limited by an element of reporting bias (119). This data will also help physicians counsel patients adequately when embarking on this therapy.

MG treatment can also be addressed by switching off complement pathways and their activation, or by altering the Fc region of the antibody such that less antibodies are available for recycling, more are destroyed and thus unavailable for pathogenic processes. Novel therapies have been developed to address both. The efficacy and safety of the terminal complement inhibitor eculizumab (a humanized monoclonal anti-C5 antibody) in MG has been rigorously studied in the REGAIN trial (120, 121). Improvements were noted in all objective MG-related scores and in the patients' quality of life scores for all those actively treated with eculizumab, and were

sustained during the 52-week study period. Patients treated in the placebo arm experienced rapid and sustained improvement in their MG status when switched to open-label eculizumab. The drug also improved fatigue scores which in turn correlated strongly with MG-specific outcome measures (122). However, the response among patients in the REGAIN trial was variable with some improving substantially, some modestly and some patients showing no response whatsoever (123). Eculizumab is now a registered therapy for myasthenia gravis. It remains an expensive drug with costs for one patient's treatment per annum amounting to $500,000. It is unclear whether this drug is cost-effective in MG. A trial of zilucoplan, a subcutaneously self-administered inhibitor of complement component 5, has been recently studied (124). The trial confirmed that zilucoplan was safe and well-tolerated and patients rapidly showed clinical improvement with this drug. The extent of clinical response correlated with the level of complement inhibition such that near-complete inhibition was demonstrated to be superior to submaximal inhibition.

Efgartigimod (also known as ARGX-113) has been trialed in generalized MG in a phase-2 randomized double-blind, placebo-controlled study in 15 centers (125). ARGX-113 is the anti-neonatal Fc receptor immunoglobulin IgG1 fragment. It has been modified to increase its normal affinity for IgGs, thus blocking the formation of disease-causing IgG. Efgartigimod was well-tolerated in this trial. In the 12 patients treated with the active drug, there was a rapid decline in total Ig levels and in AChR titers, which in turn correlated with a clinical improvement of their MG, and this was sustained in the majority.

The proteasome inhibitor, Bortezomib, depletes short-lived and long-lived B cells and is applied in the treatment of multiple myeloma (126) and plasmablastic lymphoma (127). It is likely to have a role in the treatment of refractory MG including MuSK antibody positive MG (128) but the development of a sensorimotor polyneuropathy, a recognized side-effect of this drug, is likely to be a limiting factor.

Questions remain unanswered about the long-term safety, efficacy, and tolerability of these novel therapies (meaning after several years of continuous treatment). It is unclear whether long-term complement inhibition, for instance, would pose increased general infection risks particularly in older age-groups. Determining the category of patients who are likely to benefit from these therapies is crucial. Would these therapies be aimed only for "refractory" and "severe" MG? If so, how do we precisely define these entities? Would drug holidays be considered and if so for how long? It is also less clear how cost-effective these novel therapies are, how the various global health systems would fund these drugs and how the different health insurance companies will cover the costs of these drugs. A detailed cost-utility analysis is required that will allow the diverse health systems to better understand the long-term efficacy of these therapies, how improvements in objective measurements translate into better function for the patient, and how they improve patients' quality of life. It would be imperative to ascertain and quantify the potential socioeconomic gains when using these therapies (do these therapies allow individuals to return to their employment, increase independence and reduce dependence on care-givers?) and the impact on reducing in-patient hospital care (reducing hospital admissions including to intensive care units, the requirement for regular ivIG, or frequency of attend clinic appointments due to stable disease etc.).

Fatigue in MG

Fatigue is common in all neuromuscular conditions including MG, and around 80% of MG patients will experience significant fatigue at some stage of their disease (129, 130). It is distinct from fatigability and muscle weakness and therefore it is crucial that the physician recognizes this entity since its management does not involve escalation of treatment for MG (131). Fatigue is as disabling to the patient as active muscle weakness, and may negatively impact patients' quality of life, their quality time with their family, their employment status, and their social lives. It contributes to the disease burden but is more difficult to assess or objectively measure in the clinic. Fatigue may be problematic even when MG symptoms have largely settled or when the patient has achieved minimal manifestations.

Fatigue is multifactorial. Primary fatigue occurs when muscle weakness and fatigability are active in MG and has an inherent physical component (132) contributing to fatigue. They also complain of cognitive fatigue which patients often allude to as "brain fog" (133). It is difficult to dissect out primary from secondary fatigue, with the latter occurring for various reasons. Patients with MG, often gain weight primarily due to corticosteroid treatment (134), sleep less efficiently (135), move less and develop muscle stiffness and discomfort (136). They are more likely to become anxious and depressed about their physical limitations and the variability and unpredictability of their symptoms (137). They resort to socializing less, they might discontinue their employment, which in turn may have financial consequences, and do less chores in the house or even become virtually house-bound. O'Connor et al. (138) identified that MG patients were more likely to become sedentary even when asymptomatic. It is unclear whether this is learnt behavior or fatigue-driven or simply part of a vicious cycle. Because MG patients exercise less they become quickly deconditioned and often develop breathlessness that is not secondary to respiratory muscle weakness. Their breathing becomes shallow with a tendency to hyperventilate which develops as a learned pattern and is often misinterpreted as a sign of early MG crisis. Their sleep pattern is less efficient. They may develop obstructive sleep apnoea due to weight gain. They socialize less and this in turn negatively impacts their mood further.

Fatigue is not unique to MG but is also prevalent in other neuromuscular disorders such as different types of muscular dystrophy and myotonic dystrophy (DM1). Patients with facioscapulohumeral muscular dystrophy (FSHD) often complain of fatigue and pain, and hypersomnolence is very common in patients with myotonic dystrophy. Various studies have studied the role of exercise in various neuromuscular studies including MG (139, 140). Other studies have explored using cognitive behavioral therapy in combination with graded exercise in MG, DM1, and FSHD including high intensity training and aerobic exercise which led to functional benefits in patients without evidence of damaging muscle (141–144). In a very small

and select group of patients, where fatigue is compounded by pain, anxiety and insomnia, and perhaps with an overlay of their myasthenic symptoms (i.e., true MG coexisting with an aspect of a functional neurological disorder) we have managed them also with psychology input and cognitive behavioral therapy (145).

It is challenging when prescribing exercise to MG patients or indeed to any neuromuscular patient. Different types of exercise are suitable for MG patients at different phases of their MG. Aerobic or high intensity training is not possible when MG patients are very symptomatic. In this situation, stretching exercises such as Tai chi, slow flow yoga or pilates are probably most appropriate with emphasis also on balance maintenance. When MG symptoms stabilize, physical therapy should focus on balance and muscle strengthening but physicians should also enquire specifically about other symptoms including pain, residual fatigue, sleep disturbance and mood problems and address these accordingly.

Dysfunctional Breathing in Myasthenia Gravis

It has long been observed that breathing patterns and the central ventilator drive can be altered in patients with mild or moderate MG (146). In our practice, we have observed several patients, who we deem stable or in minimal manifestations, complaining of dyspnoea as a residual prominent symptom in spite of them not having any objective evidence of respiratory muscle weakness. A very small proportion, may have had a MG crisis at some stage of their disease, which inevitably raises long-term anxiety levels to the patient and their carer, about the potential severity and sometimes unpredictability of the disease. In some, contributory factors are clear and include deconditioning or weight gain. We have identified, through collaborative work with the local respiratory team, that many of these patients have developed dysfunctional breathing (unpublished observation). Our local respiratory physiotherapist has been working with these patients, employing physiotherapy-based breathing pattern modification interventions. These include relaxation of intercostal muscles, accessory muscles and full utilization of the diaphragm thus helping them to regulate and improve their breathing pattern with good results (unpublished).

Dysfunctional breathing has been studied extensively in poorly controlled asthma (147) because it is common and is associated with significantly poor asthma control and lower quality of life. Evidence-based guidelines recommend breathing retraining interventions as adjuvant treatment in uncontrolled asthma. A multicenter randomized controlled trial is currently underway in Denmark to investigate the effect of breathing retraining on the impact on quality of life in poorly controlled asthmatics (148). In a small study (149), 12 MG patients underwent long-term respiratory muscle endurance training, which resulted in a change in their breathing pattern with prolonged expiration. Interestingly patients reported an improvement in their MG symptoms, in their respiratory symptoms and in their physical fitness. This study proves that normocapnic hyperpnea training is a useful adjuvant therapy in MG.

It is therefore imperative that physicians recognize the entity of dysfunctional breathing in MG patients and refer them on for respiratory-based physiotherapy. This is a crucial adjuvant treatment in MG patients, who complain of dyspnea, and intervention helps their overall MG symptoms, improves their exercise capacity and increases their chances of overall recovery with improved quality of life.

The End-Result—Our Practice and Comparison With Reported Outcomes

When we set up the myasthenia clinic 13 years ago, we primarily aimed this to be a regional service that manages MG patients residing in the West of Scotland. However, we were subsequently referred MG patients who were refractory to standard therapies and who came from other parts of Scotland. Our patient cohort, served over a 13-year period, is heterogeneous including ocular and generalized MG, spanning all age groups (including patients in their tenth decade), with different antibody status and thymic pathology. About 10% of our cohort is refractory to conventional treatments. Our experience, as previously reported in the literature (150), has been that most patients' MG status evolves within the first 2 years of symptom onset. Broadly, CSR has been achieved in 5–10% of our case-load, PR in 20%, MM in 25%, improvement in 35%. About 10% of our cohort's MG status remains unchanged by our therapeutic interventions. Patients were worsened by therapy in 1–2%, and 1% died from direct complications of their MG. Our rate of PR is comparable to what has been reported in the literature but it is difficult to make direct comparisons since our treatment regime has also evolved over time. Mantegazza et al. (151) reported PR in 24% and CSR in 11%. Beghi et al. (152) reported a higher chance of CSR in patients who were younger and who had a shorter disease duration. These findings were echoed in a further study by the same group almost a decade later (153). Yang et al. (154) reported a CSR rate of 60% in patients who received thymectomy for thymic hyperplasia with younger patients having a higher CSR rate. Given that we have put more MG patients forward for thymectomy in the last 3–4 years, it is likely that this would further influence our remission rates. If we were to categorize our patient cohort according to age-groups, thymus pathology, and thymectomy status this would refine our CSR and PR rates, but we have not carried out that detailed analysis to date.

CONCLUSIONS

There are various guidelines in the literature on MG management. Physicians usually adhere to and achieve confidence and familiarity with specific treatment plans. However, the "recipe" for treatment can and should be designed for the individual patient's comorbidities. The aim in MG treatment is to induce remission or MM and to enable patients to resume their normal life-style. Each patient, however, is unique with respect to their comorbidities and their social or personal circumstances. As a result, the immunosuppressive therapy prescribed needs to be "catered" for that particular individual bearing all those pertinent variables in mind. Residual

myasthenic symptoms, which physicians may perceive as minimal may have a significant impact on a patient's daily life. As physicians, we need to be mindful of the impact of patients' MG on their physical and mental health, the impact on their family or carers, and the impact of adverse effects from MG-related therapies on their general health. The development of new therapies for the severe end of the MG spectrum is exciting. We need to learn more about these drugs, gain familiarity and identify the patient groups who are more likely to benefit from them. Detailed cost-utility analysis is required for individual health-care systems to enable physicians in their process of justifying the use of these drugs to their respective hospital systems. Addressing fatigue and its management is paramount to the overall MG management. Encouraging patients to exercise should be an integral part of their treatment since this will help their overall well-being in the long-term. Finally, dysfunctional breathing should be recognized and treated accordingly.

AUTHOR CONTRIBUTIONS

MF and JG contributed equally to conceptualizing this document. MF wrote the manuscript with contributions from JG to different sections. All authors contributed to the article and approved the submitted version.

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MINI REVIEW
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MuSK-Associated Myasthenia Gravis: Clinical Features and Management

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Muscle-specific tyrosine kinase (MuSK) myasthenia gravis (MG) is a rare, frequently more severe, subtype of MG with different pathogenesis, and peculiar clinical features. The prevalence varies among countries and ethnic groups, affecting 5–8% of all MG patients. MuSK-MG usually has an acute onset affecting mainly the facial-bulbar muscles. The symptoms usually progress rapidly, within a few weeks. Early respiratory crises are frequent. The disease may lead to generalized muscle weakness up to muscle atrophy. The main bulbar involvement, the absence of significant thymus alterations, and the association with HLA class II DR14, DR16, and DQ5 alleles have been confirmed. Atypical onset, such as ocular involvement, lack of symptom fluctuations, acetylcholinesterase inhibitors failure, and negative results of electrophysiologic testing, if not specifically performed in the mainly involved muscle groups, makes MuSK-MG diagnosis challenging. In most cases, steroids are effective. Conventional immunosuppressants are not commonly able to replace steroids in maintaining a satisfactory long-term control of symptoms. However, the majority of MuSK-MG patients are refractory to treatment. In these cases, the use of rituximab showed promising results, resulting in sustained symptom control.

Keywords: muscle-specific tyrosine kinase, atypical onset, tongue atrophy, MuSK-MG therapy, rituximab

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INTRODUCTION

In 2001, serum antibodies against muscle-specific tyrosine kinase (MuSK-Abs) were identified for the first time as cause of myasthenia gravis (MG) (1), opening the way to the description of a distinct peculiar subtype of MG disease (2–5). A reliable neuromuscular junction (NMJ) transmission is guaranteed by both morphological NMJ appropriate structure and NMJ transmission efficacy. NMJ transmission efficacy is strictly related to the "safety factor," which refers to the ability of the NMJ to remain effective under several conditions. This is possible mainly because each nerve impulse releases more transmitter than is required to excite the muscle fiber, ensuring that the transmission does not fail (6). The role of muscle-specific tyrosine kinase (MuSK) in determining NMJ efficacy has been recently clarified (7). A tetrameric complex on the postsynaptic membrane results from the association between MuSK and the low-density lipoprotein receptor-related protein 4 (LRP4). The MuSK–LRP4 tetramer is phosphorylated by agrin and recruits downstream of kinases 7, which further enhances MuSK activation for postsynaptic differentiation and acetylcholine receptor (AChR) clustering. Furthermore, an interaction between MuSK and matrix proteins, such as collagen Q (ColQ), which contributes to synapsis stabilization, has been demonstrated *in vitro* (8, 9).

Recently, Huijbers et al. confirmed MuSK-Abs as pathogenetic (10). MuSK-Abs belongs mostly to the IgG4 class of immunoglobulins, which acts by the direct inhibition of protein function. In particular, MuSK-Abs interfere with MuSK–LRP4 complex and, consequently, AChR clustering is inhibited (11). The aim of this mini-review is to report on the epidemiological and major clinical features, diagnostic approach, and treatment of MuSK-MG subtype.

EPIDEMIOLOGY

MuSK-MG is reported in about 5–8% of MG patients. Its prevalence varies among countries and ethnic groups, with a higher percentage in Southern Europe, and it is clearly predominant in females, actually constituting more than 70% of patients in all studies reviewed (9, 12).

The disease has an early age of onset, with a peak of incidence in the late 3rd decade, and it rarely occurs after 70 years of age. Cohorts from different countries confirm the association with HLA class II DR14, DR16, and DQ5 (9). No significant thymus alterations have been reported in MuSK-MG patients as related to the disease (9, 12, 13).

CLINICAL FEATURES

A peculiar clinical onset picture has been described from several groups for MuSK-MG. The disease typically has an acute onset, with rapid progression within a few weeks. In the majority of cases, bulbar involvement appears in the first stage and the presenting symptoms are ptosis and diplopia.

However, some peculiarities have been demonstrated about ocular manifestations which are observed in the early stages of the disease, consisting in symmetrical ophtalmoparesis of horizontal gaze and, more rarely, of vertical gaze with rapid remittance of diplopia. Furthermore, the typical fluctuation of myasthenic symptoms may not be evident in MuSK-MG patients. Commonly, a purely ocular onset generalizes in 2–3 weeks (14–17).

Bulbar impairment has been demonstrated in up to 80% of MuSK-MG patients, consisting of dysarthria, dysphonia with nasal voice, dysphagia, and masticatory difficulty. Bulbar onset is usually related to rapid deterioration, frequently leading to respiratory crisis. Generalized weakness and fatigue have also been described as onset syndrome, resembling anti-AChR-associated MG (AChR-MG). Furthermore, MuSK-MG patients have a higher risk of myasthenic crisis (3). Usually, axial muscle weakness involves neck extensor, which may present as head drop, and it can be the only presenting sign, without bulbar involvement. Neck extensor weakness is more frequent in MuSK-MG, whereas neck flexors could be only mildly involved (18).

An unusual but distinct feature of MuSK-MG is muscle atrophy. In particular, the mainly involved muscle groups are facial muscles and the tongue (**Figure 1**). Muscular atrophy can also be observed at shoulder girdle muscles, limb, and paraspinal muscles, resulting in severe scoliosis, as reported in a few cases in literature (19).

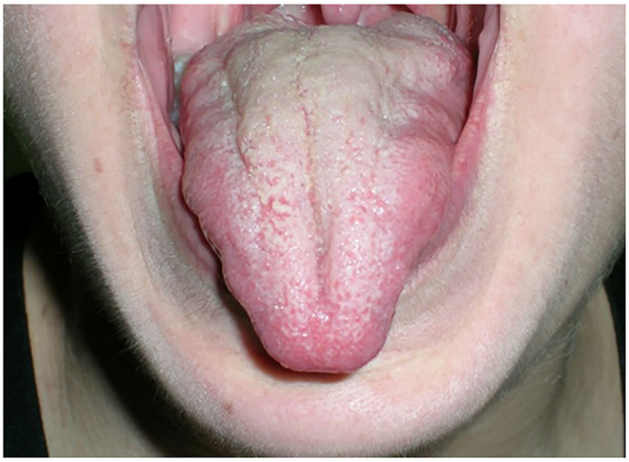

FIGURE 1 | Tongue atrophy in a young woman with MuSK-MG.

Electromyography (EMG) on atrophic muscles reveals a myopathic pattern and magnetic resonance imaging confirms muscle thinning and documents fatty replacement. There are evidences that corticosteroid treatment can improve muscle wasting; however, in some cases, atrophy becomes chronic and a significant cause of severe disability (20). The majority of MuSK-MG patients do not present relevant thymus alterations (21, 22). Hyperplasia is rarely described. Case reports incidentally documented thymoma treated with thymectomy (23). There are few data and no consensus on the role of thymectomy in MuSK-MG. In AChR-MG, a randomized, controlled trial of thymectomy in non-thymomatous acetylcholine receptor patients demonstrated a significant improvement in clinical outcomes after thymectomy, as well as a decreased requirement for immunosuppression (24). Conversely, available studies on thymectomy in MuSK-MG outline a limited improvement in clinical outcomes or immunosuppression management after thymectomy (21–24). Moreover, it has been reported that the outcome in MuSK-MG after thymectomy may not be beneficial (25). Therefore, thymectomy in MuSK-MG should not be considered as a therapeutic option.

DIAGNOSTIC APPROACH

MuSK-MG diagnosis might be challenging. In fact, muscle atrophy, dysphagia, dysarthria, and neck extensor weakness as onset clinical picture may be easily misdiagnosed, for example, with bulbar onset of amyotrophic lateral sclerosis, oculopharyngeal muscular dystrophy, and mitochondrial myopathy. The diagnostic procedure includes MuSK-Ab testing, edrophonium/neostigmine test, and electroneurophysiological studies such as repetitive nerve stimulation (RNS), single-fiber electromyography (SFEMG), and needle EMG.

A positive result for MuSK-Ab, sustained by clinical evidences, supports the diagnosis of MuSK-MG. Detection of MuSK-Ab is usually a second step for AChR-Abs-negative patients or individuals positive for AChR-Ab who do not

respond to treatment. It has been recently proposed that radio immunological assay-negative MG sera should be tested for IgG-specific antibodies by MuSK-cell-based assay to increase the detection of antibodies (26). Edrophonium or neostigmine tests, although non-routine, resulted positive in 40–75% of MuSK-MG patients; however, these tests demonstrated a higher sensitivity (97–100%) for AChR-MG diagnosis (27).

RNS sensitivity appears to be lower in MuSK-MG compared with AChR-MG, especially when performed on distal limb muscles. However, it has been reported that it is possible to increase the diagnostic sensitivity of RNS in MuSK-MG by testing proximal muscles, in particular the facial muscles, reaching a diagnostic sensitivity of 75–85% (28, 29). In AChR-MG, RNS usually show a partial recovery of the compound muscle action potential amplitude after a transient decrement during the first responses to low-frequency RNS (U-shaped pattern), not reported in MuSK-MG. On the contrary, a progressive decremental pattern after the fourth or fifth stimulation is typically revealed in Lambert–Eaton myasthenic syndrome (LEMS). It has been demonstrated that a similar pattern is usually found also in MuSK-MG, probably due to an underlying presynaptic dysfunction in MuSK-MG patients, as in the LEMS ones (28).

Needle EMG in patients with MuSK-MG may show myopathic features, rarely observed in AChR-MG, in particular in the facial muscles. These non-specific findings only partially contribute to define diagnosis (26). In cases with evocative clinical manifestations of MuSK-MG, associated with borderline antibody values, SFEMG is mandatory to diagnose MuSK-MG. Furthermore, it is worth to underline the importance to focus SFEMG on the mainly affected muscles to precociously detect alterations. In fact, in MuSK-MG, SFEMG of cervical paraspinals, deltoid, frontalis, and orbicularis oculi, which are usually the first and more frequently involved muscles, may be noticeably abnormal since the beginning of the disease. These patients may conversely have normal jitter in clinically uncompromised muscles (30). Stickler et al. reported cases of normal jitter in the extensor digitorum muscle and frontalis but markedly increased jitter with blocking in neck extensors (29). Cases with normal SFEMG at orbicularis oculi but with abnormal jitter in paraspinal muscles have been described by Padua et al. (31).

TREATMENT

Long-term pharmacological treatment is usually required to achieve an effective control of symptoms in MuSK-MG; however, it could be at least challenging.

The symptomatic treatment with acetylcholinesterase inhibitors is generally unsatisfactory and may be deleterious in MuSK-MG. Moreover, the response to pyridostigmine standard doses, used for AChR-MG, lacks efficacy and has poor tolerance because of side effects (26). Among symptomatic drugs for

TABLE 1 | Clinical features and management of MG subtypes.

	AChR-MG	MuSK-MG	LRP4-MG
CLINICAL FEATURES (9–13)			
Age of onset	Early onset <50 years Late onset ≥50 years	3rd decade	Any
Sex prevalance	Early onset: female Late onset: male	Female	Female
HLA associations	DRB1*01 DRB1*03, B*08, DRB1*09, DR2, and B7A1	DRB1*14, DRB1*16, and DQB1*05	-
Clinical features	Variable	Bulbar impairment, neck extensor weakness, muscle atrophy Higher frequency of myasthenic crisis	Variable
Thymus	Hyperplasia, AB, and B thymoma	Normal	Rare hyperplastic changes
ELECTROPHYSIOLOGICAL PROFILE (42–44)			
SFEMG	Frequently positive (∼90%) even in non-affected muscles	∼80% positive in affected muscles	Rarely positive
RESPONSE TO TREATMENT (14, 26)			
AChE-Is	Effective	No benefit, several side effects	Effective
Short-term immunotherapy	Effective PE and IVIG	Effective PE Effective IVIG (possibility of non-responders, IVIG > PE)	Effective PE and IVIG
Long-term immunotherapy	Good control achieved with PDN, AZA (or other traditional immunosuppressant)	Partial answer, difficulty to achieve symptoms control with PDN/AZA Rituximab as effective emerging drug for long-term immunotherapy	Good control achieved with PDN, AZA (or other traditional immunosuppressant)

AChR-MG, anti-acetylcholine receptor Myasthenia Gravis, MuSK-MG, anti-Muscle specific tyrosine kinase Myasthenia Gravis; LRP4-MG, anti- low-density lipoprotein receptor–related protein 4 Myasthenia Gravis; SFEMG, Single-Fiber electromyography; AChE-Is, acetylcholinesterase inhibitors; PE, plasma exchange; IVIG, Intravenous immunoglobulin; PDN, prednisone; AZA, Azathioprine.

MuSK-MG, recently 3,4-diaminopyridine (3,4-DAP), ephedrine, and albuterol have been considered. The use of 3,4-DAP in MuSK- MG patients has been described as mildly to moderately effective, with no remarkable side effects (32). There is only a report demonstrating a clinical improvement in MuSK-MG due to the administration of both ephedrine and albuterol, two sympathomimetics agents commonly used to treat some phenotypes of congenital myasthenic syndromes (33).

Immunosuppression

Immunosuppression still represents the mainstay of therapy for MuSK-MG. It is well-known that steroids have a prompt and effective response, but they are burdened by long-term side effects.

A high dose of prednisone, in combination with plasma exchange, is generally recommended for patients experiencing life-threatening weakness or suffering from severe disease deterioration. In these patients, intravenous immunoglobulin should also be considered (27).

Traditional immunosuppressants (azathioprine, mycophenolate, tacrolimus, methotrexate, and cyclosporine), in common clinical practice, have been administered with success in MuSK-MG patients as steroid-sparing agents, but it is usually more difficult to achieve and to ensure long-term and complete control of symptoms (34). It is important to consider that 10–15% of MuSK- MG patients have a refractory disease or suffer from relapses on tapering immunosuppressive medication.

The management of this percentage of patients who do not respond to steroids or traditional immunosuppressants is often difficult. In the previous years, clinical trials and evidences from observational prospective studies encourage the use of monoclonal Ab such as rituximab (RTX), a chimeric anti-CD20 monoclonal Ab (35–37). A significant number of MuSK-MG patients showed a greater and sustained improvement of symptoms after RTX administration, compared to those patients who do not receive RTX administration (37). Immunosuppressants can be reduced or even stopped (37). Topakian et al. confirmed the safety and efficacy of RTX in a large cohort of both AChR-MG and MuSK-MG patients; furthermore, these authors demonstrated a significantly higher rate of remission in patients with MuSK-MG compared to AChR-MG ones (38).

In light of common clinical practice and of the above-mentioned results, a recent consensus recommends RTX as an early therapeutic option in MuSK-MG, suggesting its possible role as a steroid-sparing agent since the beginning of the disease (39). RTX has a good safety profile; however, side effects such as myocardial infarction, spondylodiscitis, agranulocytosis, and two cases of progressive multifocal leukoencephalopathy in MG patients have been reported (40, 41).

SUMMARY

MuSK-MG is a distinctive, frequently more severe, subtype of MG. Onset is usually acute and typically bulbar, with rapid progression of symptoms within a few weeks. Clinical presentation can be atypical: neck weakness, for example, as onset symptom could be misleading, causing a delay in diagnosis. MuSK-Ab testing confirms the diagnosis when the clinical picture is highly suggestive. SFEMG plays an important role in diagnosing MuSK-MG, and we underline the importance to focus it on the mainly affected muscles to precociously detect alterations.

Response to treatment is often different from that expected in MG patients and achieving a regression of symptoms could be quite challenging. Among immunotherapies, prednisone, plasmapheresis, and RTX are the cornerstones of treatment for MuSK-MG. The main features of MuSK-MG are summarized and compared to the main other subtypes of MG (AChR-MG and LRP4-MG) in **Table 1**.

CONSENT FOR PUBLICATION

Written informed consent was obtained from the patient to acquire and publish photos.

AUTHOR CONTRIBUTIONS

CR conceived the review. CR and CB were equally involved in literature search, figure and table preparation, and drafted and wrote the manuscript. AT and GV reviewed and revised the final draft of the manuscript. All authors have both approved the submitted version of the manuscript and agreed to be personally accountable for their own contributions.

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REVIEW
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Management of Juvenile Myasthenia Gravis

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Juvenile Myasthenia Gravis (JMG) is a rare disorder, defined as myasthenia gravis in children younger than 18 years of age. While clinical phenotypes are similar to adults, there are a number of caveats that influence management: broader differential diagnoses; higher rates of spontaneous remission; and the need to initiate appropriate treatment early, to avoid the long-term physical and psychosocial morbidity. Current practice is taken from treatment guidelines for adult MG or individual experience, with considerable variability seen across centers. We discuss our approach to treating JMG, in a large specialist JMG service, and review currently available evidence and highlight potential areas for future research. First-line treatment of generalized JMG is symptomatic management with pyridostigmine, but early use of immunosuppression, where good control is not achieved is important. Oral prednisolone is used as first-line immunosuppression with appropriate prevention and monitoring of side effects. Second-line therapies including azathioprine and mycophenolate may be considered where there is: no response to steroids, inability to wean to a reasonable minimum effective dose or if side-effects are intolerable. Management of ocular JMG is similar, but requires close involvement of ophthalmology in young children to prevent amblyopia. Muscle-specific tyrosine kinase (MuSK)-JMG show a poorer response to pyridostigmine and anecdotal evidence suggests that rituximab should be considered as second-line immunosuppression. Thymectomy is indicated in any patient with a thymoma, and consideration should be given in acetylcholine receptor (AChR) positive JMG allowing time for spontaneous remission. The benefit is less clear in ocular JMG and is not advised in MuSK-JMG. Children experiencing a myasthenic crisis require urgent hospital admission with access to the intensive care unit. PLEX is preferred over IVIG due to rapid onset of action, but this needs to be balanced with feasibility in very young children. Key questions remain in the management of JMG: when to initiate both first- and second-line treatments, choosing between steroid-sparing agents, and determining the optimal dose and treatment duration. We feel that given the rarity of this disease, the establishment of national registries and collaboration across groups will be needed to address these issues and facilitate future drug trials in JMG.

Keywords: juvenile myasthenia gravis, treatment, thymectomy, immunosuppression, autoantibodies, generalized myasthenia gravis, ocular myasthenia gravis, myasthenic crises

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INTRODUCTION

Autoimmune myasthenia gravis (MG), is a disorder of neuromuscular transmission, resulting from antibodies to components of the muscle endplate that cause impaired synaptic transmission. In the majority of cases, these antibodies are directed toward the acetylcholine receptor (AChR), but they can also target muscle specific kinase (MuSK) and possibly to receptor related low density lipoprotein-4. The clinical hallmark is fatigueable muscle weakness which can be limited to the ocular muscles or more generalized. Juvenile Myasthenia Gravis (JMG) is defined as myasthenia gravis in children younger than 18 years of age. While clinical phenotypes are similar to adults, there are a number of caveats unique to JMG, that need to be considered when evaluating these patients. In this article, we will give a brief overview of the epidemiology, pathophysiology, clinical presentation, and diagnosis of JMG and a more comprehensive review of currently available therapies and approach to management, and finally outline our JMG treatment paradigm.

EPIDEMIOLOGY OF JMG

Population-based studies to determine the incidence of JMG demonstrate the rarity of this disorder, and racial variability. A large nationwide study in the UK identified 101 children (<18 years) who had antibody positive JMG (with 95% AChR and 5% MuSK antibodies), equating to an incidence rate of 1.5/million person years (1). Of this cohort only 20% were under 10 years of age when diagnosed. Similarly, a national Norwegian study of 43 incident cases, showed an incidence rate of 1.6/million person years but this cohort included both seropositive and seronegative cases (2). Again lower rates were seen in pre-pubertal children (<12 years) of 0.9/million person-years. In a long-term follow-up study in Denmark from 1996-2009, the incidence rate was 7 times higher in those aged 10–19 years compared to those aged 0–9 years (2.2/million v 0.3/million person-years) (3). The incidence in Olmstead county, USA, was determined as part of a larger cohort study as 1.2/million person years but this was only based on two confirmed cases (4). In contrast to these studies, a national study of AChR antibody positive MG from South Africa, estimated to account for 75% of cases, showed a higher incidence rate of 4.3/million person-years in those under 20 years (5). Similar rates were seen in those under 10 years and those aged 10–14 years, of 3.3 and 2.9/million person-years, respectively. A large population-based study in Southern China showed that 45% of cases had onset in childhood (<14 years) (6). In Taiwan, incidence was highest amongst the 0–4 year age group at 8.9/million cases, dropping to 3.7/million in the 10–14 year age group (7). This peak in the 0–4 year age group, was also seen in a nationwide Japanese survey of MG prevalence (8). Taken together these studies highlight racial differences in JMG, with higher rates overall, and in children under 10 years, seen in the predominantly Black South African population, and amongst Chinese, Taiwanese and Japanese populations, when compared to European studies of majority Caucasian populations (1–3, 5, 7).

In a UK cohort study of JMG, the proportion of Black and Asian children was disproportionately raised compared to the background population of the UK, supporting these differences are driven at least in part by genetics, rather than geographical location (9). Higher rates in females were seen in all studies, except in the pre-pubertal South African population, again indicating how race influences JMG epidemiology.

PATHOPHYSIOLOGY OF JMG

MG is mediated by antibodies to components of the neuromuscular junction which disrupt synaptic transmission. In the majority of cases of MG, pathogenic antibodies to the nicotinic AChR are seen, which induce loss of functional AChRs, through a number of mechanisms. The principle effect is through complement-mediated destruction of the motor end-plate (10). They also cause internalization, and subsequent degradation of the AChRs and can directly interfere with ACh-binding to the receptor (11, 12). In MuSK MG, the exact pathogenic mechanism is less clear but felt to be different to AChR-Ab (12, 13). MuSK antibodies are monovalent and largely of the non-complement fixing IgG4 sub-class, so are unable to bind complement or cross-link and internalize AChRs. Passive and active transfer experiments have shown a reduction in AChRs (14). It has been proposed that MuSK-Ab, act pre-synaptically, interfering with LRP4 function, with the consequent dispersal of AChR clusters (15).

CLINICAL FEATURES OF JMG

JMG can exist as a purely ocular form, or with more generalized skeletal muscle involvement. In children, the majority will present with ptosis and a variable degree of ophthalmoplegia, which can be markedly asymmetric which may help distinguish from genetic causes of myasthenia (16, 17). The ocular features can be mild and variable and it is important to assess for fatigue with prolonged up gaze (1 min) during the clinical examination. There are a number of useful signs on examination, that can be supportive of ocular MG, with variable sensitivity, including: Cogan's lid twitch sign (a brief twitch of the eyelid as it overshoots, as eyes return to the primary position from prolonged downgaze), improvement in ptosis after orbital cooling (placing an icepack over the eye for up to 5 min), and the "curtain sign" (worsening ptosis in the least affected eye, after lifting the worst affected eye) (18). Recognition, appropriate treatment, and prompt referral to ophthalmology is important to avoid long-term sequelae such as strabismus and amblyopia (19). In more generalized forms, patients may note proximal muscle weakness manifesting as difficulty getting up from floor, running, going up stairs, or lifting their arms above their heads. They may also have signs of bulbar and respiratory involvement including dysarthria, taking long periods to complete meals, difficulty swallowing, and shortness of breath. Symptoms may fluctuate throughout the day, but are typically better in the morning or after periods of rest. In MuSK-JMG, onset is said to be typically acute with predominant bulbar involvement and

early respiratory crises, although ocular onset which often then spreads to become more generalized is not uncommon (13). The presence of both myokymia and fasciculations can also be indicative of this condition. Similar to adult MuSK-MG there is a strong female predominance with 89% of cases female in the largest published pediatric case series (20).

Children presenting with purely ocular JMG, may go on to develop generalized JMG. The majority of cases who convert do so within 6 months of symptom onset and occurs rarely if ocular symptoms persist in isolation for longer than 2 years (17, 21). The prevalence of pure ocular JMG and the proportion who convert to generalized JMG varies across studies and appears to be strongly influenced by race, with pure ocular JMG accounting for up to 90% of Asian cohorts (8, 9, 21–23). Ocular JMG is also associated with younger age at onset with higher rates seen in pre-pubertal children, regardless of race (9, 21–24). Post-pubertal JMG more closely mirrors adult MG with a greater proportion of generalized onset and lower rates of spontaneous remission.

Remission rates vary across studies but are generally higher than in adult populations. In a large Norwegian case series 5 out of 63 experienced a spontaneous remission, accounting for 14% and 5% of the pre- and post-pubertal cohorts, respectively (21). In the same cohort 51% achieved complete stable remission (CSR) defined as off treatment for at least 1 year and no signs or symptoms of MG, again higher in pre-pubertal children. In an English series of 74 patients, 23% achieved CSR (9). Antibodies to clustered AChR proved to be the only significant predictive factor of a drug-free remission. Only 17%, of 424 Chinese children, who were followed up for a minimum of 5 years, achieved a CSR (22). Interestingly, 55% had achieved CSR for a minimum of 12 months during the course of their follow-up, but this was not sustained, highlighting the relapsing nature of MG. Discontinuing medication was reported as the commonest triggering event, although no information was available on the speed at which the treatments were withdrawn and the time from discontinuation to symptom onset varied hugely, at 1 month to 21 years.

A broad differential exists in children including congenital myopathies, mitochondrial cytopathies, acquired demyelinating neuropathies, and congenital myasthenic syndromes and careful evaluation particularly in pure ocular forms or antibody negative cases is needed. Features that may suggest an alternative diagnosis include positive family history, presence of symptoms from birth or early infancy, muscle contractures, scoliosis, and no response to symptomatic or immunosuppressive therapies.

Transient neonatal myasthenia results from the passive transfer of maternal antibodies *in utero* and has been reported in 10–20% of children born to mothers with MG (25). Infants can present with generalized hypotonia, weak cry, poor suck, ptosis, and in rare cases respiratory insufficiency that may require ventilation. It is usually self-limiting, with symptoms typically beginning 48 h after birth and in general resolve over weeks to months. In rare cases a persistent myopathy has been described (26). It is felt to be due to the loss or inactivation of AChR at a critical time during fetal development and has been termed fetal AChR inactivation syndrome (FARIS).

DIAGNOSIS

The diagnosis of JMG is primarily based on the clinical picture, but positive antibodies and abnormal neurophysiology can support the clinical impression.

Serology

Serological testing is useful adjunct in the diagnosis of JMG. Autoantibodies targeting the AChR are the most common and there are a number commercially available tests using radioimmunoprecipitation assay (RAI) or enzyme linked immunosorbent assay techniques. In JMG cohorts the frequency of AChR antibodies can vary from 70 to 80%, and is typically lower than adult MG cohorts (9, 21, 24, 27). Antibodies are more likely to be seen in generalized JMG when compared to ocular JMG, and given the increased prevalence of pure ocular MG in JMG cohorts, this likely accounts for the higher rate of seronegative cases (9, 27). It is important to repeatedly test seronegative patients at 6 monthly intervals as delayed seroconversion can be seen up to 5 years after onset and particularly in pre-pubertal children (21, 28).

Cell-based assays which detect clustered AChRs are not commercially available but can increase the diagnostic yield in antibody negative cases (29). In a UK study of 74 JMG patients, 50% of seronegative cases were shown to have these antibodies on subsequent testing (9). A similar pattern was seen amongst a Chinese cohort, where 15/34 seronegative patients were positive for low-affinity AChR antibodies using a cell-based assay (30).

Patients who are negative for AChR antibody can also be tested for MuSK antibodies which account for 5–8% of all MG patients and presents with a distinct phenotype as previously discussed (13). Recently, a MuSK cell-based assay has been developed, which when combined with an IgG Fc gamma-specific secondary antibody, detected low-affinity MuSK antibodies in 14/169 seronegative patients (31). Sensitivity and specificity will need to be confirmed in further studies but represents a promising development in reducing the number of truly seronegative cases. Autoantibodies to low density lipoprotein 4 (LRP4), agrin, and ColQ have been described in association with MG, however, their specificity remains to be determined, pathogenic mechanisms have not been fully elucidated, and there has been no animal models showing disease in response to passive transfer of antibodies which is an essential criterion in determining whether antibodies are truly pathogenic (32).

Neurophysiology

Neurophysiology can play an essential role in the diagnosis of neuromuscular dysfunction, but can be technically challenging in young children and results will depend on techniques available and operator skill (33). Both repetitive nerve stimulation (RNS) and single-fiber electromyography (SFEMG) are recognized screening tests for myasthenia. The sensitivity of SFEMG approaches 95% but requires volitional muscle activation, which is often not possible or inconsistent in young children. Stimulated potential analysis using concentric needle electrodes (SPACE), is an alternative technique where the nerve is stimulated and signals recorded, eliminating the need for patient co-operation

and has been shown to be useful in children, with a sensitivity of up to 92% achieved in patients with JMG (34). Although more sensitive the authors find that the specificity of the SFEMG is lower than for RNS (the latter requiring >10% decrement with 3 Hz stimulation), such that a negative SFEMG is strong support against, and the presence of decrement on RNS strong support for, myasthenia. The sensitivity of RNS is increased if performed in a weak muscle when a negative test suggests the weakness may not be myasthenic and can be useful in the clinic when a MG patient has superimposed functional weakness.

Edrophonium Testing

Edrophonium is a quick-acting, short-lasting, anticholinesterase inhibitor. It can be administered intravenously as a diagnostic test for JMG, where you would expect to see, transient improvement of symptoms. It is most useful, in the setting of ptosis, ophthalmoplegia or dysarthria, as these symptoms can be easily and quickly assessed. Its use in clinical practice is now limited, due to the potential side-effects including bradycardia, and increased reliability of neurophysiological tests and antibody testing. In order to carry out the test the child needs continuous cardiac monitoring with appropriate resuscitation equipment at the bedside.

TREATMENT OF JMG

There are no internationally accepted standards of care for JMG, although this issue was recently addressed and recommendations published by the European Neuromuscular Center workshop study group (35). Management should be delivered by a multidisciplinary team, encompassing pediatric neurology and ophthalmology services with expertise in JMG as well as physiotherapy, occupational therapy, speech and language therapy, dietetics, and psychology input. Treatment typically involves a combination of symptomatic and immunosuppressive therapies, with thymectomy in appropriate cases.

Supportive Therapy

Despite limited published evidence, we feel supportive management should be initiated early in the disease course, both to manage the impact of the disease itself on physical and mental health but also to mitigate the potential medication side-effects, particularly of corticosteroids. It is important to consider early input from allied health, with regard to diet and lifestyle. Both children and adults need to be cautious to avoid excessive weight gain in the context of reduced physical activity and advised with regard to healthy snacks and increased fruit and vegetable intake. The benefits of physical activity need to be highlighted, usually in the form of a graded exercise program, being mindful to avoid excessive fatigable weakness. Close communication with schools is important, and educational care plans may need to implemented to ensure that these children do not become unduly disadvantaged in accessing the educational curriculum. Varicella vaccination should be considered prior to initiation of immunosuppression, if clinically safe to delay. Annual influenza vaccination with inactivated vaccine is recommended. A recent study, suggested an association between a live-attenuated Japanese encephalitis vaccine and the high prevalence rate of JMG in China (36). The hypothesis was supported by a mouse model but has not been replicated by other groups and at this time, we would not contradict the use of live-attenuated vaccines where required. Intercurrent illnesses should be managed promptly. Families need to be provided with a list of medications that affect the neuromuscular junction, with potential to worsen the condition, and should therefore be avoided (**Table 1**). Regular ophthalmology input is needed, given the risk of amblyopia in this population. Discussing the potential psychological impact in the early stages may lead to early recognition and management, preventing low mood, depression-related fatigue and tiredness being mistaken for myasthenic symptoms and subsequent overtreatment.

TABLE 1 | List of medications that may cause worsening of underlying myasthenia gravis.

Type of medication	Medication
Antibiotics	Aminoglycosides—gentamicin, amikacin, streptomycin, telithromycin* Quinolones—ciprofloxacin, levofloxacin Tetracyclines—tetracycline, doxycycline, minocycline
Antimalarials	Chloroquine, hydroxychloroquine
Anesthetic agents	Muscle relaxants—succinylcholine
Antihypertensives	Beta-blockers—propranolol, bisoprolol, sotalol, metoprolol Calcium-channel blockers—verapamil, lercandipine, amlodipine
Antiarrhythmics	Procainamide, Quinidine
Rheumatic drugs	Penicillamine
Immunotherapy	Checkpoint inhibitors
Antipsychotics	Chlorpromazine, risperidone
Miscellaneous	Magnesium salts, Botulinum toxin

*It is important to note that this list is not exhaustive and should serve as a guide. Not all medications will exacerbate myasthenic symptoms to the same degree, and some of these medications can be used cautiously in patients with MG without deleterious effects, particularly is their MG is well-controlled. Common examples in each class of medication are listed but any medication within a class should be considered as carrying the same risk. *Has been associated with deaths in MG and should never be given.*

Symptomatic Therapy

Cholinesterase inhibitors (ChE-I) are used first-line as symptomatic treatment in JMG. Pyridostigmine is a non-selective ChE-I and is the most widely used. These drugs act at the neuromuscular junction, where they interfere with the breakdown of acetylcholine (ACh) increasing its availability to bind to post-synaptic nicotinic receptors. A Cochrane review in 2014 concluded that the evidence from observational studies clearly show significant benefit in MG, and it would not be justified to conduct placebo-controlled study in this patient group (37). We advocate starting at 0.5–1 mg/kg, taken 3–4 times per day, and this can be increased to up to 1.5 mg/kg 5 times per day (maximum 450 mg/day). Times and doses can be adjusted to an individual child's time-table, such as taking their dose 30–60 min before significant physical activity. Slow-release forms of pyridostigmine are available but they come in high dose

preparations (180 mg tablets) which limits their use in children and long acting preparations may build up over time and make it difficult to assess immediate response to medication. Failure to suppress symptoms with doses around 1 mg/kg 4 times per day, should lead to consideration of immunosuppressive therapy, taking into account the severity of symptoms. It should be noted that pyridostigmine works within hours and thus the drug effectiveness can be assessed within a couple of weeks. Decision to initiate immunosuppression, should not be delayed for several months, whilst increasing pyridostigmine to maximal tolerated dose. This can lead to a delay in resolution of weakness, and subsequently, impact on the social, and educational activities of the child.

Side-effects relate to excessive cholinergic stimulation, and include abdominal cramps, diarrhea, hypersalivation, sweating, blurred vision, bradycardia, hypotension, and bronchoconstriction. Anti-cholinergic medications that do not bind to the nicotinic receptor, such as propantheline and glycopyrrolate can be a useful adjunct to manage these symptoms and increase tolerability. It should be noted, that a poorer response to ChE-I has been seen in MuSK-MG patients with higher rates of side-effects on standard doses and in some cases clinical deterioration (13).

Immunosuppressive Therapy

There are no formal guidelines for the use of immunosuppressive therapy in JMG and current practice has been taken from adult guidelines and expert opinions based on individual experience (35, 38, 39).

Despite the lack of clinical trials, prednisolone is accepted as the first-line immunosuppressive therapy in JMG (38). The recommended starting dose is 0.5 mg/kg alternate days. Higher doses can be associated with worsening of MG symptoms and should only be attempted in the in-patient hospital setting. Doses are gradually uptitrated, pending response, to a maximum of 1.5 mg/kg alternate days (maximum: 100 mg) or 1 mg/kg/day (maximum: 60 mg). Lower doses may be required in pure ocular JMG, although outcomes in patients with ophthalmoparesis were better, if patients were treated earlier with higher dose steroids (40). A benefit is usually seen within weeks but it can take up to 6 months or longer to see the full effect of a treatment dose. The goal of therapy is to induce remission and then to wean off ChE-I first and then slowly reduce the corticosteroids monthly to the lowest effective maintenance dose. We would typically reduce by 5 mg every month to 15–20 mg alternate days and then reduce by 1 mg per month to stop. If the maximum prednisolone dose has been used, then this wean can take over a year. Sequential or concurrent initiation of corticosteroids with ChE-I was discussed at a recent expert workshop in JMG, with the majority favoring a short trial of ChE-I prior to the introduction of steroids in mild JMG and a consensus that they should be commenced concurrently in moderate or severe JMG particularly if bulbar symptoms were present (35).

There a numerous adverse effects associated with steroid use, including mood and behavioral disturbance, sleep disruption, weight gain, growth restriction, hypertension, diabetes, osteoporosis, infections, and gastric-esophageal reflux disease (GORD). In order to mitigate these effects all children should be commenced on vitamin D as per local guidelines for bone health and consideration given to gastric protection. Children and their families need to be given advice on potential for weight gain, and healthy eating (increasing vegetable portions and healthy snacks) and exercise, discussed to prevent this. These side-effects can cause psychological stress, particularly in adolescents and psychological supports should be offered. Regular monitoring of blood pressure, growth velocity, and weight should be carried out while on treatment and those on long-term steroids should have a bone density assessment.

Second-line therapies or steroid-sparing agents may be introduced when (1) there is no response to steroids, (2) an inability to wean steroids to a reasonable minimum effective dose, or (3) if side-effects of steroid treatment become intolerable. These include but are not limited to azathioprine, mycophenolate mofetil, tacrolimus, rituximab, cyclosporine, and cyclophosphamide and use may vary depending on an individual center's experience. Intravenous immunoglobulin (IVIG) and plasma exchange (PLEX) have also been used as maintenance therapy (41).

Use of azathioprine in JMG is largely guided by expert opinion (35, 38, 39). A number of case series confirm its use, in both generalized and ocular JMG but none were designed to assess its efficacy (17, 27, 42, 43). It is a purine analog that acts by suppressing B cell and T cell proliferation. It is converted to its active metabolite 6-mercaptopurine by the enzyme thiopurine methyltransferase (TPMT). All patients should be screened for TPMT activity prior to starting azathioprine, as reports suggest enzyme deficiency, is more likely to be associated with myelosuppression. It is commenced at a dose of 1 mg/kg either daily or twice daily and can be increased by 0.5 mg/kg every 2–4 weeks to 2.5 mg/kg/day (35, 39). Azathioprine is typically used in combination with steroids and has been shown in a clinical trial of adult MG patients to be a useful steroid-sparing agent but its effects can take up to 12 months to become fully effective (44). The use of azathioprine may also allow a reduction in dose or tailing off of prednisolone.

The side-effects seen with azathioprine use include GI disturbance, liver dysfunction and myelosuppression. Patients should have a full blood count and liver function tests weekly, until on the maintenance dose for 8 weeks, and then 3 monthly if test parameters remain stable. Azathioprine is felt to be safe in pregnancy and thus is a good choice for female children of all ages, likely to need long term treatment (45).

Mycophenolate mofetil selectively inhibits B cell and T cell proliferation, by targeting cells that rely on the *de novo* pathway for purine synthesis. A large international phase III trial in adult MG patients failed to reach its primary endpoint, although the study period was likely too short at 36 weeks (46). This is supported by a retrospective study of 102 AChR-Ab positive patients, in which 80% of patients who were followed for longer than 24 months had improved, and 56% had been able to discontinue steroids (47). Some pediatric patients were included in this cohort but no breakdown of response was given. Given the lack of evidence, mycophenolate is considered a second-line agent and used if patients are intolerant or fail to respond to

azathioprine. Common side-effects include nausea, vomiting, diarrhea, and less frequently leukopenia. Mycophenolate has been shown to be teratogenic and use is generally avoided in females of childbearing age as a long term option (48).

Tacrolimus is a calcineurin inhibitor which provides an immunosuppressive effect by modulation of T cell activity and support of antibody production in B cells. It is from the same class as cyclosporine but is felt to be less nephrotoxic. A systematic review of all prospective studies in adult MG suggested a beneficial effect on MG symptoms and facilitated the reduction of overall steroid dose (49). The studies were largely carried out in Asian populations which may limit the generalizability of these results. Long-term follow-up was available in some studies with no safety signal generated. An open-label trial in China, looked at the safety and efficacy of tacrolimus in 13 steroid-refractory JMG patients (50). The majority of patients had ocular MG, were aged 7–13 years and mean disease duration was 42 months. At 12 months follow-up, 10 patients were able to discontinue steroids and an improvement was seen on QMG and other quality of life measures. There is also a number of case reports that suggest a benefit in treatment refractory patients (51–54). Side-effects of tacrolimus include hypertension, headache, tremor, renal impairment, new-onset diabetes mellitus, diarrhea, malignancy (e.g., lymphoma and dermatologic), and increased risk of infection.

Rituximab is a chimeric monoclonal antibody, which acts by binding to CD20 on B cells and triggering cell death. It is given intravenously, at a dose of 375 mg/m^2/week for 4 weeks or two doses of 750 mg/m^2 (up to maximum 1 g) 2 weeks apart. If required, repeat doses may be given, typically when the CD19+ CD20+ B cells begin to rise (usually around 6 months). Its use in MG is typically reserved for treatment refractory cases. A recent systematic review of rituximab use in adult MG identified 108 adult MG patients, treated with rituximab in 9 case series and one uncontrolled trial (55). The review concluded that all studies demonstrated an improvement in MG symptoms and the majority of patients were able to reduce concomitant immunosuppressive drugs. MuSK-MG patients tended to respond better.

The pediatric literature for rituximab is limited to case series or case reports, and generally shows favorable results in treatment refractory JMG (28, 42, 56–58). The largest series, reported on rituximab use in 5 children with refractory JMG, 3 AChR-Ab positive, and 2 MuSK-Ab positive (42). It was described as well-tolerated and two children improved significantly, while the remainder had a partial response. Complete remission has been reported in one case of MuSK-JMG (59). Side-effects were not reported but rituximab has been associated with higher rates of infection including opportunistic infections such as progressive multifocal leukoencephalopathy (PML), and may cause long-term B cell depletion and hypogammaglobulinaemia. These studies, taken together, support a role for rituximab in the treatment of refractory JMG, particularly MuSK-JMG, but there a number of issues around its use that are unresolved including: duration of treatment, timing of future doses, and repeating cycles in cases where there is no clear response.

Cyclosporine, methotrexate and cyclophosphamide can be used as alternative immunosuppressive agents in MG but typically in treatment refractory cases, where other options have failed, due to lack of confirmed efficacy or concern around the side-effect profile. Cyclosporine has been shown to be effective as a steroid-sparing agent in adult MG patients but high rates of side-effects, particularly renal toxicity limit its use (60, 61). Efficacy of methotrexate as a steroid-sparing agent in adult MG was shown in an uncontrolled trial, but a more recent randomized controlled study failed to achieve this primary end-point, although the authors argue the study methodology may have been flawed (62, 63). Cyclophosphamide has been shown to be effective in inducing remission in treatment refractory adult MG patients, but is associated with high relapse rates unless used in conjunction with other immunomodulating therapy (64–66). There are significant side-effects associated with cyclophosphamide use, including bladder cancer and hematological malignancies, as well as possible implications on fertility. The risk is determined by the cumulative dose over time, and all patients need to be counseled with regard to these risks prior to initiating therapy. There are no studies looking at outcomes of these medications in JMG.

Intravenous immunoglobulin (IVIG) is used in many neurological conditions due to its diverse mechanisms of action (67). It has been shown to inhibit complement binding, neutralize pathogenic cytokines, downregulate antibody production, enhance remyelination and modulate Fc-receptor-mediated phagocytosis, and T cell function. Response to treatment is typically seen within days but can take a couple of weeks to be maximal. These characteristics mean it is useful in treating exacerbations or to optimize function prior to thymectomy as demonstrated in case series of JMG patients (41, 68, 69). It has also been shown to be effective as a maintenance therapy but availability due to a worldwide shortage and resource implications due to high cost need to be considered (41). The typical dose is 1 g/kg given intravenously and repeated over 2 days. In general the maintenance dose is 1 g/kg repeated at 4–6 weekly intervals but this will depend on patient response. Side-effects include infusion reactions, rash, headache, hypertension, increased risk of thrombosis and aseptic meningitis.

The principal mechanism of action of plasma exchange (PLEX) is the removal of pathogenic autoantibodies from the circulating blood stream (70). It has also been suggested that it may affect lymphocyte proliferation and function. The indications for use are similar to IVIG, mainly in the treatment of exacerbations and inducing stability pre-surgery. Although studies in adult patients, have shown no difference in clinical outcomes with either IVIG or PLEX (71) most myasthenia experts feel PLEX is probably more effective in most patients and may be quicker acting in practice. In a study comparing outcomes between generalized JMG patients treated with either PLEX or IVIG as a maintenance therapy, a higher response rate to treatment was seen in PLEX group but numbers in each group were small (17 in total) (41). Favorable outcomes have also been seen in MuSK-MG. In a multicentre study of 110 patients, improvement was seen in 93% of those who were treated with PLEX compared to 61% who received IVIG (72). The response

to PLEX was described as rapid and thus is preferred in the treatment of exacerbations. A typical course of treatment is 3–5 exchanges on alternate days and often requires placement of a central venous catheter. The major limiting factor in small children is inadequate venous access and as such IVIG may be more practical in that setting.

Thymectomy

The role of the thymus in MG pathogenesis is supported by a number of factors, including the high rates of thymic pathology seen in MG patients, correlation between anti-AChR antibody levels and the degree of follicular hyperplasia and favorable outcomes post-thymectomy (73). Thymic hyperplasia is not uncommon in JMG but thymoma is rare as demonstrated by a number of large cohort studies (22, 74, 75). All children should have thymic imaging (CT or MRI) regardless of clinical presentation.

The presence of a thymoma is an absolute indication for thymectomy, but its role in non-thymomatous MG depends on the antibody status, the age and disease duration, and subtype of MG. A benefit has been shown in a single international randomized controlled trial of transsternal thymectomy of adult MG patients (aged 18–65 years), which demonstrated better clinical outcomes and reduced medication requirements, in those who underwent thymectomy and corticosteroids compared to corticosteroids alone (76). All patients within this study had AChR-Ab positive generalized MG and were within 5 years of diagnosis. Now that VATs thymectomy is the surgical technique of choice, with lower associated morbidity, it may be considered in patients outside those included in the trial i.e., those with ocular MG, and those without detectable AChR-Abs who are MUSK antibody negative (such patients may have undetectable AChR-Abs and thymic hyperplasia) (77). While there has been no trials in pediatric patients, a recent systematic review, which included 488 patients who underwent thymectomy, showed that the procedure was well-tolerated and 77% symptomatically improved after the surgery (78). Furthermore, sustained remission was seen in 29%. Patients with pure ocular symptoms accounted for half of the total cohort. A small number of studies, have attempted to compare surgical and non-surgical management, with discordant results, but these retrospective cohorts were not matched for age, sex, disease features, duration of symptoms, etc. limiting the generalizability of results (21, 78). A recent study showed that thymectomy did not influence conversion from ocular to generalized disease (22).

Despite the lack of prospective studies evaluating thymectomy in JMG, it is generally accepted that thymectomy is considered as part of the initial management of all AChR-Ab positive generalized JMG patients. Its role is less clear in children with milder disease due to the higher rate of spontaneous remission in this group. In our opinion, it should also be considered in AChR-Ab positive ocular JMG patients, who fail to respond to a reasonable trial of immunosuppression, to avoid the long-term sequelae of these treatments. Earlier surgical intervention (within 2 years of symptom onset) has been associated with better outcomes (79). This needs to be balanced against patient age; with higher rates of spontaneous remission seen in pre-pubertal children and also allowing time for immune maturation in very young children. A recent review, of neonatal thymectomy for congenital heart disease, has shown that in the short-term, the rate of infections and autoimmunity do not appear to be increased in this patient cohort but long-term follow-up studies are lacking (80). Patients with MG are at an increased risk of developing other autoimmune diseases, in particular autoimmune rheumatological disease (ARD) including rheumatoid arthritis (RA) and systemic lupus erythematous (SLE) (81). In a large Taiwanese study looking at 6478 MG patients, the risk of developing an ARD was 6 times higher than age- and sex-matched controls. Analysis of those who underwent thymectomy demonstrated risk was 10 times higher than age- and sex-matched controls and no increased risk was seen in those who underwent PLEX. It needs to borne in mind, that those undergoing thymectomy are more likely to be antibody positive and have thymic hyperplasia, which may be independent risk factors for developing other autoimmune diseases. There is no indication for thymectomy in MuSK-JMG and its role in seronegative cases is unclear.

Further advances have seen a move toward minimally invasive techniques which demonstrate similar clinical outcomes and have the advantage of lower morbidity and shorter length of stay (82, 83). The limitation of these techniques is that it may not always be possible to achieve a complete resection.

Other Potential Treatments

3,4-diaminopyridine (3,4-DAP) is a non-specific voltage-dependent potassium channel (Kv1.5) blocker, which causes depolarization of the presynaptic membrane at the NMJ and delays nerve repolarization, thus increasing quantal release of ACh. It is used in the treatment of Lambert-Eaton myasthenic syndrome (LEMS) and congenital myasthenia (84, 85). A recent phase IIb study in 10 adult patients with MuSK-MG, showed that it was safe and an improvement was seen across both objective measures of muscle strength and patient reported outcomes (86). The only treatment-related side-effect was transient paresthesia, which were reported in 60%, but did not lead to discontinuation of treatment.

Eculizumab is a humanized monoclonal antibody that targets complement protein C5 and inhibits terminal complement-mediated damage at the neuromuscular junction. It is licensed by both the Food and Drug Administration (FDA) in the United States and the European Medicines Agency (EMA), for use in adults with refractory AChR-Ab positive generalized MG, following a large multi-center RCT (87). Despite the study not achieving its primary end-point, benefit was suggested in the secondary outcomes including QMG scores and quality of life measures. The high cost will likely limit its use. A clinical trial in pediatric patients is currently underway (NCT03759366). As MuSK antibodies are predominantly IgG4, which do not activate complement pathways, Eculizumab may not be an effective treatment in this group.

Management of Myasthenic Crises

Myasthenic crises result from significant neuromuscular weakness causing respiratory failure and a need for respiratory

support (35, 38, 39). The frequency of myasthenic crises in JMG is unknown but accounted for 10% in one case series (21). Features which are suggestive of an impending crisis include worsening bulbar dysfunction, drowsiness, dyspnoea, and marked global weakness. Children who display any signs of impending crisis need to be urgently assessed in a unit where ventilatory support is available if needed. In the early phase of respiratory failure non-invasive ventilation may be an option, but with established infection and atelectasis endotracheal intubation is likely to be needed (88). The first step is to identify any potential triggers. They need to be screened for underlying infections including aspiration pneumonia. Any recent changes in medications must be noted. A number of commonly used medications including some antibiotics can exacerbate myasthenic symptoms. A list has been outlined in **Table 1**. Recent dose adjustments and compliance with myasthenia therapy should also be assessed as a rapid up-titration or withdrawal can also trigger a flare-up of symptoms. Both PLEX and IVIG can be used acutely and the regime has been discussed in the above paragraphs. PLEX may be favored due rapid onset of action but venous access can be an issue in small children. Cholinesterase inhibitors can be held while ventilated, and steroids can be started at the top dose without concern of deterioration because they will have respiratory support.

Prescribing Considerations in Females of Child-Bearing Age

Given the high proportion of JMG in adolescent females, this is an important consideration when prescribing long-term immunosuppression in women of childbearing age. Clinical guidelines have been endorsed and published by the Association of British Neurologists (89). Pyridostigmine does not cross the placenta and has not been associated with fetal malformations and can be continued during pregnancy. The use of prednisolone, azathioprine and ciclosporin is also felt to be safe during pregnancy but mycophenolate and methotrexate should be avoided. The evidence on tacrolimus is less clear and the current license advocates use in pregnancy only, when no safer alternative is available. Data from transplant registries suggest no increased risk of congenital malformations, but high rates of pre-term delivery and low birthweight were seen, although both are common in this population who are often treated with multiple immunosuppressants (90). There are also a number of reports of transient hyperkalaemia in the neonate.

Summary of Treatment Recommendations

- JMG needs to be managed by a multidisciplinary team.
- Supportive management should be instituted early to improve both physical and psychological outcomes.
- First-line symptomatic treatment is with ChE-I, most commonly pyridostigmine starting at a dose of 0.5–1 mg/kg, taken 3- 4 times per day, and this can be increased to up to 1.5 mg/kg 5 times per day (maximum 450 mg/day).
- Corticosteroids are used as first-line immunosuppressive therapy. They should be gradually increased to a maximum of 1.5 mg/kg alternate days (maximum: 100 mg) or 1 mg/kg/day (maximum: 60 mg). In any child with significant weakness or bulbar symptoms, admission to hospital for rapid escalation of steroid treatment may need to be considered.
- Second-line therapies including azathioprine and mycophenolate may be considered where there is: no response to steroids, an inability to wean to a reasonable minimum effective dose or if side-effects are intolerable.
- There is evidence to suggest rituximab may be more effective in MuSK-MG and may be considered as second-line therapy.
- While IVIG and PLEX can be used as maintenance therapies, they are generally reserved for treatment of acute exacerbations or to optimize function prior to surgery due to accessibility and resource constraints.
- Thymoma is an absolute indication for thymectomy and consideration should be given to all patients with generalized JMG who are AChR positive. The role in ocular and seronegative cases is less clear and there is no indication in MuSK-JMG.
- Children experiencing a myasthenic crisis or with significant weakness require urgent hospital admission with access to the intensive care unit. PLEX is preferred over IVIG due to rapid onset of action, but this needs to be balanced with feasibility in very young children.

CONCLUSIONS

JMG is a rare disease, and evidence-based guidelines are lacking. In this review, we have critically assessed the currently available literature, and outlined a treatment paradigm, incorporating our experience in managing these patients as a specialist referral center (**Figure 1**). A number of questions remain, including when to initiate both first- and second-line treatments, choosing between steroid-sparing agents, and determining the optimal dose and treatment duration. There is a need for prospective studies to properly evaluate treatment regimes, but the rarity of the disease combined with the diversity of the condition itself, and the influences of race and gender, are likely to make this challenging. In order to achieve this, collaboration across centers and the establishment of international patient registries will be needed.

Case Studies

Case 1

A 2 year old Afro-Caribbean girl was referred to a pediatric ophthalmologist with unilateral resting ptosis of 30%. Her parents reported it had been present for 3 months and varied from day to day. There was evidence of fatigueability on prolonged upgaze but eye movements appeared full. She was unable to tolerate electrodiagnostic testing but AChR-Ab was positive. She was started on pyridostigmine with a good symptomatic response. She was reviewed by her ophthalmologist 6 months later and her eye exam was noted to be normal. Her parents still reported intermittent ptosis, especially when tired and at times though her left eye "drifted out." Due to the normal exam, a decision was made to wean her pyridostigmine, however, upon discontinuation, she experienced an acute worsening of her symptoms, with new onset double vision and bilateral ptosis. At this point she was prescribed 20 mg of prednisolone daily,

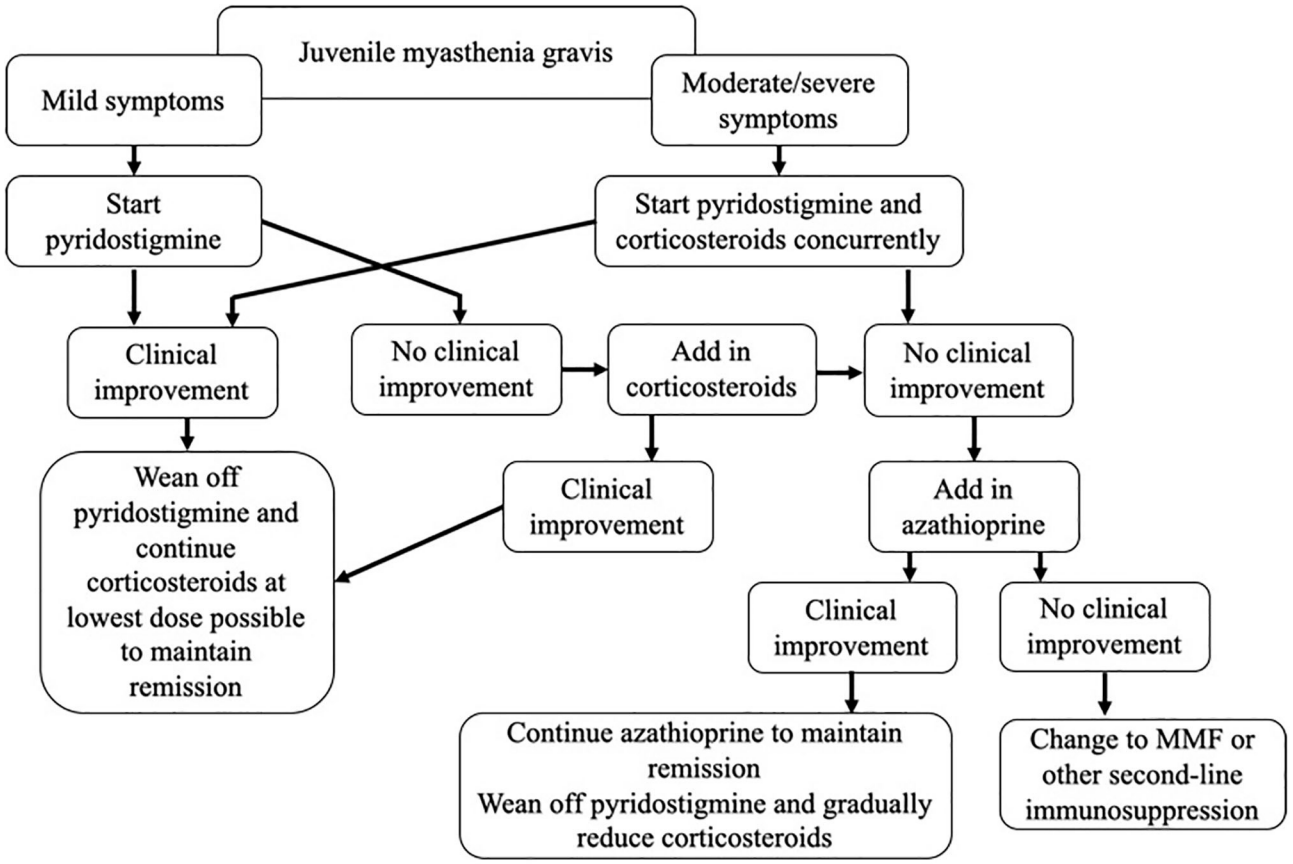

FIGURE 1 | Our approach to pharmacologic therapy in juvenile myasthenia gravis.

which was stopped at her 3 month review as she was felt to be in remission. Once off steroids, her symptoms quickly returned. She was referred to the pediatric neurology service and on exam, she was also noted to have features of generalized MG with difficulty getting up from the floor and lifting her arms above her head.

Comment

This case highlights a number of issues in managing JMG: the importance of not weaning treatment too early, especially when the history suggested breakthrough disease; the need for an adequate course of steroids and gradual tapering of the dose prior to discontinuation; the need for combined neurology and ophthalmology input as subtle signs of more generalized disease may have been missed at earlier assessments; and patients with ocular JMG are at greatest risk of converting to generalized disease within the first 2 years and need regular review over this time.

Case 2

A 13 year old Caucasian girl was referred to the neurology service with slurred speech, generalized weakness and fatigue, that had worsened over 6 weeks. She now became breathless on minimal exertion. Examination showed mild bilateral ptosis, normal eye movements, dysarthria (unable to count aloud to 10), and weakness of neck flexion and shoulder abduction. Her forced vital capacity was 50% normal. Her symptoms and exam were felt to be consistent with generalized MG and the severity raised concern for an impending myasthenic crisis. She was admitted to the neurology ward and anesthetic review was arranged. She was commenced on PLEX and concomitant pyridostigmine and oral steroids. She had a good symptomatic response to treatment and was discharged on a slow oral steroid taper, reducing by 10 mg every month to an initial maintenance of 20 mg on alternate days. Subsequent investigations showed she was AChR-Ab positive, her neurophysiology was consistent with a neuromuscular junction disorder and CT thorax was reported as showing no evidence of a thymoma.

Her first relapse occurred when her prednisolone was reduced to 30 mg alternate days, necessitating an increase in medication. She had developed a number of side-effects including weight gain and low mood. A decision was made to commence Azathioprine as a steroid-sparing agent. Her TPMT levels were normal. While her dosage was being uptitrated, her liver function became deranged, leading to discontinuation. She had another significant flare of symptoms requiring a further cycle of PLEX and her steroids were again increased. At this point she was 12 months into her diagnosis, while higher-dose steroids induced remission she was developing intolerable side-effects and was becoming

depressed and withdrawn. A decision was made to refer her for thymectomy and continue intermittent PLEX prior to surgery.

Comment

This is a challenging case. While not treatment-refractory, our patient is steroid-dependent and was intolerant of first-line immunosuppression. We considered the addition of mycophenolate at this point, but were concerned about teratogenicity, now that she was of child-bearing age. She responded well to PLEX and given she was AChR-Ab positive with generalized disease, we felt thymectomy was the appropriate next step in management.

Case 3

An 8 year old Caucasian boy presented acutely with generalized weakness, shortness of breath on minimal exertion, marked dysarthria and difficulty swallowing with nasal regurgitation of fluids. His symptoms has progressed rapidly over a few weeks. Clinically his symptoms were felt to be consistent with MG. He was admitted to the pediatric ward and reviewed by the anesthetic service. He was maintained under close surveillance but a decision was made to hold off invasive ventilation. He was commenced on ChE-I, oral prednisolone and IVIG. He made good progress and was discharged home with a plan for a further course of IVIG in 4 weeks in his local hospital due the severity of his initial symptoms, and lag time for steroids to take effect. He was seen in clinic 12 months later and at this time was on maximum alternate day steroids. He was also receiving 4-weekly IVIG infusions at his local hospital. Both him and his parents reported a dramatic response to the IVIG but felt the effect wore off after about 3 weeks and his symptoms particularly fatigue became "as bad as ever." On examination he had no weakness. Prior to his diagnosis he was said to be an outgoing boy and very involved in sports. His parents now reported he was refusing to go to school most days and no longer engaging in any extra-curricular activities. They felt the slightest thing could have him in tears. A decision was made to assess him neurologically at the time of his next infusion. While on the ward strength was noted to be normal and on further questioning he said that he kept reliving his initial hospital admission and felt that the "only reason he didn't die was because of the special protein drip." He was felt to have evidence of post-traumatic stress disorder and appropriate psychological supports were put in place. IVIG was withheld and a gradual improvement was seen.

Comment

This case highlights the importance of the multidisciplinary team in managing young patients with JMG. Psychological issues need to be addressed early and the necessary supports put in place. It is not uncommon for young patients to report fatigue rather than true muscle weakness, and this is often a manifestation of an underlying mood disorder rather than their MG. Careful assessment needs to be carried out in all patients prior to using IVIG to ensure that it is being used in the appropriate setting.

AUTHOR CONTRIBUTIONS

KO'C was involved in the planning of the manuscript, wrote the original draft, and subsequent changes. SR was involved in the planning and review of the manuscript. JP conceived the original topic idea, and was involved in the planning and review of the manuscript. All authors contributed to the article and approved the submitted version.

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Myasthenia Gravis and Physical Exercise: A Novel Paradigm

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The benefits of physical exercise for healthy individuals are well-established, particularly in relation to reducing the risks of chronic lifestyle related diseases. Furthermore, physical exercise has been seen to provide beneficial effects in many chronic diseases such as multiple sclerosis, rheumatoid arthritis, and chronic obstructive pulmonary disease and is therefore recommended as part of the treatment regimen. Myasthenia Gravis (MG) is a chronic autoimmune disease that causes neuromuscular transmission failure resulting in abnormal fatigable skeletal muscle weakness. In spite of this fluctuating skeletal muscle weakness, it is reasonable to assume that MG patients, like healthy individuals, could benefit from some of the positive effects of physical exercise. Yet exercise-related research in the field of MG is sparse and does not provide any guidelines on how MG patients should perform physical training in order to obtain exercise's favorable effects without risking disease deterioration or more pronounced muscle fatigue. A handful of recent studies report that MG patients with mild disease activity can adhere safely to general exercise recommendations, including resistance training and aerobic training regimens, without subjective or objective disease deterioration. These findings indicate that MG patients can indeed improve their functional muscle status as a result of aerobic and high-resistance strength training. This knowledge is important in order to establish collective as well as personalized guidelines on physical exercise for MG patients. This review discusses the present knowledge on physical exercise in MG.

Keywords: myasthenia gravis, physical exercise, resistance training, neuromuscular, physical activity

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INTRODUCTION

Myasthenia Gravis (MG) is an autoimmune disorder of the neuromuscular junction characterized clinically by fluctuating skeletal muscle weakness and fatigue (1). Muscular weakness in MG can affect ocular, limb, respiratory, and bulbar muscles, varies over time and is often exercise induced. Physicians caring for patients with neuromuscular disorders have in the past been reluctant to actively encourage physical exercise, postulating that one could overwhelm already weak muscles with overwork (2). This "overuse weakness" (3) concern is theoretically understandable considering the exercise induced muscle weakness and fatigability seen clinically in MG, but has not been confirmed in any controlled studies. In addition, advances in modern immunosuppressive, symptomatic and supportive treatments mean that today the vast majority of well-regulated patients with MG have a good prognosis, with normal life expectancy and modest effects on activities of daily living (1).

The benefits of physical exercise for the human body and mind are unequivocal. The available evidence shows at least 20–30% risk reductions for premature mortality and chronic disease in people who exercise according to international recommended guidelines (4). More recent literature challenges earlier threshold based recommendations and shows clinically relevant benefits by simply becoming more active (4). Furthermore, time spent sedentary is an independent risk factor for all-cause mortality, cardiovascular disease, cancer, and diabetes even after statistical adjustment for the amount of deliberate exercise taken (5). Given that regular physical exercise reduces the risks for more than 25 chronic debilitating diseases including cardiovascular disease, stroke, diabetes, and various cancers (6), one would assume that a solid body of evidence showing harm would be necessary before cautioning patients with MG against reaping the benefits of physical activity.

The lack of clinical consensus guidelines on exercise for patients with MG therefore represents a conundrum for patients and caregivers alike (7). Patients want to know if they can exercise safely, what kind of exercise they should perform and how it will affect their disease.

This review describes the few studies that exist on the topic of physical exercise and MG, thereby informing patients and clinicians seeking to establish physical exercise routines, and providing a base on which to guide the necessary development of future larger randomized controlled trials.

THE EFFECTS OF EXERCISE ON AUTOIMMUNE AND NEUROMUSCULAR DISEASES

Autoimmune disorders represent a wide range of heterogenous chronic diseases caused by failure of the immune system to distinguish self from non-self and mounts therefore an immunologic response against the body's own tissues. Physical exercise is considered safe in many autoimmune diseases including for example systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), multiple sclerosis (MS), and inflammatory bowel disease (IBD). In several of these autoimmune conditions, physical exercise is even an established part of the treatment regimen (8). As a general trend, patients with autoimmune conditions have lower physical activity levels than the general population (8). The incidence of RA, MS, IBD, and psoriasis is also higher in people who are less physically active (9–11). Advances in biological treatment of autoimmune conditions have improved quality of life (QoL) for many of these patients, however self-modifiable lifestyle factors also play an important role in patients' well-being and immune system function.

Physical exercise leads to an immune response, with a rise in T regulatory cells, decreased immunoglobulin secretion, and a shift in the Th1/Th2 balance toward decreased Th1 cell production (8). In addition, physical exercise causes release of the myokine (cytokine released by skeletal muscle) IL-6, which induces an anti-inflammatory response through IL10 secretion and IL-1β inhibition. Additional beneficial effects of physical activity are improvement in mood, reduction in fatigue, and positive effects on cognition and mobility, seen for example in patients with MS (8). Furthermore, physical activity improves QoL and reduces co-morbid cardiovascular disorders in SLE and RA patients (8).

General fatigue and cardiovascular deconditioning are more prevalent amongst patients with neuromuscular diseases compared with the general population (12). Regarding neuromuscular disorders in general, few well-designed studies have been conducted on the benefits or disadvantages of physical exercise (12). In inflammatory muscle diseases, including polymyositis and dermatomyositis, exercise enhances aerobic capacity, improves muscle function, and reduces disability (13). In patients with inflammatory polyneuropathy, significant improvement in muscle resistance, functional activities, and physiological adaptations following exercise are reported (14). In addition, reduction in chronic fatigue has been reported in patients with facioscapulohumeral muscular dystrophy type 1 (15).

A detailed review on exercise in relation to a broad spectrum of neuromuscular diseases concluded that a regular exercise regimen is beneficial in neuromuscular disease, whether aerobic/endurance or strength/resistance training (16). This review recommends that patients should establish an exercise program with their physician and that those with neuromuscular junction disorders and metabolic myopathies should combine strength training and submaximal aerobic exercise on alternating days (though it is unclear exactly what evidence this was based on), aim to slowly increase the number of repetitions and achieve 65% of maximal heart rate (220-age/min) during aerobic training.

CHALLENGES OF MEASURING FATIGUE IN MYASTHENIA GRAVIS (MG)

One challenge regarding physical exercise evaluation in MG is the objective measurement of fatigue. Several of the studies mentioned in this review fail to define fatigue and there is no standard terminology. Fatigue involves both performance fatigability and fatigue perception, and is a distinct primary symptom which must be differentiated from pre-existing muscle weakness (17). A unified taxonomy of fatigue has been proposed for neurological diseases, which may be useful in future research studies to differentiate between performance fatigability and fatigue perception (18). Fatigability is defined as performance decline during prolonged cognitive and physical activities and muscle fatigability is an objective decline in strength as the routine use of muscle groups proceeds (19). In the early stages of MG, impaired neuromuscular transmission causes muscle weakness, physical exhaustion, and tiredness and this is evidenced by pathological decrement at neurophysiological evaluation with repetitive nerve stimulation (RNS). However, routine clinical MG score and RNS are suboptimal at detecting fatigue in proximal muscles (20) and the persistence of fatigue in patients with stable longstanding generalized disease who lack muscle fatigability on bedside testing requires deeper explanations. The quantitative MG score (QMG) encapsulates fatigability in the early stages of disease by measuring endurance

over a fixed time period of for example outstretched arms and legs and neck flexion. However, neither QMG nor RNS are sufficiently sensitive to capture fatigability in longstanding stable generalized MG (17). The Fatigue severity scale (FSS) has been employed in some studies (21, 22), however this was developed primarily for use in MS and SLE and may not reflect the fatigue of MG, which is commonly of a different nature. Self-report fatigue scales can broadly be classified as measuring perceptions of fatigue. Nevertheless, recent literature suggests that research questions regarding fatigue may best be assessed using multiple measures (18). Also, one should take into account other disease unrelated potential confounders, for example body mass index (BMI) which can be a confounder for the presence of fatigue.

Functional evaluations include the 6-min (6MWT) and 2-min walk tests. The 6MWT is an established simple assessment tool for aerobic capacity and endurance and represents a submaximal test of exercise capacity. It measures the maximum distance a patient can walk in 6 min, oxygen saturations and pulse are often also monitored (23). The shortened version of the 2 min walk test has recently been described as a valid alternative to describe walking capability in patients with neuromuscular diseases and is chosen in patients who cannot complete the 6MWT due to fatigue or dyspnoea (24). An evaluation of the reliability of these tests was conducted on 31 patients with MG (MGFA class II or III) (25). On the first admission for testing, timed walk tests were performed at 3-h intervals on the same day, 1–2 h after pyridostigmine intake. Three to seven days later patients were admitted for a second time and the tests were repeated in the same fashion. Both timed walk tests were found to be reliable between test and retest conditions and they had good construct validity. They performed similarly in their ability to reflect which MGFA severity class (II or III) the patients fitted into. The study also noted the difficulty in practically measuring maximum oxygen consumption in a clinical setting (25). Other potential confounding factors which may influence result analysis in these types of exercise studies are medication effects, for example corticosteroids could mask the antihypertensive benefits of exercise and can induce weakness due to steroid myopathy.

FATIGUE, FATIGABILITY, AND DECONDITIONING IN MG

Exercise capacity in MG may be restricted by proximal muscle weakness, fatigability, and impairment in respiratory muscle function (26). Furthermore, the inherent muscle weakness and the subsequent risk of increased sedentary behavior in MG may in turn increase the risk of becoming overweight, developing respiratory infections, and osteoporosis which in turn leads to falls and fractures (1). Poor physical fitness in healthy individuals as well as MG patients may result in a "vicious circle" where physical deconditioning causes lethargy and fatigue (27) and in younger individuals, non-specific fatigue disorders are part of the differential diagnosis for MG (1). In the child and adolescent population, the objective measurement of fatigue is complex and there are no guidelines on how much exercise children and adolescents with MG can and should take, representing a conundrum for the patients themselves, their parents and physicians. Bearing this in mind, it is intriguing how little is known about baseline fitness and conditioning levels in MG patients. One study monitored the baseline activity patterns of 27 MG patients with mild to moderate MG (13 female, mean age: 62 years) using an accelerometer worn consecutively for 7 days (28). Amounts of moderate and vigorous intensity activity were measured in terms of metabolic equivalent of task minutes (MET-min), physical activity level (PAL), number of steps/day and sedentary time, and the results were compared with the American College of Sports Medicine guidelines for exercise (28, 29). Participants were found to be engaged in sedentary activity 78% of their time and reached a mean number of 7,462 steps/day, with only 22% (all women) achieving the recommended level of 10,000 steps/day. Despite this, when all types of moderate and vigorous activities of at least 10 min duration were counted for, 78% still achieved the minimum average physical activity output of 64 MET min/day as recommended by the American Heart Association (30). The results regarding time spent sedentary mirror findings in the general population, showing undesirably long periods of sedentary time despite deliberate efforts to take exercise. In comparison to data on healthy individuals the MG patients were less physically active and were more often sedentary. MG disease severity as measured by MG Composite score (MGC) interestingly did not correlate with any of the different measures of physical activity. This lack of correlation emphasizes the complexity of factors which can lead to sedentary behavior, including disease perception of the individual with MG and their physician.

Objective measures of neuromuscular function that reflects the entire motor unit include compound motor action potential (CMAP) (31), which differs between trained and untrained individuals, and neuromuscular ultrasound. However, structural measures of exercise effects are not well-established areas of extensive evaluation, and in relation to neuromuscular disease they have not been previously studied (31–33).

One study which focused specifically on fatigue and fatigability in MG patients, assessed the time dependent physical performance of 32 individuals with stable generalized MG and compared with 17 healthy controls (17). A gradual performance decline within a given time period was proposed to be suitable for quantifying fatigability in MG patients, especially in those without neuromuscular deficits on routine clinical assessment. The MG patients had low QMG scores and no pathological decrement on RNS, indicating stable disease. Both patients and controls were assessed with the 6MWT and the arm movement test (AMT), where subjects hold a weight with the arm horizontally outstretched and move it between 2 points. Fatigability was assessed by performing these repetitive movement tasks of proximal muscles and calculating a linear trend to reflect fatigability. Subjects also filled out fatigue questionnaires to assess fatigue perception including MG fatigue scale, MG activity of daily living scale, MG quality of life (MGQoL), Pittsburgh sleep quality index, and center for epidemiological studies depression scale. In MG patients the mean value for the linear trend for both AMT and 6MWT was negative, indicating that a gradual decrease in performance

was an objective parameter of fatigability even though only two out of 32 patients had mild pathological decrement in RNS of the trapezius muscle after AMT exercise. Controls had a positive linear trend, indicating physiologically stable performance. Interestingly, the performance decline in AMT correlated with current elevation in AChR antibodies and was not correlated with BMI. The perception of physical fatigue was significantly higher in MG patients than controls, although there was no correlation between the subjective fatigue parameters and the objective linear trend results. This underlines the multifactorial etiology of fatigue and reinforces the importance of considering psychological and lifestyle factors in patients instead of always increasing the dosage of acetylcholinesterase inhibitors in patients experiencing worsened fatigue.

Another pilot study investigated whether a combination of psychological and physical therapy could reduce fatigue in stable MG patients (22). Nine patients completed a 10-week program involving breathing and relaxing exercises, muscle stretching exercises, and teaching sessions on the management of stress and fatigue. Fatigue was measured using the modified fatigue impact scale (MFIS), the visual analog fatigue scale (VAFS), and the FSS at the start of the study, at various intervals during the study and 3 months after study completion. There was a slight improvement in the physical and psychosocial subscale of the MFIS during the program and a significant improvement in the VAFS at the end of the program, with no improvement in FSS. The improvement was minor and unsustained with all fatigue scores returning to baseline 3 months after study completion.

CLINICAL TRIALS ON PHYSICAL EXERCISE IN MG: CHALLENGES AND SHORTCOMINGS

The available studies on MG and physical exercise are few, with methodological shortcomings mainly due to small sample sizes, MG being a rare condition, which further limits subgroup analyses according to age, gender, MG severity, and subtype. Furthermore, the studies cannot be directly compared with each other due to the different methodologies employed.

Most training intervention studies to date have relatively short training and follow-up periods, although the health and strength benefits of an exercise intervention are transient unless patients keep up their physical training. Muscular adaptions in response to strength training such as muscle fiber hypertrophy and increased protein synthesis take 6–8 weeks to develop. On the other hand, neural adaptions such as increased activation and motor unit synchronization occur as early as 2 weeks and account for early strength gains during a training program (3). Cross transference is a phenomenon observed whereby neural adaptation in a trained single limb causes strength increases in the contralateral limb, and must be considered when reviewing studies where the design involves one limb being trained and the contralateral limb acting as a control (3). However, longer interventions and follow-up times could lead to higher numbers of dropouts due to the time-consuming nature of the intervention, and time for more confounding factors coming into play. A control group is desirable, but it is challenging to design a comparable group of healthy individuals in relation to exercise.

There is moreover an obvious inability to double blind patients to exercise as an intervention.

Which outcome measures to choose in exercise studies on MG patients is far from clear. There are general recommendations regarding evaluation of MG disease severity (34). However, when it comes to measuring functional outcome and physical improvement there are a huge number of measurements of varying potential significance to choose from, none of them having been evaluated for MG patients. Therefore, previously performed studies on physical exercise in MG often have used different outcome measures (as presented below), making them difficult to compare.

Outcome measures such as fatigue and QoL are inherently subjective and also fluctuate.

A flaw in many studies is the use of strength or force as an outcome rather than functional performance, which may be a more useful indicator of benefit in neuromuscular disease (12).

Despite a variety of modalities being available for the objective evaluation of functional exercise capacity, there is no "gold standard" physical performance test in clinical practice, rather one must choose between a high tech complete assessment of all systems involved in exercise performance or a simpler more practical test, based on the clinical question at hand (23). Similarly, the benefits of physical exercise can be more difficult to quantify than in healthy individuals given the fluctuating nature of muscle weakness characteristic to MG.

POTENTIAL INFLUENCE OF MG TREATMENT, COMORBIDITIES, AND AGING FOR PHYSICAL EXERCISE

Most patients with mild to moderate MG are treated with symptomatic treatment, acetylcholinesterase inhibitors (AChEIs) and/or low dose of corticosteroids (e.g., prednisone). When this medication fails to clinically stabilize their MG, corticosteroid sparing agents are used, including azathioprine, cyclosporine, mycophenolate mofetil, and in more severe cases rituximab and other medications are used (35). Unfortunately, most of the studies on physical exercise and MG described below fail to clearly describe the effects of medications and comorbid conditions on these patient's ability to perform exercise. In particular, glucocorticoids (e.g., prednisone) have a range of effects which may interfere with an MG patient's ability to perform exercise. These medications are associated with weight gain, partly due to increased appetite and redistribution of body fat causing Cushingoid appearance even at low doses (36). Furthermore, glucocorticoids are associated with a variety of adverse cardiovascular effects, including fluid retention, premature atherosclerotic disease, and arrhythmias. Cardiovascular disease risk is dose-dependent and may be low or absent in patients on low-dose glucocorticoid therapy (37), whereas hypertension is a poorly understood dose related adverse effect of glucocorticoids (38). Glucocorticoids cause increases in serum glucose levels, although the development of *de novo*

diabetes in a patient with initially normal glucose tolerance is rare (39). Other co-morbidities related to the side effects of corticosteroid treatment are osteoporosis and less commonly dose-related myopathy and neuropsychiatric conditions. MG patients with established osteoporosis should continue with weight bearing exercises to prevent bone loss and muscle atrophy in combination with bone protecting medications in those taking long term glucocorticoids. The more uncommon side effect of myopathy impairs exercise tolerance and could be initially mistaken for a worsening of the patients MG status. Additionally, physical exercise has a well-established role in reducing weight gain and improving cardiovascular risk profiles, which may help to negate some of these cardiovascular side effects in MG patients. Physical exercise also has beneficial effects on mood which may help to combat the neuropsychiatric side effects of glucocorticoids.

The symptomatic medication pyridostigmine is a parasympathomimetic reversible cholinesterase inhibitor, which enhances the efficiency of cholinergic transmission at the neuromuscular junction. Rare side effects include transient bradycardia and hypertension. However, to our knowledge, no evidence exists to caution patients with cardiac disease against exercise in combination with AChEIs. Rather, in non-MG patients with cardiovascular disease pyridostigmine delays the onset of myocardial ischaemia by inhibiting the submaximal chronotropic response to exercise on a treadmill (40). Furthermore, in non-MG patients with chronic heart failure, the effects of pyridostigmine on parasympathetic tone lead to improved heart rate recovery 1 min after exercise (41).

Azathioprine and mycophenolate mofetil have a wide range of side effects including gastrointestinal upset, bone marrow suppression and possible increased susceptibility to infection and malignancy; however, to the best of our knowledge these drugs have no direct effects which would contraindicate physical exercise.

Coexisting conditions are rather common in MG and must therefore be considered (35). Many patients suffer from comorbid cardiac disease, and the benefits of exercise for cardiovascular health are unequivocal. Approximately 15% of MG patients have a second autoimmune disease (35). Thyroid disease is the most common coexisting condition, and the effects of exercise on circulating thyroid hormone values remains controversial (42). SLE and RA are the next most common disorders. Therapeutic exercise programmes appear to be safe in SLE patients and result in improvements in physical fitness, fatigue, and depression (43). In RA patients, exercise has been seen to be effective in improving disease related outcomes, including functional ability and systemic manifestations, such as the increased cardiovascular risk (44).

In addition, normal aging in MG patients and an already high age in late onset MG patients, can naturally give rise to difficulties exercising. Although many of the studies on MG patients considered below included some patients over 70, none were targeted at an older population. One study employed balance strategy training in MG patient, exercises that address the functional needs of patients, which has been shown to be beneficial in the elderly (45). The results from this study were positive but the study only included one patient over the age of 70. Studies of exercise in frail older adults without MG have shown beneficial effects, although the optimal program remains unclear (46).

ACTIVE PHYSICAL EXERCISE INTERVENTION STUDIES IN MG

In regard to studies with a primary focus on exercise interventions in MG patients, the literature comprises of only a handful of studies and these are therefore considered in relative detail below, followed by a summary (**Table 1**).

In a two arm randomized and stratified study by Rahbek et al. (47), on the feasibility of exercise training in MG, 15 patients were randomly assigned to either a progressive resistance training (RT) or an aerobic training (AT) intervention over 8 weeks. Six MG patients completed the RT program and six patients completed the AT program. The primary outcome was feasibility of these two types of training, based on adherence dropout rates and adverse events, and secondary outcomes included increases in muscular strength, oxygen uptake (VO2 max), and various functional capacity measurements such as 6MWT. Patients completed three sessions with a battery of tests; *before run in* to familiarize themselves, *before intervention*, and *after intervention*. Patients continued on their routine medications throughout the study and the three test sessions were conducted at the same time of day and 1 h after pyridostigmine intake to reduce diurnal variation and fluctuation in medication effects. A focused 25 repetition isokinetic fatigability test of knee extensors was also performed unilaterally on the dominant side in order to assess the muscular fatigability of the subjects.

The training programs are described in **Table 1**. In terms of feasibility, the moderate to high intensity level of the exercise program did not appear to deter participants, with over 90% adherence rates observed, in line with adherence rates often reported in studies on exercise interventions in MS (51). Three patients (20%) dropped out during the intervention which is in line with dropout rates observed in exercise studies of people with neurological disorders and healthy individuals (33). One patient dropped out due to bulbar symptoms requiring prednisone 4 weeks into the study, while the other two dropped out due to reasons unrelated to the study. The dropout who deteriorated clinically may have worsened due to the training, but it is also possible that this deterioration began before the exercise intervention, as the individual was noted to have an increase in QMG score from 2 to 4 primarily in the categories of speech and facial muscle scoring in the lead up to the intervention. Both groups of patients reported adverse events including temporary worsening of fatigue and bulbar symptoms, but this did not affect participation except for the dropout described above who dropped out from the RT group.

The psychological tests of MGQoL15 questionnaire, major depression inventory, and modified fatigue impact scale showed lower scores in the RT group after the intervention, indicating no negative effects. However, in the AT group there was a significant deterioration in MGQoL15 score and scores almost doubled

TABLE 1 | Summary of active physical exercise intervention studies in myasthenia gravis (MG).

Study	Design	Aim	Participants	Training protocol	Results
Rahbek et al. (47)	2 arms randomized+ stratified 20 supervised training sessions over 8 weeks	1. Feasibility of AT and RT 2. Muscular strength, oxygen uptake, functional capacity, psychological well-being	15 in total MGFA class II+III 3 dropouts (1 = worsened bulbar symptoms, 2 = other unrelated reasons) 6 completed AT 6 completed RT	• AT protocol (6 patients, moderate to high intensity AT): Incremental cycle test to exhaustion 75–80% of max HR by 8 weeks • RT protocol (6 patients): Full body progressive resistance exercises e.g., weighted step ups, bench press Progressive increase in reps	• Exercise feasible for most patients with mild MG-over 90% adherence to protocol • Improved knee extensor+shoulder abductor strenth, 30SSS, BBT, less fatigability of knee extensors in RT group • Stable V02 peak in both groups • Deterioration in MGQoL score in AT group
Westerberg et al. (48)	Prospective pilot study. Tailored, supervised AT+RT twice weekly over 12 weeks	Safety and efficacy of physical exercise training in MG	10 in total MGFA class I+II 8 AChRAb+ 2 AChRab-	• AT protocol-bicycle interval training • RT protocol-8 resistance exercises e.g., biceps curl, sit ups max 2 sets of 10 reps • Balance training	• All patients completed the program • No change in disease activity (MGC and RNS decrement) • Improved 6MWT and 30SSS • No change in muscle force (dynamometer) • Increased muscle+reduced fat mass • Subjective improvement (ESES) • Serum-significant decrease in disease specific miRNAs
Westerberg et al. (21)	Non-blinded observational study. Supervised AT and RT over 12 weeks	Safety and efficacy of physical exercise training in MG Effects of exercise on functional muscle parameters	14 in total MGFA class I-IV 3 dropouts (all unrelated) 8 AChR Ab+ 1 Musk Ab+ 2 AChR/MuSK- 5 EOMG 6 LOMG	• AT protocol-bicycle interval training • RT protocol-7 resistance exercises e.g., rowing, • Biceps curl, sit-ups max 2 sets of 10 reps	• No clinical MG deterioration, MGC and QMG slight decrease, no RNS decrement deterioration • Majority exceeded 70% of pulse max during training • 10 increased resistance weights on 4 of 7 strength exercises • 4 increased their bicycle resistance • 30SSS improved • Muscle thickness increased in biceps and quadriceps (CMAP amplitude, neuromuscular ultrasound) • 12MWT, TUG, handgrip strength unchanged • MGQ0L15 improved • FSS+ESES unchanged
Wong et al. (45)	Pilot study 16 session workstation intervention training 1–2 times/week	Improve balance and functional mobility in MG patients	7 in total	• Balance strategy training-16 tailored balance strength and endurance exercises e.g., heel to toe walking, sit to stand, ball catching, and throwing	• Clinically significant improved QMG score • Clinically significant improved TUG, partially maintained at follow up
Lohi et al. (49)	Pilot study 10-week training period	Determine whether MG patients can increase muscle force or resistance to fatigue with physical training	11 in total aged 25–50 mild to moderate severity MG	• Subjects randomized to dynamic strength training of right arm and left leg or vice versa, contralateral extremity = within subject control • Muscle fatigue assessed using peak values achieved during reps of 3 s max contractions	• 9 could not complete all reps in each set and 8 could not increase workload as planned • 23% improvement in maximal voluntary muscle force (dynamometer) compared with 4% on the untrained side • Inconclusive results of fatigue test
Lucia et al. (50)	Case report 3-month training program 5 sessions weekly	Restore the subject's capacity for independent living by improving exercise tolerance and limb weakness	29-year-old female with MG (diagnosed age 24) and McArdles disease since childhood and obesity AChR Ab+, thymoma	• Low to moderate intensity AT walking, cycling or swimming increasing duration from 10 to 60 min Carbohydrate ingestion to prevent rhabdomyolysis	• Regained ability to live independently • Increased exercise time by 44% • Increased V02 peak by 50% • Increased peak heart rate and peak workload (watts) post intervention • Subjective improvement in well-being and ability to perform ADLs

AT, aerobic training; RT, resistance training; 30SSS, 30 second sit to stand; BBT, box and block test; MGQoL15, MG quality of life-15; MDI, major depression inventory; MFIS, modified fatigue impact scale; MGC, MG composite scale; ESES, exercise self-efficacy scale; Reps, repetitions; EOMG, early onset MG; TUG, timed up and go; LOMG, late onset MG; QMG, quantitative myasthenia gravis score; ADL, activities of daily living.

across all three psychological tests (where higher scores indicate worse symptoms), although this was not statistically significant. The authors suggest that rise in core temperature may have worsened symptom severity in the patients, as has been seen before in studies of AT vs. RT in MS patients (52, 53). While no adverse effects on the clinical status of the patients were noted, this observation should be examined further in future studies.

In terms of secondary outcomes, the RT group showed improved muscle strength post intervention but not the AT group, as expected. The 10% increase in maximal knee extensor strength seen in the RT group may have clinical relevance as deficits in knee extensor strength have been described in MG patients (54). There was a 23% increase in shoulder abductor strength in the RT group, the shoulder abductors being the most affected muscle group in MG patients (55). The study was unable to demonstrate increase in the VO2peak test (which measures maximum oxygen uptake during incremental exercise and is a quantitative measurement of aerobic fitness) in either group. This was surprising as previous AT interventions in neuromuscular disease have given rise to improvements in aerobic capacity (12), and a case report on exercise in MG increased aerobic capacity (50). Improved 30-second-sit-to-stand test performance (30SSS), which measures functional leg strength and box and block, which measures manual dexterity was seen in the RT training group post training intervention, almost reaching the minimal level considered necessary for clinical difference in neurological disorders (50, 56). The fatigability test of knee extensor muscles revealed intriguingly less fatigability of these muscles post exercise intervention in the RT group.

In summary 8 weeks of moderate to high intensity AT and PRT were feasible for most patients with mild MG. Secondary outcomes revealed improved muscle strength and functional capacity in the RT group, whereas the AT group did not show these improvements.

In a prospective pilot study by Westerberg et al. (48), 10 MG patients performed supervised AT and RT twice weekly for 12 weeks. Patients were examined at the same time of day before and after the study to reduce the effects of diurnal variation. These patients followed exercise guidelines recommended for healthy adults, 150 min of medium intensity aerobic exercise weekly and strength training twice a week (30) under physiotherapist supervision. Participants completed tailored 90-min training programs as described in **Table 1**.

After 12 weeks there was no change in disease activity, as measured by MGC, and RNS decrement remained unchanged. None of the patients discontinued the training program due to increased muscle fatigue, and peak expiratory flow rates remained constant.

A significant improvement was seen in the physical performance measures of 6MWT and 30SSS. Improvement in the function of proximal muscles was seen in the form of an enhanced ability to bear increasing weights with these muscles. Furthermore, increased CMAP amplitude, which correlates with isometric muscle strength (31), was seen in the biceps and quadriceps muscles. There was however no significant change in muscle force as measured by hand held dynamometer and handgrip strength test, maximum repetitions of toe rise endurance test and balance as measured by time in Romberg's test. Increased muscle mass and reduced fat mass was seen in the subjects, with no significant change in BMI. Pulse (% of max) remained consistent in the patients over the course of the training period, despite a gradual increase in bicycle load resistance, demonstrating positive aerobic effects of the training program.

Patients also subjectively reported improved ability to perform physical training after completion of the training program on the exercise self-efficacy scale. Serum analysis in the patients revealed a transient rise in CKMB and myoglobin after exercise without reaching abnormal levels, it remains to be elucidated whether this transient rise represents muscle damage or rather disruption in energy control processes at a molecular level (57). Intriguingly, a significant long term decrease was observed in the disease specific micro RNAs miR-150-5p and miR-21-5p, which have been proposed as potential MG biomarkers (58).

A prospective unblinded observational study by the same group (21) recruited 14 MG patients to a similar 12 week aerobic and resistance strength training program involving cycling and strength training. Eleven patients completed the program, with three dropouts, none of which discontinued due to MG deterioration. This study examined the effects of exercise on disease activity but also focused on the effects of physical exercise on functional skeletal muscle parameters in the participants. Medications were unchanged during the training period, except for three patients who were able to lower their doses of acetyl cholinesterase inhibitors during the training period.

The participation rate of the 11 participants who completed the study was between 75 and 96%. The vast majority of patients exceeded 70% of pulse maximum during training periods with high resistance loads. Ten participants increased their resistance weights in at least four of the seven strength training exercises and eight patients increased their bicycle resistance in the second half of the training period.

The clinical markers of disease activity, median MGC and QMG scores, decreased slightly during the training period, indicating slight improvement in MG status, and no patients described any subjective negative effects of the training program. MG specific quality of life assessment MGQOL 15 tended toward higher scores i.e., improved quality of life however not significantly. There were no significant changes in FSS or on the subjectively measured exercise self-efficacy scale, which was scored highly before beginning the intervention. RNS did not deteriorate after the 12-week-program, one patient having abnormal decrement after the program compared with four patients before, and respiratory muscle function as measured by peak expiratory flow remained unchanged. BMI, blood pressure, resting pulse, and body composition fat vs. muscle mass did not change significantly. The physical performance-based measure of 30SSS improved. Twelve-minute-walk-test, timed-up-and-go, which assesses mobility and falls risk, and handgrip strength tests remained unchanged. An improvement was noted in the functional muscle measures of isometric muscle force as measured by hand held dynamometer recording from the biceps brachii and quadriceps muscles. Muscle thickness increased as

measured by CMAP amplitude and neuromuscular ultrasound increased in the rectus femoris muscle.

Serum analysis revealed a modest few changes; a significant increase in apolipoprotein A1 levels, plasma-25-hydroxyvitamin D decreased significantly with unchanged calcium, and phosphate and HbA1c levels were non-significantly lowered. All functional outcome measures of the proximal leg muscles improved in this study, with no improvement seen for arm muscles. This arm leg difference remains unexplained, though it was noted in one previous study (59) and may have been influenced by the female preponderance in these two studies.

While this study shares the common shortcomings of small sample size and short intervention time, beneficial effects on subjective, and objective muscle outcomes especially in proximal leg muscles were noted. There was no evidence of clinical MG deterioration in these subjects with well-controlled MG.

In a pilot study specifically aimed at improving balance and functional mobility in MG patients, seven MG patients underwent a 16 session workstation intervention, completing 1 or 2 sessions per week (45). Balance strategy training is based on exercises that address the functional needs of patients by targeting the function of neural sensorimotor processes involved in postural control (45). These exercises have been shown to increase balance strength and functional ability in several populations, particularly the elderly as postural instability increases with age due to deteriorating function of dynamic sensorimotor processes and cognitive processing (60). Subjects performed a total of 16 tailored balance strength and endurance training exercises, training once or twice a week according to their ability. The training regimen was developed to address the functional needs of subjects, has been shown to be of benefit in osteoporosis and falls prevention and involves a range of exercises such as heel toe walking, sit to stand, ball catching, and throwing (45). The intervention resulted in a clinically significant improvement in QMG core >15% in subjects post intervention and additional improvements at follow up. A clinically significant improvement was seen in the timed up and go test post intervention which was partially maintained at follow up and reflected improvements in dynamic balance and functional ability. The distance mobilized in the 6MWT increased, but this was not statistically significant.

In a study published in 1993, 11 MG patients with mild to moderate MG underwent a strength training program of ∼30 sessions over 10 weeks (49). Six patients had mild symptoms, two had moderate symptoms from the limbs and three had mostly ocular and bulbar symptoms. Eight patients were medicated with acetylcholinesterase inhibitors and these were tested at a fixed time after the last dose intake. Subjects were randomized to dynamic strength training using weights of either their right arm and left leg or vice versa, the contralateral extremity serving as a within subject control.

Voluntary maximal muscle force was measured in three muscle groups: those involved in knee extension, elbow flexion, and extension. Muscle fatigue was assessed using the peak values achieved during repetitive three second maximal contractions. The subjects experienced slight muscular pain during the run-in period as to be expected but none complained of adverse effects during the training period. However, nine MG patients could not complete all 10 repetitions in each training set and eight patients could not increase their work load as planned. Six patients managed well-training elbow flexion, the remaining four having trouble with the number of repetitions and increasing work load. Only one patient was unable to use the initial predicted training weight for knee extension but managed well-later, as did all the others. The results showed a significant 23% increase in maximal voluntary muscle force in knee extension compared to 4% on the untrained side. All patients reported subjectively that they improved their strength and resistance to fatigue during the training period. The fatigue test employed showed large test-retest variability and most subjects experienced muscle pain after testing. The authors concluded that dynamic training with small loads is relatively well-tolerated and provides some improvement in strength in patients with mild MG.

The largest randomized controlled trial (RCT) to date on the subject is ongoing and plans to evaluate the benefits of a home-based physical exercise program compared to usual care in 42 MG patients with stable disease (61). This multicentre interventional single-blinded two arm parallel group RCT will see patients aged 18–70 years undertake a 40-min home-based exercise program using a rowing machine 3 times a week for 3 months as an add-on to usual care. Patients will be observed for 3 months prior to commencing the intervention and followed up for 3 months after completing the intervention. The control group will receive usual care without the addition of the exercise intervention. The primary outcome is mean change in MGQoL and secondary outcomes include measures of functional limitations e.g., MG activities of daily living scale as well as clinical scores and measures of respiratory function, muscle force, fatigue, anxiety, and depression.

Another interventional trial to be completed in 2020 (NCT01047761) aims to characterize the fitness level and cardiovascular disease risk profile in 30 generalized MG (GMG) patients and determine whether a 3-month moderate intensity home exercise program is safe and provides benefits in deconditioned stable MG patients. The objective is to investigate whether the exercise program can reduce cardiovascular risk and improve physical activity levels, strength and fitness. Primary outcome measures are cardiovascular fitness, gait and physiological reserve. Secondary outcomes are ambulatory function as measured by 6MWT and accelerometer, muscle strength (dynamometer), MGQoL, QMG and pulmonary function tests. Volunteers will undergo 3-month home-based exercise intervention 3 days a week of progressive intensity involving aerobic training (walking), resistance training and breathing exercises.

Of interest, a study on physical exercise in MG with focus on patients undergoing thymectomy concluded that exercise is not a contraindication in MG, and rehabilitation can be safely performed before and after thymectomy, reducing operative risks and decreasing recovery time (62). Forty-six MG patients who underwent thymectomy for MG during the years 2005–2010, completed pre- and post-operative rehabilitation programs and were matched with a "control patient" who underwent thymectomy without preoperative rehabilitation

TABLE 2 | Summary of available case reports on MG and sports participation.

Author	Case description: clinical status, medication	Type of sport	Outcomes
Birnbaum et al. (63)	36-year-old female; GMG: MGFA II B; AChR Ab+ Pyridostigmine, Azathioprine (100 mg daily)	Marathon running Trained 5–10 km weekly runs during the first 2 years after MG onset	• Stable MG status i.e., persistent right-handed weakness, occasional ocular, and bulbar symptoms at the end of pyrodistigmine dose • Discontinued azathioprine • Normal respiratory function • Stable limb strength in knee flexors and extensors • Improved MGQoL
Scheer et al. (65)	55-year-old male; GMG: MGFA IIA diagnosed 5 years prior to race Pyridostigmine 60 mg 6 times daily; Prednisone 10 mg daily	Ultramarathon in a hot environment 35°C ambient temp	• Completed 220 km ultramarathon (his 5th ultramarathon) • Fluctuating leg weakness, dysphasia, dysphagia, and dyspnoea during the race which subsided with rest or pyridostigmine
Stout et al. (66)	26-year-old male; GMG Running, baseball, weightlifting athlete Pyridostigmine 60 mg daily; Azathioprine 150 mg daily; Prednisone 60 mg daily	15-week resistance training program e.g., bench press, leg curls +creatinine supplementation	• Increased body weight • Increased fat free mass • Increased peak strength for leg extension and leg flexion
Leddy et al. (67)	17-year-old male; GMG (MGFA IIA), AChR Ab– Prednisone discontinued by the patient himself-	Collegiate football player Continued to train football while experiencing mild left ptosis and mild decrease in tolerance to intense exercise	Retired from football with a back injury, continued to play recreational sports

GMG, generalized myasthenia gravis; AChR+, acetylcholine receptor antibody seropositive; AChR-, acetylcholine receptor antibody seropositive; MGQoL, Myasthenia Gravis quality of life 15 score.

(control patients were retrospectively chosen from within 5 years preceding the active study). The program involved aerobic training, mild resistance training, and pulmonary rehabilitation. All patients but two completed the program and those completing the program had reduced operative risk, decreased early postoperative morbidity, lower rates of admission to intensive care, and shorter hospital stays. Measures of disease activity such as QMG score as well as 6MWT and forced vital capacity (FVC) measured a significantly faster recovery at 3 months. There was however no significant difference in complete stable remission.

MG AND SPORTS PARTICIPATION

There is a paucity of information on MG patients and sports participation and no clear guidelines for athletes with MG (7, 63, 64). To our knowledge, four case reports exist on athletes with MG and are summarized in **Table 2**. In addition, one case report (referred to in **Table 1**) describes a 29-year-old lady with both MG diagnosed at age 29 and Mc Ardle's disease (muscle glycogen phosphorylase deficiency) since childhood, who showed a significant increase in her exercise capacity and a regained ability to live independently after completion of a 3 month aerobic exercise training program (50).

RESPIRATORY MUSCLE TRAINING IN MG

Respiratory insufficiency due to weakness of the diaphragm can be a threat to patients with GMG (35). Patients with GMG often have restrictive spirometry and may exhibit a "myasthenic pattern" of decremental respiratory volumes during maximal voluntary ventilation (68). They may also demonstrate obstructive spirometry, with lower FEV1/FVC ratio than controls, even in well-regulated disease (26). Patients may complain of dyspnoea on extreme effort due to muscle weakness, and ventilatory muscle impairment impairs physical activities and patients' activities of daily living due to perceived fatigue. Level III evidence (indications of effectiveness) exists for the benefits of breathing exercises for MG patients (69).

One study randomized 27 stable MG patients into training and control groups in an 8-week- intervention. The training group participated in training of diaphragmatic breathing and pursed lips breathing and improved their respiratory muscle endurance, maximum inspiratory and expiratory pressures, and thoracic mobility in comparison with their own baseline levels and compared with controls (70). Smaller studies have shown the benefit of long-term respiratory muscle endurance training on lung function and respiratory endurance in mild to moderate MG (71, 72).

A recent cross-sectional trial showed that expiratory muscle strength is also a predictor of functional exercise capacity in GMG (73). Twenty-eight GMG patients (15 women, median age 53.5 years) of MGFA class II-III were tested with 6MWT, pulmonary function tests, respiratory strength and endurance assessment. Nearly 40% of the patients had expiratory muscle strength (as measured by maximal expiratory pressure) below the lower limit of normal. Multiple linear regression analysis revealed that the percentage of predicted expiratory muscle strength was a

significant and independent predictor of the achieved percentage of predicted 6MWT distance (according to age and gender).

Despite speech difficulties being a symptom of MG, to our knowledge there is a paucity of evidence on the benefits of tailored exercise regimens to improve speech in these patients. One case report of a patient whose dysphonia contributed to the diagnosis of MG showed benefit from drug therapy combined with speech therapy, which improved voice quality and great impact on quality of life (74).

CONCLUSION

Existing research on exercise in patients with MG is limited in scope. Despite infrequent cautionary observations that arise from the literature, such as the single patient who dropped out due to deteriorating bulbar symptoms and the deterioration in MGQoL observed in the AT group of the Rahbek study (47), the conclusions are overwhelmingly positive in favor of the benefits of exercise in MG. Clinically stable MG patients, just like healthy individuals, should be able to reap the benefits of physical exercise and we suggest that a reasonable program to begin with is to follow the minimum recommended international guidelines on exercise for healthy adults, i.e., at least 150 min of moderate intensity exercise a week (30). As MG by its nature can involve fluctuations in symptoms dependent or independent of physical exercise, patients should always contact their physician if experiencing sustained worsening of symptoms, to receive supportive advice on further management.

Thus, based on the current knowledge described in this review we propose that stable MG patients are encouraged to perform physical exercise. However, it remains to be determined to what extent physical exercise should form part of routine treatment regimens in MG and if there are any specific training protocols of particular benefit to individuals with the disease. In order to establish tailored training recommendations for MG patients, further studies are warranted. To improve the impact of such studies the recommended MG outcome measures for clinical MG trials should be used (34). However, this set of recommendations does not specifically address exercise studies and, as illustrated by the studies in this review, there remains a lack of consensus on what outcome measures of fatigue and physical performance status should be used. It would be desirable to have a fixed battery of validated outcome measures customized for MG trials, that cover disease activity and QoL as well as physical performance status and measures of physical and mental fatigue.

A unified taxonomy of fatigue has been proposed for neurological diseases, which may be useful in future research studies to differentiate between performance fatigability and fatigue perception (18).

We await with interest the results of ongoing trials on the topic of exercise in MG (61) (NCT01047761), and hope that future research in this area will inspire even clearer guidelines on exercise for MG patients and their caregivers.

Exercise is a self-modifiable lifestyle factor which plays a vital role in preventing the development of a range of chronic diseases from potentially fatal illnesses such as cardiovascular disease to debilitating conditions such lumbago and fatigue. Based on the evidence documented in this review, we conclude that MG patients and their caregivers can be encouraged to commence tailored exercise programs in stable well-controlled MG, while bearing in mind that simply being more active and reducing overall sedentary time is just as important.

AUTHOR CONTRIBUTIONS

All authors contributed to manuscript drafting and revision, read and approved the submitted version.

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REVIEW
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Diagnosing Myasthenia Gravis With Repetitive Ocular Vestibular Evoked Myogenic Potentials

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Timely and accurate diagnosis of myasthenia gravis, particularly in patients with fluctuating, isolated ocular involvement, remains challenging. Serological antibody testing and repetitive nerve stimulation of peripheral muscles usually have low sensitivity in these patients. Edrophonium testing may cause adverse events, single-fiber electromyography (SFEMG) is time-consuming and both tests are often unavailable outside specialized institutions. Repetitive ocular vestibular evoked myogenic potential (roVEMP) stimulation has recently been introduced to facilitate the diagnosis of myasthenia gravis. Similar to repetitive nerve stimulation, roVEMPs detect muscle decrements with the benefit of being non–invasive and allowing for direct measurement of the extraocular muscles. This review summarizes the clinical evidence of the diagnostic value of roVEMP for myasthenia. Prospective clinical trials have demonstrated high sensitivity and specificity. RoVEMPs are of particular interest in challenging myasthenia subgroups with isolated ocular involvement, negative serology, and/or negative conventional electrophysiological results. Optimal roVEMP repetition rates of 20–30 Hz have been identified. This promising novel diagnostic tool merits further attention and investigation to establish its value as a clinical test for myasthenia.

Keywords: myasthenia gravis diagnosis, electrophysiology, vestibular evoked myogenic potentials, repetitive nerve stimulation, ocular myasthenia

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OCULAR MYASTHENIA GRAVIS – A DIAGNOSTIC CHALLENGE

Ocular myasthenia gravis (OMG) is a rare, but potentially sinister autoimmune condition, that affects neuromuscular transmission. For various reasons extraocular muscles are particularly susceptible to transmission deficits at the neuromuscular junction, hence diplopia and/or ptosis are the initial complaint in up to 75% of patients (1). Progression to a potentially life-threatening state can occur unexpectedly. In 20–70% of patients, OMG generalizes to involve the peripheral, bulbar and/or respiratory musculature (2–4). Furthermore, myasthenia gravis can be associated with thymoma and other autoimmune conditions (5). Hence, early diagnosis and adequate treatment is of utmost importance. "Fluctuation," the hallmark of the disease and its clinical signs, often impedes the diagnostic process. Moreover, serologic antibody testing, as well as

repetitive nerve stimulation of peripheral muscles is reported to be less sensitive in OMG as compared to generalized myasthenia gravis (MG), with sensitivity rates of approximately 50% vs. up to 90% (6–12). Edrophonium testing may cause fatal adverse events and is often unavailable outside specialized institutions. With sensitivity and specificity levels above 85%, single fiber electromyography (SFEMG) of the orbicularis oculi muscle currently is the gold standard for the diagnosis of OMG, but it is time-consuming and examiner dependent (9, 13–15).

So far, several diagnostic methods, mainly using oculographic, orthoptic and tonographic parameters, have attempted to utilize eye movement fatigability for the diagnosis of OMG. For various reasons (availability, reliability, accuracy and difficulties assessing diplopia due to yoke muscle activation) none of these have been implemented in clinical practice (16). Repetitive ocular vestibular evoked myogenic potential (roVEMP) stimulation, as a non-invasive, non-pharmacological test may have the potential to fill this gap.

OCULAR VESTIBULAR EVOKED MYOGENIC POTENTIALS (OVEMPS) - OVERVIEW AND CLINICAL UTILITY

Ocular vestibular evoked myogenic potentials (oVEMPs) are biphasic myogenic responses to utricular stimulation representing crossed vestibulo-ocular reflexes (17). The oVEMP reflex has been shown to originate from the inferior oblique muscle and is elicited in response to otolith stimulation via bone-conducted vibration or air-conducted sound (18). After activation of the vestibular nerve and nucleus the oVEMP pathway is thought to travel through the medial longitudinal fasciculus, oculomotor nuclei and nerves to reach the extraocular muscles. They are recorded via surface electrodes from the contralateral inferior oblique muscle. In recent years oVEMPs have gained clinical significance, now forming an essential component of routine neuro-otological workup (19). Their clinical value lies in allowing for specific assessment of utricular function. OVEMPs are useful parameters for the diagnosis of diverse neuro-otological disorders e.g., Menière's disease, vestibular neuritis, vestibular Schwannoma or superior semicircular canal dehiscence (20). OVEMPs are a well-tolerated, rapid and simple diagnostic method, which can effortlessly be implemented in centers equipped for electrophysiological testing.

REPETITIVE OCULAR VESTIBULAR EVOKED MYOGENIC POTENTIALS (ROVEMP) AS A NOVEL DIAGNOSTIC TEST FOR OCULAR MYASTHENIA – A REVIEW OF THE LITERATURE

Patients with MG typically show a decrementing response to repetitive nerve stimulation. As mentioned above, in patients with isolated ocular involvement this characteristic decrement is often absent in the peripheral musculature. RoVEMP mirrors repetitive nerve stimulation, but has the key advantages of direct and non-invasive measurement of the extraocular muscles, along with exceptionally fast repetition rates.

We performed a literature search in the PubMed and Medline databases through to April 2020. The search query in PubMed was phrased as follows: ("ocular myasthenia vestibular evoked myogenic potentials" [Mesh]) OR (repetitive ocular vestibular evoked myogenic potentials* [Title] AND myasthenia [Title]) OR (ocular myasthenia [Title] AND VEMP [Title]). An equipollent search query was used to search the Medline database. The references in eligible papers identified in the initial search were also screened. Four original papers of relevance were identified.

In 2016 Valko et al. published on the first application of roVEMPs for the diagnosis of ocular myasthenia gravis.

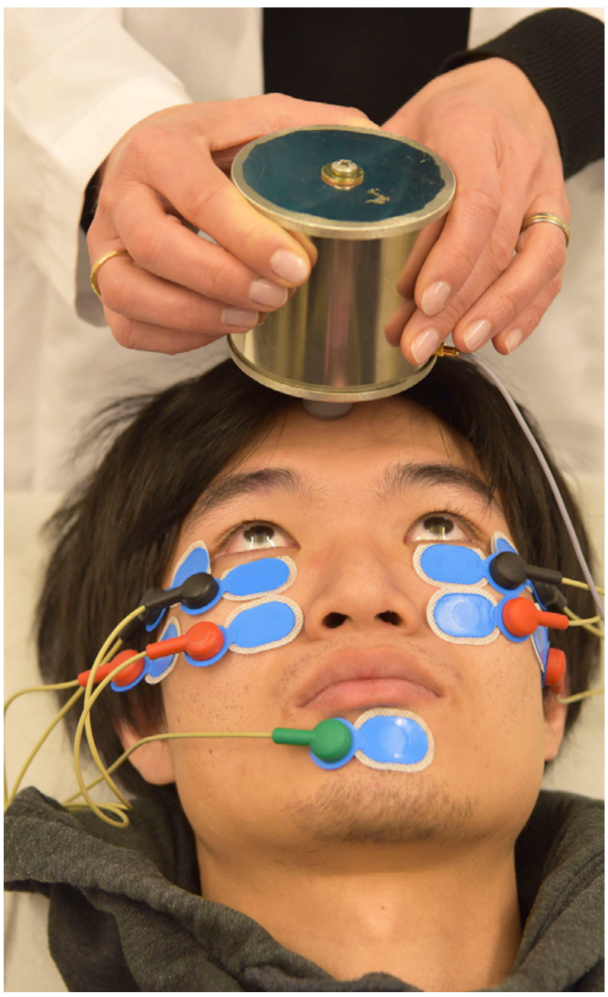

FIGURE 1 | Experimental setup of roVEMP stimulation. The mini-shaker delivers bone-conducted vibration to the skull. Responses from inferior oblique extraocular muscles are recorded using surface electrodes (black: active, red: reference, green: grounding). Reprinted with permission from Wirth et al. (23).

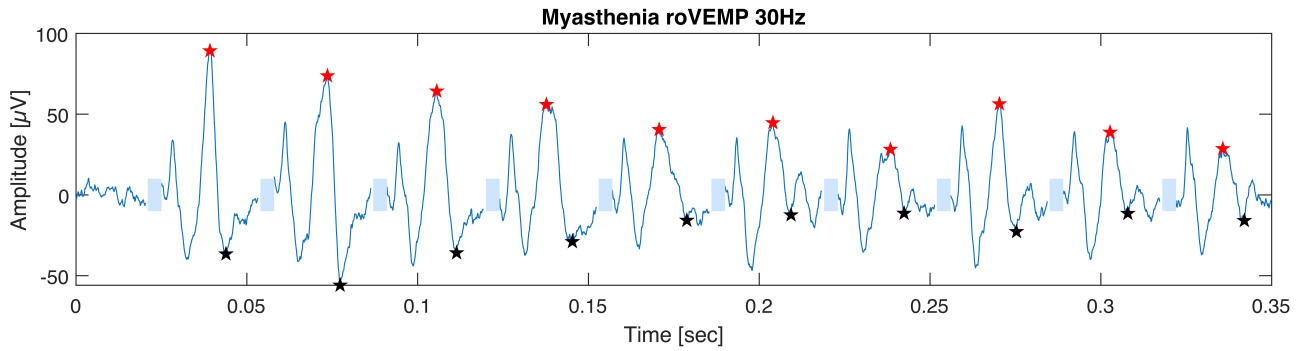

FIGURE 2 | RoVEMP responses of a myasthenia subject examined with stimulus trains of 30 Hz.

Our study included 27 myasthenic patients and 28 healthy controls. Stimulation with 4 ms bursts of 500 Hz bone-conducted skull vibration at repetition rates of 3, 10, and 20 Hz were applied, with 20 Hz yielding the most effective results. The setup for roVEMPs was similar to the standard oVEMP montage (21, 22). A train of 10 repetitive vibration bursts were delivered via a shielded hand-held mini-shaker to the forehead at the hairline in the midline (the skull location identified as standard AFz'). Responses were acquired using surface electrodes mounted at the infraorbital margins, with the reference electrodes directly below and the patient holding maximal up gaze (**Figure 1** RoVEMP setup). This proof-of-concept study reported sensitivity levels of 89% when a unilateral decrement (whenever at least one of the two eyes showed a decrement) and 63% when a bilateral decrement (whenever both eyes showed a decrement) was considered. Specificity levels were 64% (for unilateral decrement) and 100% (for bilateral decrement) (24).

In 2018 El-Sayed Mojahed et al. studied whether oVEMP stimulation (without repetitive stimulation) allows for differentiation between healthy controls and various myasthenia subgroups (25). In their prospective study, the authors used air conducted oVEMP stimulation to examine a treatment naïve myasthenia group ($n = 10$), a symptomatic myasthenia group on treatment ($n = 15$) and an asymptomatic, treatment-controlled myasthenia group ($n = 15$) vs. healthy controls ($n = 10$). The authors found a significant difference of oVEMP response rate between healthy controls and myasthenia subjects ($p = 0.002$; $p = 0.001$); however no difference between the various myasthenia subgroups ($p = 0.895$) and when comparing ocular vs. generalized myasthenia patients ($p = 0.895$) was found. In conclusion, they state, that oVEMPs are a useful diagnostic parameter, yet have no value in differentiating various myasthenic subgroups or in monitoring therapeutic response.

In 2019 our group published another study with the purpose of optimizing the stimulation parameters of roVEMP. 18 MG patients and 20 healthy controls underwent testing for this study. A heterogeneous group of MG patients, of whom 44% reported isolated ocular symptoms, 22% bulbar weakness and 50% generalized muscle weakness, were included. Fourteen patients were on treatment at the time of testing. The experimental setup was similar to the initial description of our group in 2016 (24). We found that repetitive stimulation at 30 Hz resulted in highest sensitivity and specificity values, whereby repetition rates at 20, 40, and 50 Hz also led to a robust decrement in the inferior oblique muscles of myasthenia patients (23). (**Figure 2** Single patient RoVEMP result at 30 Hz) When using the smaller decrement of the two tested eyes 30 Hz repetitive stimulation resulted in sensitivity and specificity values of 71 and 94% (area under the curve (AUC) 0.88) and 82% sensitivity and 78% specificity when considering the larger decrement for analysis (AUC 0.81) [**Figure 3** Results with ROC curves of 30 Hz repetitive stimulation, modified and reprinted with permission (23)] Recordings from the inferior oblique muscle were superior to recordings from the lateral rectus muscles and continuous 100 Hz stimulation was not found to be useful for the differentiation between diseased participants and healthy controls.

A recent prospective case-control study examined whether roVEMP allows for differentiation of MG from relevant differential diagnoses, such as Lambert-Eaton myasthenic syndrome (LEMS), genetically confirmed congenital myasthenic syndrome, inclusion-body myositis, facioscapulohumeral muscular dystrophy, myotonic dystrophy, myopathy, oculopharyngeal muscular dystrophy (OPMD), chronic inflammatory demyelinating polyneuropathy, cranial nerve palsies (III, IV, VI), mechanical diplopia, and Graves' orbitopathy (GO) (26). The study included 92 MG patients, 22 healthy controls, 33 patients with a neuromuscular disease other than MG (as mentioned above), 4 LEMS patients and 2 congenital myasthenic syndrome patients. Results showed a significantly larger decrement in MG patients (28.4% $\pm$ 32.2) as compared to healthy controls (3.2% $\pm$ 13.9; $p < 0.001$) and neuromuscular controls (3.8% $\pm$ 26.9; $p < 0.001$). When considering neuromuscular controls as reference, roVEMPs resulted in a sensitivity

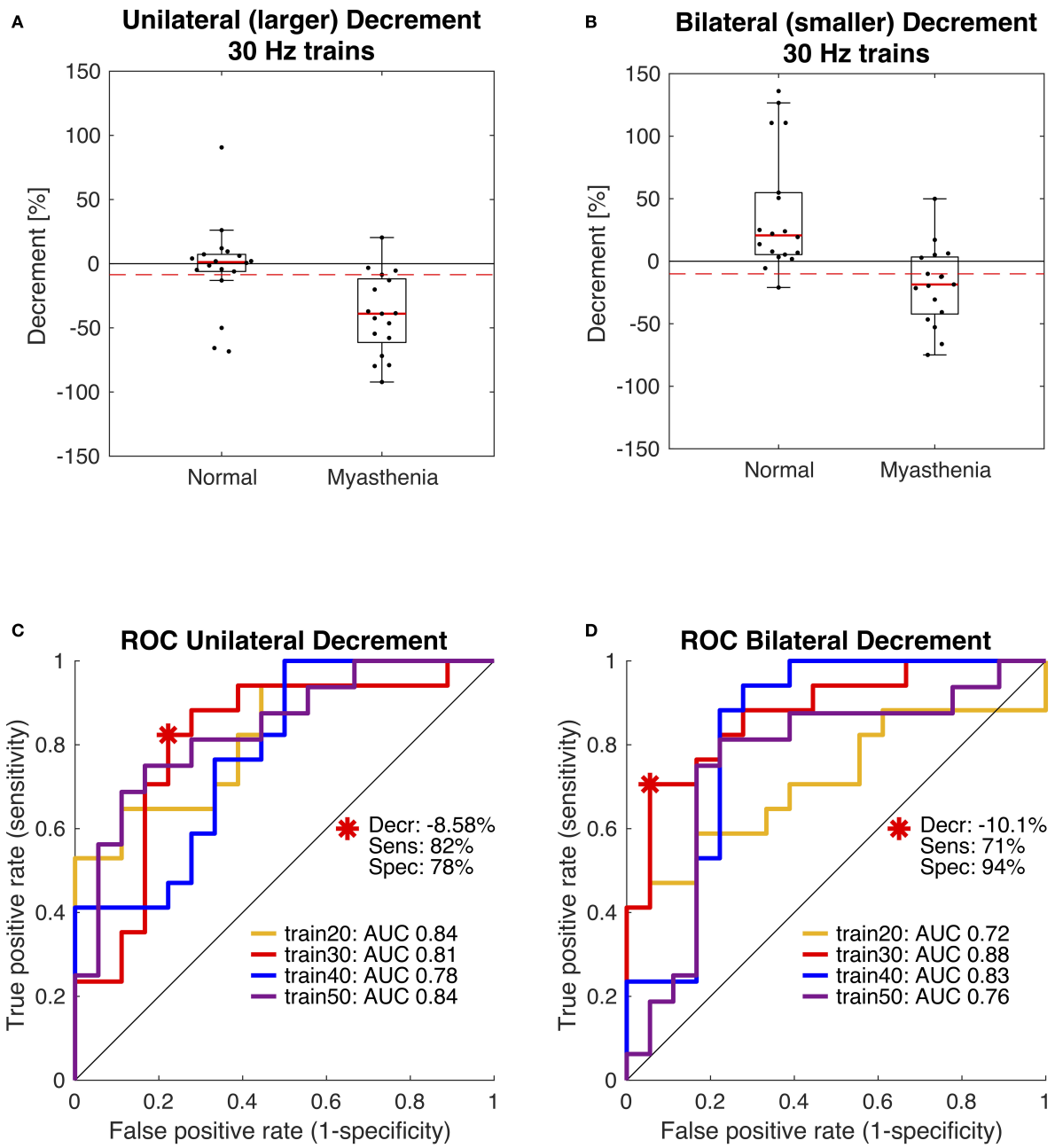

FIGURE 3 | Receiver operating characteristic curve (ROC) statistics for optimal cut-off determination. Box plots in panels **(A,B)** compare the distribution of participants and show data of the 30 Hz paradigm. The dashed red lines indicate the optimal diagnostic thresholds for eyes with the larger decrement, i.e., unilateral **(A)** and for eyes with the smaller decrement, i.e., bilateral **(B)**, as derived from the red ROC curves shown in panels **(C,D)**. Area under the curve (AUC) was largest using 30 Hz trains (red). Modified and reprinted with permission from Wirth et al. (23).

of 67% and a specificity of 82%. The mean decrement in ocular MG (32.1% ± 23.7) and generalized MG patients (27.1% ± 34.9) was comparable. A subgroup analysis of seronegative (Acetylcholine receptor antibody) and SFEMG negative patients showed abnormal roVEMPs in 86 and 73%, respectively.

DISCUSSION AND FUTURE PERSPECTIVES

Current literature suggests that roVEMP may serve as a valuable, well-tolerated and inexpensive test for the diagnosis of MG. The vibration bursts used for bone-conducted oVEMP allow

for stimulation at high repetition rates to drive the response of the small extraocular motor units into a decrement. Based on this unique property, roVEMP stimulation represents an ideal examination technique for detecting a decrement in extraocular muscles. This facilitates the diagnosis of seronegative and SFEMG negative ocular myasthenia, the most challenging subgroup of patients. RoVEMP stimulation has been proven useful in a number of clinical studies. Data suggest its value in distinguishing MG from other rare neuromuscular and ophthalmic diseases (i.e., LEMS, OPMD, GO etc.) and its usefulness in generalized MG.

Although usually clinically distinguishable, it is not yet clear, whether roVEMPs are also capable of differentiating other causes of ptosis (e.g., involutional/aponeurotic, congenital, ptosis in the context of Horner's syndrome) and diplopia (e.g., supranuclear palsies, mitochondrial myopathies, Duane's syndrome, strabismus etc.) from MG. Moreover, there are currently limited data about the utility of roVEMP in additional MG subgroups (e.g., patients on immunomodulatory treatment vs. treatment- naïve patients, patients post thymectomy etc.).

Further prospective studies are warranted to establish the definitive value of roVEMP in clinical practice.

CONSENT STATEMENT

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

MW: concept and visualization, literature research, and manuscript preparation. YV and FF: literature research, manuscript revision and editing, and supplemental material. KW: supervision, concept, resources, and manuscript revision and editing. All authors contributed to the article and approved the submitted version.

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PERSPECTIVE
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Management Issues in Myasthenia Gravis Patients Living With HIV: A Case Series and Literature Review

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South Africa is home to more than seven million people living with human immunodeficiency virus (HIV) and a high prevalence of tuberculosis. Human immunodeficiency virus–infected individuals may develop myasthenia gravis (MG), which raises questions regarding their management. An MG database, with 24 years of observational data, was audited for HIV-infected persons. Case reports of MG in HIV-infected persons were reviewed. We identified 17 persons with MG and HIV infection. All had generalized MG with a mean age at onset of 37.8 years. Eleven had acetylcholine receptor antibody–positive MG; one had antibodies against muscle-specific kinase. Six developed MG prior to HIV infection (mean $CD4^+$ 361 cells/mm^3); four worsened <6 months of starting antiretrovirals. Eleven developed MG while HIV-infected (mean $CD4^+$ 423 cells/mm^3); five presented with mild MG; three in MG crisis requiring rescue therapies (intravenous immune globulin or plasma exchange and/or intravenous cyclophosphamide). Two were diagnosed with HIV infection and MG at the same time. Fifteen required maintenance steroid-sparing immune therapies, predominantly azathioprine, or methotrexate. Plasma HIV viral loads remained below detectable levels on antiretrovirals during immunosuppressant treatment. Over the average follow-up of 6 years, 10 achieved minimal manifestation status, and the remainder improved to mild symptoms. Three cases had tuberculosis before MG, but none developed tuberculosis reactivation on immunosuppressive therapy; one used isoniazid prophylaxis. Herpes zoster reactivation during treatment occurred in one. Conclusions include the following: MG in HIV-infected patients should be managed similarly to individuals without HIV infection; half develop moderate–severe MG; MG symptoms may worsen within 6 months of antiretroviral initiation; safety monitoring must include plasma HIV viral load estimation. Isoniazid prophylaxis may not be indicated in all cases.

Keywords: HIV, myasthenia gravis, autoimmune, immune restoration, immunosuppressive therapy, rituximab, methotrexate

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INTRODUCTION

Myasthenia gravis (MG) has a similar incidence worldwide (1). However, South Africa is also home to more than seven million people living with human immunodeficiency virus (HIV), and therefore the co-occurrence of MG in some persons living with HIV infection is expected. In the early 2000's, South Africa rolled out the largest governmental-sponsored antiretroviral treatment

(ART) program globally. Initially, ART triple therapy was provided only to those with $CD4^+$ count of <200 cells/mm^3, but since 2016, ART has been available to all with HIV infection regardless of $CD4^+$ count. Present first-line ART comprises efavirenz, tenofovir (TDF), and emtricitabine. South Africa provides treatment to ≈4.4 million HIV-infected people (2).

In Africa, HIV spreads predominantly through heterosexual transmission, and hepatitis B (HepB) coinfection is rare, but tuberculosis is common (3). As HIV-infected people are at risk of opportunistic infections, and this risk may be increased with comorbid autoimmune diseases requiring immunosuppressive therapies, we audited the results of our HIV-infected patients with MG. We have summarized our results with reported cases to develop empiric management guidelines.

MATERIALS AND METHODS

The diagnosis of MG was based on clinical criteria of fatigable weakness and responsivity to anticholinesterases, repetitive nerve stimulation (RNS), and/or acetylcholine receptor (AChR)–antibody (ab) testing as previously reported (4, 5). Although routine muscle-specific kinase (MuSK)-ab testing is unavailable, AChR-ab–negative sera were tested for MuSK-abs between 2006 and 2015 (6). Observational data have been collected using standardized forms since 1997. Data captured MG Foundation of America (MGFA) disease outcomes (7), MG composite scores (8), drug dosages, and complications thereof, hospitalization events, and opportunistic infections. The registry (R004/2014) and audit (HREC 611/2013) were approved by the institutional ethics committee.

Although screening for HIV was not routinely performed before 2012, since then HIV, HepB, and HepC infection screening occurred prior to starting immunosuppression.

To review the literature, PubMed was searched for articles published in English (1997–2019) with the terms "HIV" or "AIDS" and "myasthenia gravis" and from manual searching reference lists.

RESULTS

Seventeen patients were identified in the MG database ($n = 844$ entries) who were also living with HIV (2003–2019); six were diagnosed with MG and subsequently became HIV-infected; nine were HIV-infected on effective ART [viral load (VL) <20 copies/ml or lower than detectable level (LDL)] prior to developing MG; and two were diagnosed with HIV and MG at the same time (**Table 1**).

MG Patients on Immunosuppressant Therapy Becoming Infected With HIV (MG-HIV Group)

Six women with AChR-ab–positive MG were diagnosed with HIV infection between 1.5 and 40 years after developing MG. Four had thymectomies <3 years of symptom onset. All were receiving immunosuppression at the time of presumed seroconversion: five had reached MGFA minimal manifestation status (MMS), and one had mild symptoms (grade 2A).

Three developed skin rashes, which prompted HIV testing between 1, 2, and 10 years after MG diagnosis. One patient developed a flulike illness 12 years after azathioprine initiation, which was followed by a declining leukocyte count on routine monitoring when HIV infection was confirmed. After stopping azathioprine, the $CD4^+$ count rapidly increased from <100 to >500 cells/mm^3. She remained off all immune therapy for 9 years requiring only pyridostigmine for MG symptoms. Antiretroviral treatment was started when the $CD4^+$ count declined to ∼200 cells/mm^3. Myasthenia gravis symptoms subsequently worsened over several months, and 12 months after ART initiation, a lower dose of azathioprine was reinitiated ($CD4^+$ ∼250 cells/mm^3), resulting in symptomatic improvement. Another patient developed unexplained weight loss of 20 kg, which prompted HIV testing ($CD4^+$ ∼154 cells/mm^3). As she was asymptomatic, azathioprine was discontinued, and the $CD4^+$ count recovered to ∼650 cells/mm^3. Within 6 months, azathioprine was reinitiated, at a lower dose, because of recurring bulbar symptoms. The remaining patient's MG was in remission, and her immunotherapy was being weaned when she tested HIV-positive.

These patients have been followed up for an average of 11.8 years since their HIV diagnosis. Four remain on azathioprine, although the doses required to control their disease before HIV infection was detected were significantly higher compared to the doses required to control MG after ART was reintroduced (2.6 mg/kg; *SD* ±0.1 vs. 1.2 ± 0.1; $p < 0.0001$). Two patients were in MG-MMS and were weaned off azathioprine when testing positive for HIV infection; one has remained in remission for 14 years, but the other developed bulbar symptoms after 10 years and was reinitiated on azathioprine (VL-LDL). The two patients without thymectomies were weaned off prednisone maintaining MMS on maintenance treatment. One patient had pulmonary tuberculosis on two occasions, more than 3 years prior to MG, but was not started on isoniazid prophylaxis when ART was commenced.

Patients Living With HIV Who Subsequently Developed MG (HIV-MG Group)

Eleven HIV-infected people developed MG. Nine were receiving ART [mean, 5 years (*SD*, ±3.9)] prior to developing MG symptoms, whereas two tested HIV-positive at the time of MG diagnosis. The mean $CD4^+$ count at MG diagnosis was 423 cells/mm^3, although three, who had been on effective ART (VL-LDL) had $CD4^+$ counts of <200 cells/mm^3 (range, 173–190 cells/mm^3).

Three patients presented in MG crisis after 6–12 months of symptoms. Three had a history of tuberculosis 2–11 years before manifesting with MG, but none developed reactivation of tuberculosis on immunosuppressive therapy; isoniazid prophylaxis was used in one case. One individual developed herpes zoster reactivation during MG treatment.

TABLE 1 | Clinical characteristics of patients with concomitant MG and HIV infection.

	MG-HIV ($n = 6$)	HIV-MG ($n = 11$)	Case reports 1998–2019 ($n = 13$)
Sex, female, *n* (%)	6 (100)	8 (73)	7 (54)
Age at MG symptom onset, mean ± *SD*, years	30.8 ± 14.9*	39.6 ± 8.6*	38.2 ± 18.6
$CD4^+$ count, mean ± *SD*, cells/mm^3	361 ± 133#	423 ± 76#	428 ± 315
Diagnostic Criteria, *n* (%)			
AChR ab^+	6 (100)	5 (45)	4 (31)
MuSK ab^+		1 (9)	4
AChR ab^-/RNS^+		4 (36)	5 (38)
AChR ab^-/RNS^-/$CHEI^+$		1 (9)	0
MGFA Grade Nadir, *n* (%)			
2a/b	2 (33)	5 (45)	9 (69)ϒ
3b	1 (17)	3 (27)	
4b/5	3 (50)	3 (27)	
Concomitant autoimmune disease		2 (PM/IBM, ATD)	0
MG treatments, average doses when HIV^+			
Prednisone (max doses), (*n*) mg/kg	0.14 (1)	0.52 ± 0.3 (10)	(6)
Azathioprine, mean ± *SD* (*n*), mg/kg	1.2 ± 0.1 (4)##	2.1 ± 0.3 (5)##	(2)
Methotrexate, weekly, mean ± *SD* (*n*), mg		15.6 (4)	
Mycophenolate mofetil (*n*)		2 × 1,250 mg (1)	(1)
Cyclosporine (*n*)		2 × 150 mg (1)α	(1)
Cyclophosphamide pulses, (*n*)		5 × 250 mg (1)	
Rituximab cycles (*n*)		4+2 (1)	(1)
IVIG/Plasma exchange, *n* (%)		3β/1χ (4)	(5/2)
MG crises after MG diagnosis/treatment in HIV^+	0	1	1
Minimal manifestation status, *n* (%)	4 (80)	6 (55)	UK
Patients on continued IS therapy, *n* (%)	3 (60)	10 (91)	UK
Follow-up since comorbid MG/HIV diagnosis, mean ± *SD* (*n*), years	11.8 ± 5.2**	3.9 ± 3.1**	1.2 ± 0.8 (12)
HIV viral load <20 copies on follow-up	6 (100)	11 (100)	UK

MG-HIV refers to the patients with MG who later became infected with HIV; HIV-MG refers to patients living with HIV who later manifested MG. Four HIV-MG cases developed MG ≥45 years. No cases had thymoma-MG.

**Refers to MG diagnosis before the patient became HIV-infected vs. HIV-MG ($p = 0.14$).*

***$p = 0.009$.*

#$p = 0.53$.

##$p = 0.033$.

ATD, autoimmune thyroid disease; PM/IBM, polymyositis/inclusion body myositis overlap; HIV^+, known to be HIV-infected; AChR ab^+, acetylcholine receptor ab–positive; MuSK ab^+, muscle-specific kinase ab^+; AChR ab^-, not tested for MusK-abs; $CHEI^+$, responsivity to cholinesterases; IVIg, intravenous immunoglobulin; RNS^+, 3-Hz repetitive nerve stimulation >10% decrement; IS, immunosuppressive.

α3 months before renal dysfunction.

βThree of the IVIg course were administered at MG diagnosis in crisis (MGFA grade 5).

*χPlasma exchange during MG relapse months after diagnosis (**Figure 1**). Rituximab cycles: 375 mg/m^2 2 weekly × 2, monthly × 2, and then at 6 months.*

ϒMGFA grade 2a/b assigned to descriptions of mild disease. Rituximab cycles: 375 mg/m^2 4 weekly × 2, monthly × 2.

Case reports (1998–2019) (9–20).

Cases Who Were Concurrently Diagnosed With HIV and MG

A patient was diagnosed with HIV infection ($CD4^+$ ∼160 cells/mm^3) when admitted in MG crisis following symptoms for 12 months. Acetylcholine receptor antibody testing was negative (MuSK-abs not tested), but with decremental RNS. She required ventilation, but responded rapidly to intravenous immunoglobulin (IVIg) and prednisone (0.8 mg/kg). She was initiated on ART within 1 week and a month later on azathioprine (1.4 mg/kg). At 12 months, she was asymptomatic, and ART was effective (VL-LDL).

Another case presented with generalized MG (grade 3B), which developed over 4 weeks. Acetylcholine receptor antibody testing was negative (MuSK-abs not tested), but RNS showed a decremental response, and he responded to pyridostigmine. He was found to be infected with HIV ($CD4^+$ ∼700 cells/mm^3) and HepB (HepC-negative). Antiretroviral treatment and prednisone were initiated, and he improved so rapidly that steroid-sparing therapy was omitted. After 6 months on ART (VL-LDL), he

was only mildly symptomatic, and prednisone was successfully weaned over several months.

Cases Living With HIV Infection Developing MG

The ages of these patients ranged between 28 and 53 years. Five cases had circulating AChR-abs, one had MuSk-abs, and three were AChR-ab–negative (MuSK-abs not tested) but responded to anticholinesterases.

One case who had been virally suppressed on ART for 5 years developed a detectable VL as a result of an inability to swallow the ART tablets. Three months later, she was diagnosed with MG grade 3B and was initiated on pyridostigmine, increasing prednisone doses (0.9 mg/kg) and azathioprine (1.9 mg/kg). At 12 months, the MG was in MMS, and ART was effective (VL-LDL).

The drug dosages in newly diagnosed HIV-MG cases were similar to those who were diagnosed with MG and became HIV-infected years later [azathioprine 2.1 vs. 2.6 mg/kg in MG (pre-HIV); $p = 0.12$]. Four cases were treated with weekly methotrexate (range, 10–20 mg) and one with mycophenolate mofetil 2,500 mg daily for 5 years. The average follow-up since MG diagnosis has been 3.9 years (range, 0.5–10 years). Five achieved persistent MMS, one without treatment, and the other improved to mild MG on maintenance therapy, and therefore none with AChR-abs underwent thymectomy.

Special Case Scenarios

MuSK-MG

This woman with severe oculobulbar MG manifesting over 6 months was reported previously (6). She had received effective ART for 4 years. She was admitted in myasthenic crisis (**Figure 1**) and showed a transient response to pyridostigmine and IVIg. However, she developed steroid-induced psychosis resulting in her refusing plasma exchange. Instead, 5 monthly cyclophosphamide infusions [one-third of 500 mg/mm^2 (21)] were administered together with azathioprine and isoniazid prophylaxis. During this time, she had improved slowly, until she relapsed into MG crisis precipitated by pneumonia. She agreed to plasma exchange, which was followed by rituximab infusions (375 mg/mm^2) and a steady recovery. She currently remains asymptomatic on azathioprine and ART. Interestingly, within 6 months of starting azathioprine (2.3 mg/kg), her γ-glutamyl transferase (GGT) increased to 3× the upper limit of normal, and isoniazid was discontinued. Subsequently, hepatic transaminases (aspartate transaminase and alanine transaminase) and GGT increased to 4× the upper limit, which normalized after an azathioprine dose reduction (1.1 mg/kg). During the stormy course of MG requiring cyclophosphamide and rituximab infusions, leukocytes remained $>3 \times 10^9$/L, polymorphs $>2 \times 10^9$/L, lymphocytes $\geq 0.7 \times 10^9$/L, CD4$^+$ ∼222/mm^3, and VL-LDL.

MG With HIV Inflammatory Myopathy/Inclusion Body Myositis

This middle-aged woman developed proximal weakness over several years since HIV infection was diagnosed and ART initiated. She presented to the neurology service after developing, over 2 years, additional symptoms of fatigable diplopia and bulbar symptoms accompanied by limb fatigability. At presentation, she was receiving effective ART, and serum creatine kinase was raised (1.5× upper limit). Although the serum AChR-abs (and muscle autoimmune panel) were negative, MG was confirmed by a decremental response on RNS and responsivity to intramuscular neostigmine (swallowing and leg strength). A muscle biopsy showed endomysial fibrosis and fiber size variation, but no rimmed vacuoles. Treatment for MG was started viz. pyridostigmine, steroids, and methotrexate. The MGC score improved by 50% over 12 months, but she developed moderate weakness of hand flexors and knee extensors. The prednisone has been weaned, and she remains on methotrexate 20 mg weekly. The working diagnoses include MG, which is at present minimally symptomatic, and HIV-associated inflammatory myopathy overlapping with inclusion body myopathy (22). The current goal is to wean the methotrexate to the lowest dose maintaining control of MG.

Drug–Drug Interactions

Another case was diagnosed with AChR-ab–positive MG (grade 2B) after 3 months of symptoms. She had been on effective ART for 4 years (CD4$^+$ 200 cells/mm^3; VL-LDL) and had been treated for tuberculosis twice, at least 2 years prior to the diagnosis of HIV and the onset of MG symptoms. Prednisone and methotrexate were started together with isoniazid prophylaxis for 18 months because of associated bronchiectasis. Her CD4$^+$ count remained stable (VL-LDL), but deteriorating bulbar MG symptoms required high-dose prednisone. After 12 months, methotrexate was replaced with cyclosporine, and although the MG responded within 4 months, her kidney function deteriorated (rising urea/creatinine). The ART regimen contained TDF, which was replaced with zidovudine. The cyclosporine dose was initially reduced, but eventually replaced with azathioprine. Although the renal function improved, she developed aplastic anemia. The ART regimen was then altered to include abacavir, nevirapine, and lamivudine, and given that she had achieved MMS, the azathioprine was discontinued. Within weeks, her bulbar symptoms recurred, and she was cautiously started on mycophenolate mofetil (MMF) (500 mg daily) with prednisone. The MMF was increased to 2,500 mg daily. After 15 months, she started improving, and the prednisone dosing could be reduced by 50%. Four years later, she remains in remission on MMF.

Literature Review

Thirteen case reports were identified and summarized in **Table 1** (see legend). One patient had MG-HIV, two were diagnosed with HIV when they presented with mild MG (CD4$^+$ 250–350 cells/mm^3), and 10 had HIV-MG. More than 80% had mild–moderate MG symptoms. Only one case presented with MG and a CD4 count of <200 (CD4$^+$ 63 cells/mm^3). At least five were on effective ART (VL-LDL) when they developed MG. Five showed mild symptomatic deterioration 3 weeks to 3 months after ART initiation or adjustment (for improved efficacy).

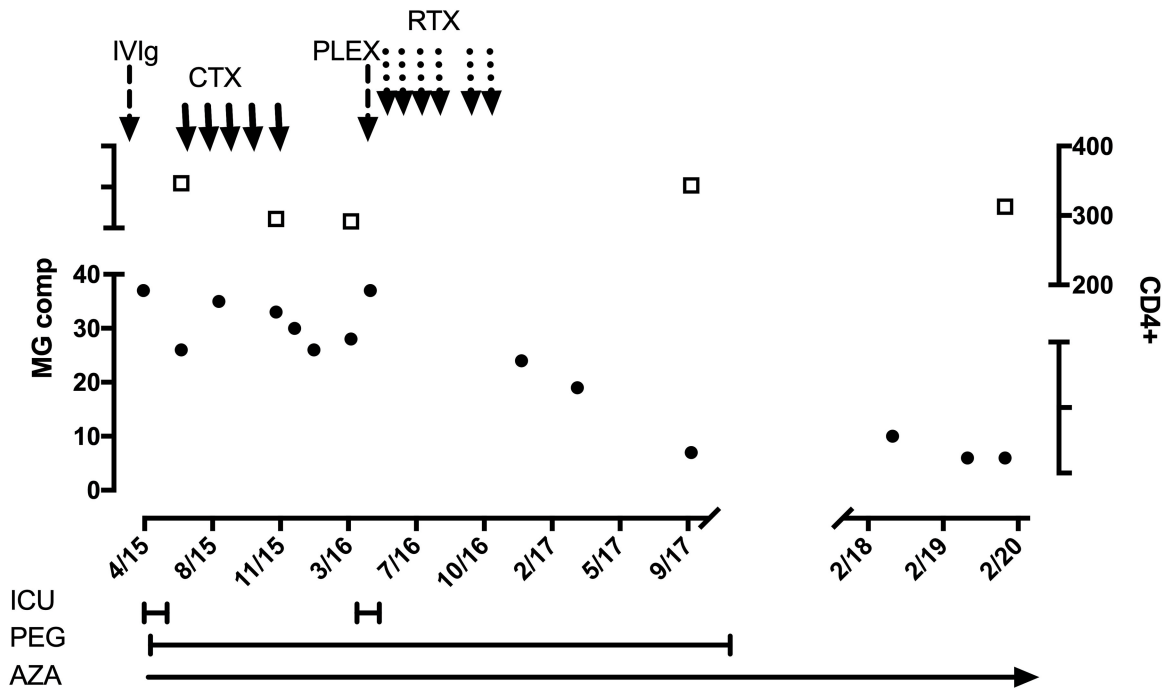

FIGURE 1 | HIV-infected patient with MuSK-MG. Black circles and left-sided *y* axis refers to MG composite score. Open squares and right-sided *y* axis refers to $CD4^+$ count. ICU, intensive care unit; PEG, percutaneous endoscopic gastrostomy; AZA, azathioprine; IVIg, hyperimmune intravenous globulin; CTX, cyclophosphamide monthly pulses (250 mg); PLEX, plasma exchange; RTX, rituximab infusions (500 mg weekly × 4; and 2 weekly × 2 after 3 months).

DISCUSSION

We describe a cohort of HIV-infected MG patients, followed for many years, unlike previous cases reports with <2 years follow-up. Several findings need highlighting. Most cases known with HIV infection develop MG with relatively preserved $CD4^+$ counts and/or mild disease at onset. However, HIV-MG may present in crisis and with lower $CD4^+$ counts [$\leq$222 cells/mm^3 in three cases, and (23)].

Immunomodulatory Therapy in HIV-Infected MG Cases

Four HIV-MG patients required intensive and multiple immune therapies to gain control of their MG despite ART, which included "rescue" therapy with plasma exchanges/IVIg and/or induction therapy with cyclophosphamide or rituximab in addition to maintenance therapies (prednisone and steroid-sparers). Methotrexate is a cost-effective alternative in generalized MG (24), and four HIV-infected people, who were not potentially child-bearing, were managed with methotrexate, and two obtained MG remission status within 6 months.

In addition to our case, one other reported HIV-infected MuSK-MG patient was treated with rituximab. In both, the protocols comprised 4 weekly, followed shortly after by 2 weekly infusions. Jing et al. (25, 26) and our experience (unpublished) in HIV-uninfected patients have found excellent responses after a single ≈500–600 mg rituximab dose, which may last for 9 to more than 42 months; this regimen should be explored in HIV-infected cases with MG.

In total, 16 of 17 patients received prednisone and steroid-sparing immunomodulatory therapies. With the exception of one patient who developed a herpes zoster reactivation rash, none developed opportunistic infections during such treatment. It should be noted that all patients receiving ART remained virally suppressed on immunomodulatory therapy. Maintaining effective ART (VL-LDL) while taking immunosuppressive therapies is critically important. New guidelines advise that if the $CD4^+$ count was >200 cells/mm^3 before starting immunosuppressive therapies, monitoring of the HIV-VL is the most cost-effective (27). Despite effective ART, MG symptoms may recur years later. It is prudent to then consider additional autoimmune thyroid disease, hormonal-related fluctuations (pregnancy, menopause), and drug–drug interactions. Overall, HIV-infected patients with MG should be managed similarly to HIV-uninfected cases: immunomodulatory therapies should be administered according to the severity of MG.

Although it was shown that thymectomy in AChR-ab generalized MG resulted in lower prednisone doses required to improve MG and maintained for 5 years (28), it was not performed here mainly because these patients appeared to improve and maintain MMS with successful weaning of prednisone.

Effects of Level of Immunosuppression and Immune Recovery on MG

Two main groups were encountered: MG patients who were well-controlled on immunosuppressive therapy when they became

infected with HIV and could subsequently be managed on lower doses of azathioprine compared to pre-HIV dosing (1.3 vs. 2.6 mg/kg; $p < 0.0001$) and patients who developed MG after HIV acquisition and who required similar doses of immunotherapies to that used in HIV-uninfected populations (29) (2.1 vs. 2.6 mg/kg, respectively; $p = 0.12$).

Antiretroviral treatment initiation within the first 3 months may be associated with a subclinical "cytokine storm" (30), and recovery of the immune system may take many months as shown by $CD4^+$ count recovery (31). During this period, MG symptoms may deteriorate as was evident from four MG-HIV cases who were weaned off immunosuppressants when diagnosed with HIV, but had to be reinitiated within 6 months of starting ART, albeit with lower doses. A previous report (19) described an HIV-infected man ($CD4^+$ 290 cells/mm^3) who developed bulbar MG shortly after the addition of ritonavir to his two-drug ART regimen. The authors suggested that MG occurred as a side effect from ritonavir. At present, in South Africa, it is estimated that there are 200,000 patients on second-line ART (2), which encompasses protease inhibitors (ritonavir). One of our cases received ritonavir without MG deterioration. Our experience with this class has shown few neuromuscular side effects (32). It is more likely that the reported patient (19) worsened due to immune "recalibration" with more effective ART.

Isolated cases presenting with MG at the same time as HIV infection have improved alongside ART with or without a short course of prednisone in addition to anticholinesterases and not requiring long-term immune therapy.

Investigations Prior to Immunomodulatory Therapy

Coinfection with HepB/HepC must be excluded. Baseline blood laboratory values should be determined prior to starting or adding immune therapies to the medication list so that drug-induced complications are easily identifiable. If an expected drug-associated side effect occurs, such as raised transaminases with azathioprine, a lower dose may be all that is required to safely continue the drug (33). Prior to rituximab, screening for previous HepB infection (anti-HepB surface-ab^- and anti-HepB core-ab–positive, but HepB surface-antigen negative) must be performed because 25–40% of these cases may seroconvert to active HepB after rituximab (34). The WHO estimates that only 10% of HepB-infected people are aware of their infection status (who.int/news-room/fact-sheets/detail/hepatitis-b).

It is important to screen for active tuberculosis prior to starting immunosuppressive therapy. Among HIV-infected people, cases at particular high risk include those with prior tuberculosis exposure and/or diabetes (35). Screening should include a chest X-ray to exclude active tuberculosis or identify evidence of fibrotic scarring. Human immunodeficiency virus–infected people have 3–20 times higher risk of reactivation of latent tuberculosis compared to the general population (36). In resource-rich areas with low background prevalence of tuberculosis, diagnostic tests, such as the interferon γ release assays (QuantiFERON-TB Gold Plus; T-SPOT test), can be useful to identify infected individuals. However, the results have to be interpreted with caution in areas with high tuberculosis prevalence. These tests may be falsely negative after starting immune therapies (37). False-positive tests may be a response to BCG-vaccinations, environmental exposure to non-tuberculous mycobacteria (35), and in autoimmune diseases (37).

Prophylactic Treatment for Tuberculosis During Immunomodulatory Therapy

While some have suggested using prophylactic antifungal and antituberculosis therapies in patients on immunosuppressive therapy (38), we do not follow this practice routinely, although we screen for active tuberculosis by questionnaire and chest radiograph. We recommend that, in tuberculosis-endemic areas, each patient's comorbidities be considered for possible isoniazid preventive therapy. With fibrotic lung lesions, isoniazid preventive therapy for 6–12 months reduced the odds for reactivation of latent tuberculosis by ≈50% (35), but must be given with 25 mg pyridoxine (39).

The conclusions that can be drawn from these cases include the following: MG in HIV-infected people should be managed similarly to HIV-uninfected individuals; transient worsening of MG may occur weeks to months following ART initiation; monitoring should comprise HIV VLs estimation; and isoniazid prophylaxis is not indicated in all cases.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/supplementary material.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by University of Cape Town Human Research Ethics Committee. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

JH performed the case, literature review, and drafted the manuscript. SM edited the manuscript drafts and contributed to the literature review. All authors contributed to the article and approved the submitted version.

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Pharmacodynamic Properties of Subcutaneous Immunoglobulin in Myasthenia Gravis: Sub-analyses From an Open-Label Trial

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Background: We previously reported an open-label prospective trial of subcutaneous immunoglobulin (SCIg) in mild to moderate exacerbations of myasthenia gravis (MG). The effective dose of SCIg in MG and whether measured immunoglobulin G (IgG) levels correlated with measures of disease burden were not reported.

Objectives: To understand the relationship between SCIg dosing and serum IgG levels on measures of disease burden: quantitative MG (QMG), MG activities of daily living (MG-ADL), MG composite (MGC), and manual muscle testing (MMT) scores.

Methods: We performed *post-hoc* analyses of variance to assess change in oculobulbar and generalized sub-scores. We assessed the improvement in QMG, MG-ADL, MGC, or MMT over intervals from baseline to week 2, weeks 2–4, and week 4 to end of study. Improvement was either greater than (coded 1) or was equal to or less than (coded 0) the previous 2 weeks. Binaries were assessed in binary logistic regression as a function of SCIg dose over the two-week interval as the independent variable. We also performed linear regression analyses with change in the clinical scores as the dependent variable and change in IgG level over the entire study period and over the interval from weeks 2 to 4, during which change in IgG level was maximal, as the independent variables.

Results: Subanalysis of QMG and MG-ADL scores demonstrated significant reductions in the oculobulbar and the generalized portions of both measures. Binary logistic regression analyses did not find any statistically significant correlations between the odds of improvement and weight-adjusted dose of SCIg over 2-week intervals. There were no significant relationships between changes in scores and IgG level over the entire study period or over the interval from weeks 2 to 4.

Conclusions: Although SCIg dose varied over the study period, the odds of improvement were not significantly correlated with this, which suggests that the current dose of 2 g/kg for SCIg should be compared to different, possibly lower, dosing regimens head-to-head. The change in clinical scores was not significantly associated with IgG levels suggesting a complex relationship. SCIg may be effective for both ocular and generalized presentations of MG.

Keywords: subcutaneous immunoglobulin, pharmacodynamics, myasthenia gravis, hizentra, serum level

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INTRODUCTION

Autoimmune myasthenia gravis (MG) is a disorder of the post-synaptic neuromuscular junction characterized by fluctuating, fatigable weakness that can affect extraocular, bulbar, limb, and respiratory muscles (1). Immunomodulatory therapy is a central pillar in the treatment of autoimmune MG. Preparations of exogenous human immunoglobulin, which include subcutaneous immunoglobulin (SCIg) and intravenous immunoglobulin (IVIg), have been used in various autoimmune disorders, but the role of SCIg in treating neuromuscular disorders was defined in chronic inflammatory demyelinating polyradiculoneuropathy, while the data in MG are limited but encouraging (2). We previously reported the results of an open-label prospective trial of SCIg 2 g/kg total in mild to moderate exacerbations of MG that was conducted over 6 weeks with assessments at baseline, weeks 2, 4, and 6 (end of study) wherein we demonstrated that SCIg is effective, safe, and tolerable (3). A case report demonstrated stabilization and maintenance with SCIg alone in one of two patients with MG (4), while a growing body of evidence supports the efficacy of SCIg in maintenance of stable MG (2, 5–8).

Reported below are the results of *post-hoc* analyses that we conducted on data collected for our previously reported open-label prospective trial (3). The rationale for further analyses are as follows: we observed continued improvement over the study period; however, there was a robust early response, which we hypothesized would meet thresholds of clinically meaningful responses in the quantitative MG (QMG) (9), MG activities of daily living (MG-ADL) (10), and MG composite (MGC) (11) scores. We also sought to assess the effect of SCIg on oculobulbar and generalized manifestations of MG, which are captured by portions of the QMG (12) and MG-ADL (13). Furthermore, while the total dose in our study was 2 g/kg, we dosed the study drug in a dose-escalating manner (3), such that the weekly interval dose varied across the study. We therefore analyzed whether the dose was associated with the rate of improvement in the aforementioned clinical scores along with manual muscle testing (MMT) score, as the effective dose for SCIg has not been defined and the current recommendations have been generated by extrapolation from other conditions and from IVIg dosing (2). Also, one of the drawbacks of SCIg as compared to IVIg is the time required to infuse the full dose (2 g/kg), which may not be practical in MG exacerbations where rapid treatment is required. The safety and tolerability of SCIg demonstrated in the trial results suggests that the dose may be given over a shorter period (3). It is plausible that faster infusion of SCIg may result in earlier peak in the clinical response. To that end, we undertook the analysis to assess the impact of the rate of change of serum IgG levels on the clinical parameters.

METHODS

Full methodology concerning the recruitment and assessment of trial participants is described in our original report (3). In summary, this was a phase 3, open-label, prospective trial with a single study arm that included a total of 22 participants who successfully completed the trial. Of the 22 that completed the trial, three had a subsequent exacerbation and were re-enrolled, such that we studied 25 instances of MG exacerbation treated with SCIg in addition to other standard therapies. MG exacerbation was defined as transitioning of a patient to a higher class as per Myasthenia Gravis Foundation of America (MGFA) clinical classification i.e., from Class I to II or III, or from Class II to III.

All statistical analyses were completed using SPSS version 26. We performed *post-hoc* responder analyses using assessments from study weeks 2, 4, and 6 (end of study). Based on previous reports, a clinically meaningful response was defined as $\geq$3-point improvement from baseline to week 6 for QMG (9, 14), MG-ADL (10), and MGC (11). Additionally, a more stringent $\geq$5-point cut-off was applied for QMG (15). We analyzed sub-scores for oculobulbar and generalized weakness for QMG (12) and MG-ADL (13). Sub-score analyses were performed with one-way repeated measures analysis of variance where Mauchly's test was used to assess sphericity. In cases where sphericity was not met, the Greenhouse-Geisser correction was used for $\varepsilon < 0.75$ and the Huynh-Feldt correction was used for $\varepsilon > 0.75$. The results of statistical analyses are reported in-text and graphical representations in the form of line and dot plots are presented as figures.

Our protocol was that of flexible dose-escalation based on body weight and patient tolerability (3), and participants administered their weekly dose divided over multiple days. As a result of participants starting their study drug mid-week, their dosing schedules were not necessarily aligned with their assessment days, which occurred on set weekdays. We thus calculated dose totals at the three study assessment points: week 2, week 4, and end of study (week 6). Peak dosing occurred during the interval from weeks 2 to 4. We analyzed whether the dose of SCIg during the three assessment intervals—baseline to week 2, weeks 2–4, and week 4 to study end—correlated with the change in the four clinical scores studied, QMG, MG-ADL, MGC, and MMT. We explored the question as a binary whereby the answer was that improvement in a given score was greater (coded 1), or that it was equal to or less than (including no improvement or worsening; coded 0) as compared to the previous 2-week interval. We thereafter performed binary logistic regression analyses where the independent variable was SCIg dose over the 2-week interval in question expressed in g/kg and the dependent variable was the binary for score improvement. The results of statistical analyses are reported in-text and graphical representations in the form of binary fitted plots are presented as figures.

We performed univariate linear regression analyses where the independent variables were the change in IgG level from baseline to end of study and change in IgG level from weeks 2 to 4, and the dependent variables were the changes in clinical scores over the same intervals. The results of statistical analyses are reported in-text and graphical representations in the form of scatter plots with lines of best fit generated using the least-squares method are presented as figures.

RESULTS

The mean dose from baseline to week 2 was 0.47 $\pm$ 0.15 g/kg (range 0.26–0.96 g/kg), and the mean dose from weeks

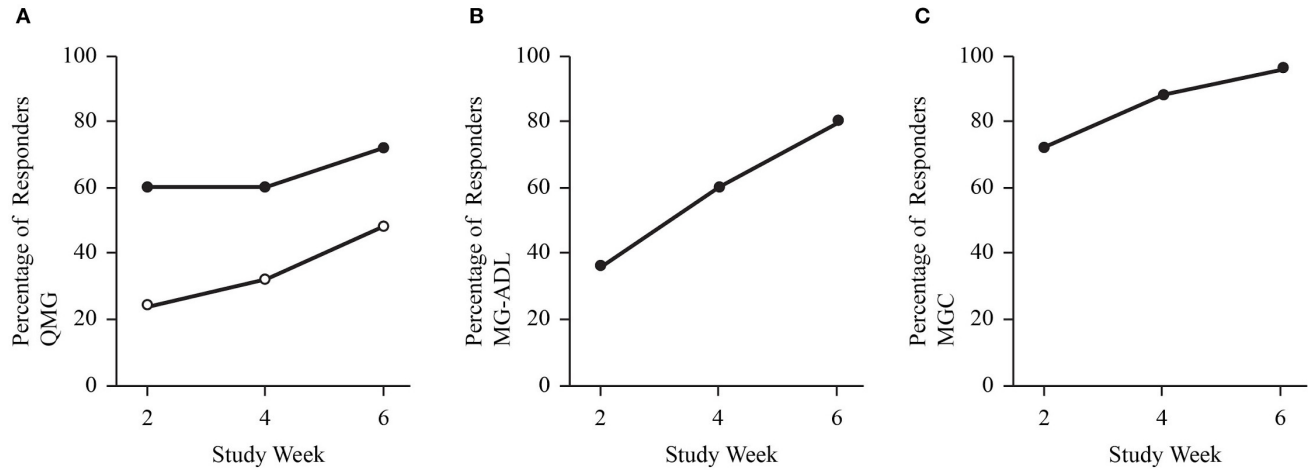

FIGURE 1 | Dot plots demonstrate the proportion of study participants with a clinically meaningful improvement in QMG **(A)**, MG-ADL **(B)**, and MGC score **(C)**. Solid dots represent a cut off of 3-or-more point improvement. Open dots represent a cut off of 5-or-more point improvement for QMG.

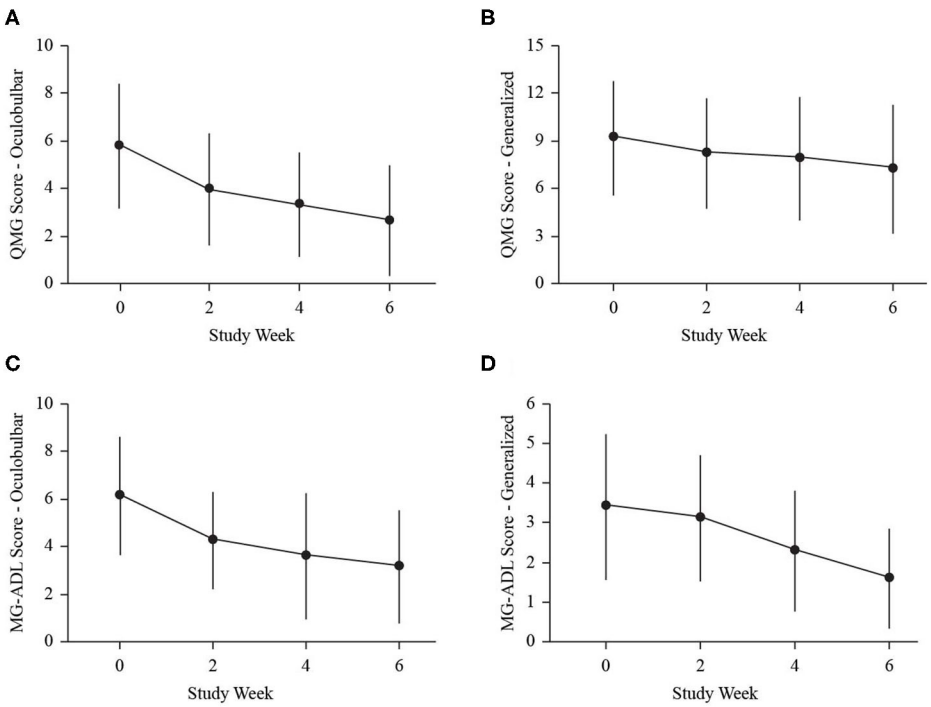

FIGURE 2 | Dot and line plots demonstrate change in mean clinical sub-scores: oculobulbar QMG **(A)**, generalized QMG **(B)**, oculobulbar MG-ADL **(C)**, and generalized MG-ADL **(D)** at baseline (0), week 2 (2), week 4 (4), and end of study (6). Error bars represent standard deviation.

2 to 4 was 1.07 ± 0.14 g/kg (range 0.79–1.39 g/kg). In 22 exacerbations (88%), the dosing was completed after the week 4 assessment, such that the mean dose after week 4 was 0.35 ± 0.14 g/kg (range 0–0.55 g/kg). The proportion of individuals with a clinically meaningful response increased at each assessment (weeks 2, 4, and end of study; **Figure 1**). At study end, 72% (3-point) and 48% (5-point) of exacerbations for QMG (**Figure 1A**), 80% for ADL (**Figure 1B**), and 96% for MGC (**Figure 1C**) had a clinically meaningful response. The maximum improvements in QMG, MG-ADL, MGC, and MMT were 12 points, 11 points, 22 points, and 45 points, respectively. Sub-score analysis of QMG and MG-ADL scores demonstrated statistically significant reductions in the oculobulbar portion of QMG [$F_{(3,72)} = 17.92$, $p < 0.001$, **Figure 2A**] and MG-ADL [$F_{(2.23,53.44)} = 23.94$, $p < 0.001$, **Figure 2C**], as well as the generalized portion of QMG [$F_{(1.83,43.84)} = 5.43$, $p = 0.009$,

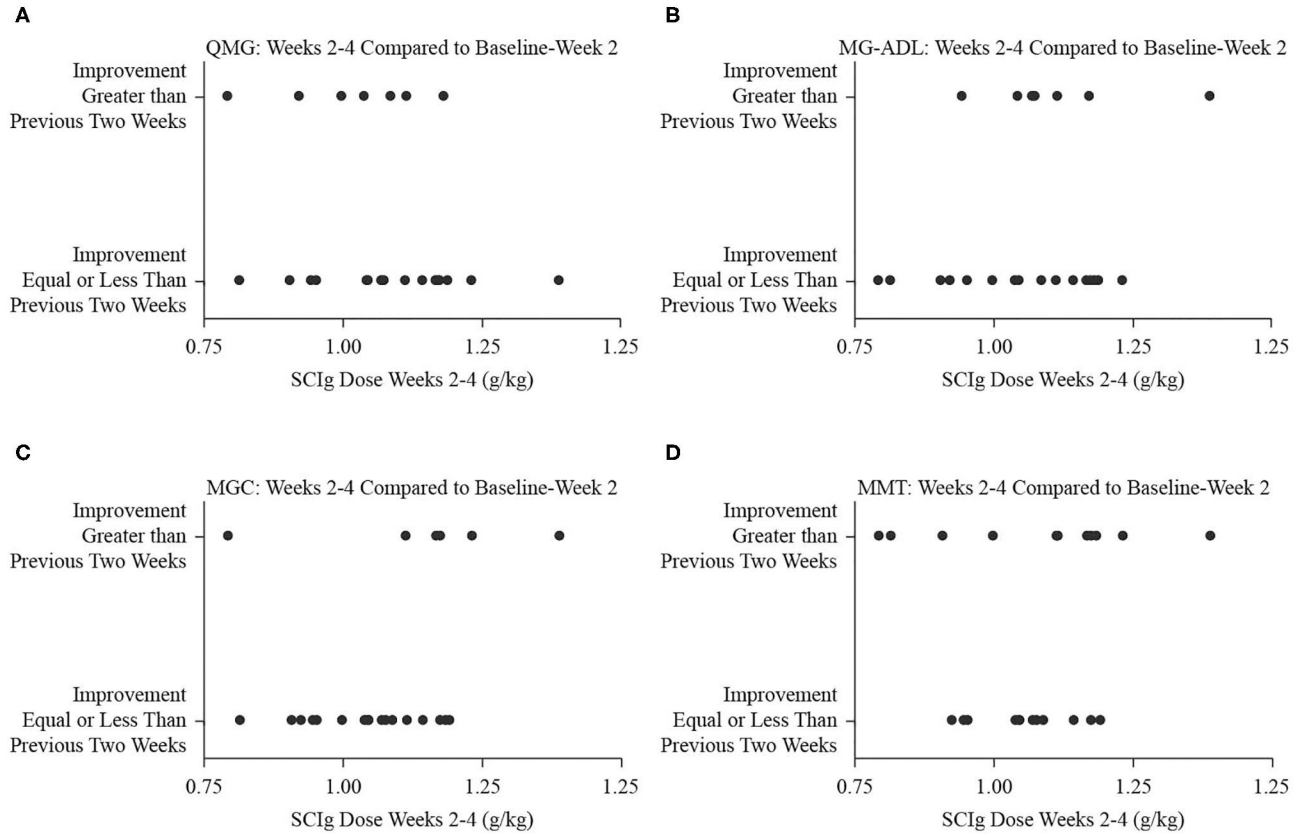

FIGURE 3 | Binary fitted plots comparing the improvements in QMG **(A)**, MG-ADL **(B)**, MGC **(C)**, and MMT **(D)** scores over the interval from weeks 2 to 4 to the interval from baseline to week 2. The binary answer was that improvement was greater, or that it was equal to or less than, including no improvement or worsening. These results (dependent variable) were plotted on the SCIg dose over the interval from weeks 2 to 4 adjusted for patient weight (independent variable).

Figure 2B] and MG-ADL [$F_{(2.17,51.96)} = 23.25$, $p < 0.001$, **Figure 2D**].

Comparing the study period from weeks 2 to 4 to the period from baseline to week 2, the number of participants who experienced greater degree of improvement was 8 (32%) for QMG, 9 (36%) for MG-ADL, 7 (28%) for MGC, and 12 (48%) for MMT. The dose of SCIg from weeks 2 to 4 was not significantly associated with the odds of improvement for any of QMG (B = −3.97, $p = 0.268$, **Figure 3A**), MG-ADL (B = 3.95, $p = 0.283$, **Figure 3B**), MGC (B = 6.57, $p = 0.138$, **Figure 3C**), or MMT (B = 1.27, $p = 0.684$, **Figure 3D**). Comparing the interval from weeks 4 to 6 (study end) to the interval from weeks 2 to 4, the number of participants who experienced greater degree of improvement was 12 (48%) for QMG, 11 (44%) for MG-ADL, 8 (32%) for MGC, and 5 (20%) for MMT. The dose from week 4 to end of study was not significantly associated with the odds of improvement for any of QMG (B = 9.28, $p = 0.065$, **Figure 4A**), MG-ADL (B = −3.97, $p = 0.250$, **Figure 4B**), MGC (B = −2.95, $p = 0.366$, **Figure 4C**), or MMT (B = −0.52, $p = 0.897$, **Figure 4D**).

Serum IgG levels significantly increased at end of study (18.3 ± 3.6 g/L) compared to baseline [9.3 ± 2.3 g/L, $t_{(22)} = 12.74$, $p < 0.001$], and the largest change occurred over the period from weeks 2 to 4, when IgG levels increased by 6.0 ± 1.8 g/L. There were no significant relationships between the magnitude of change in IgG level from baseline to end of study (independent) and improvement in any of QMG (dependent, B = 0.09, $p = 0.746$, **Figure 5A**), MG-ADL (dependent, B = −0.37, $p = 0.064$, **Figure 5B**), MGC (dependent, B = −0.54, $p = 0.085$, **Figure 5C**), or MMT (dependent, B = −0.56, $p = 0.334$, **Figure 5D**). There were no significant relationships between the magnitude of change in IgG level from weeks 2 to 4 (independent) and improvement in any of QMG (dependent, B = 0.47, $p = 0.088$, **Figure 6A**), MG-ADL (dependent, B = 0.23, $p = 0.477$, **Figure 6B**), MGC (dependent, B = −0.29, $p = 0.951$, **Figure 6C**), or MMT (dependent, B = 0.18, $p = 0.795$, **Figure 6D**).

DISCUSSION

We performed *post-hoc* analyses that demonstrated continued improvement over the study period, despite peak SCIg dosing occurring in the middle of the study. We also found statistically significant reductions in the oculobulbar and generalized subscores of both the QMG and MG-ADL. Several of our patients were already treated with prednisone, and this study did not compare SCIg to other treatment modalities. Previous

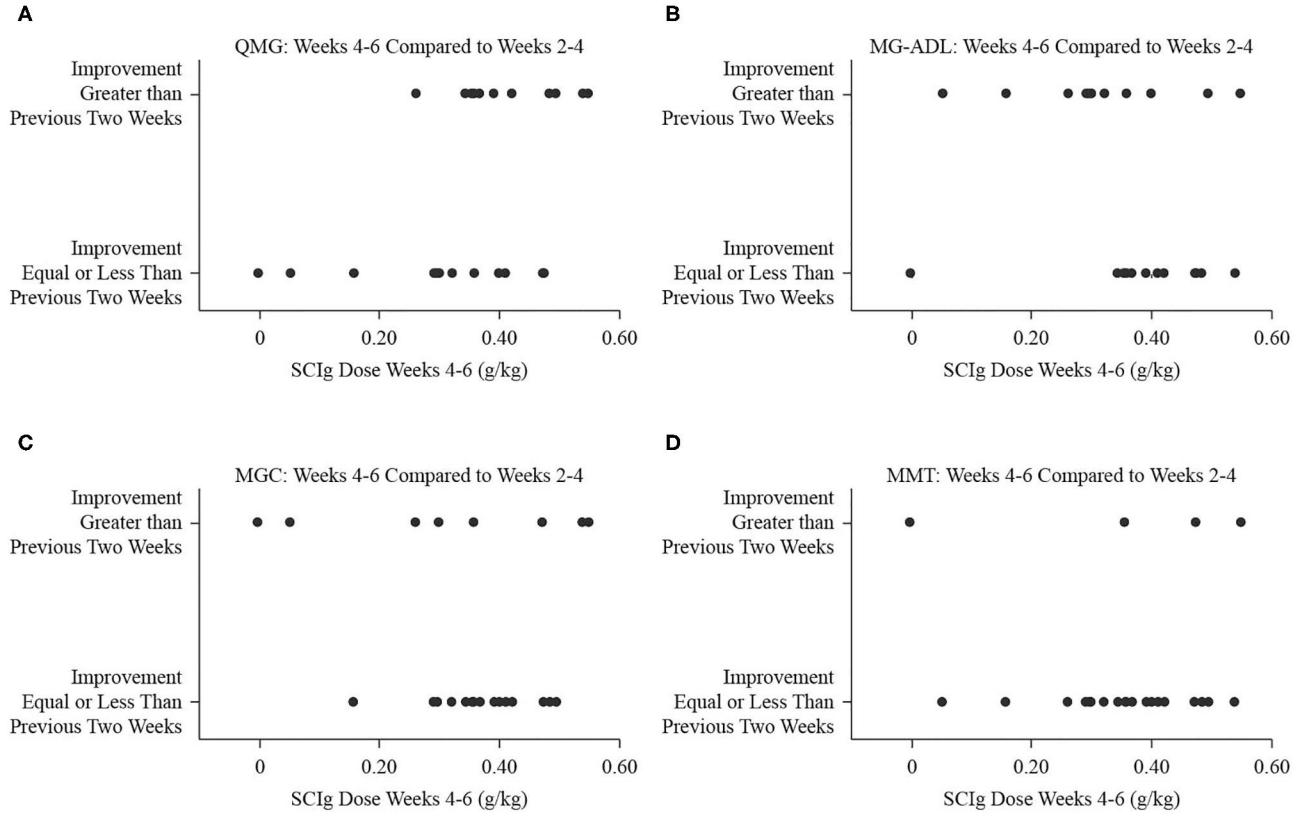

FIGURE 4 | Binary fitted plots comparing the improvements in QMG **(A)**, MG-ADL **(B)**, MGC **(C)**, and MMT **(D)** scores over the interval from weeks 4 to 6 (end of study) to the interval from weeks 2 to 4. The binary answer was that improvement was greater, or that it was equal to or less than, including no improvement or worsening. These results (dependent variable) were plotted on the SCIg dose over the interval from weeks 4 to 6 adjusted for patient weight (independent variable).

evidence has implicated a differential whereby prednisone was more effective for ocular manifestations, and IVIg or therapeutic plasma exchange were more effective for generalized manifestations (16). Our data are encouraging that SCIg may be used as a therapy in various presentations of MG.

Binary logistic regression analyses did not find a relationship between the dose, expressed in g/kg, and the odds of improving more or less relative to a previous 2-week interval. When comparing the interval from baseline to week 2 to the interval from weeks 2 to 4, increasing the dose of SCIg did not portend further improvement. Similarly, as the dose tapered in the interval from week 4 to end of study, the odds of improvement, or lack thereof, did not significantly correlate with the dose. Extrapolating the doses in the intervals from baseline to week 2 or from week 4 to end of study over three dosing intervals yields total doses below 2 g/kg (1.41 g/kg for weeks 2–4 and 1.05 g/kg for week 4 to end of study, respectively), suggesting that lower doses of SCIg may be sufficient to both initiate and maintain a response in patients with mild to moderate MG exacerbations. In view of the large volumes required and the resultant time over which 2 g/kg needs to be administered, head-to-head comparisons of 1 g/kg and 2 g/kg regimens are warranted. To date, this has not been formally evaluated in MG for SCIg *per se*. A single reported case achieved stabilization of MG with 0.16 g/kg per week (4). In looking beyond SCIg in MG, there is no evidence of a difference in efficacy between 1 and 2 g/kg dosing regimens for IVIg in MG exacerbation (17). Furthermore, a phase 3 trial in maintenance treatment of chronic inflammatory demyelinating polyradiculoneuropathy with SCIg that compared high dose (0.4 g/kg per week), low dose (0.2 g/kg per week), and placebo found significantly less relapses in the treatments groups compared to placebo, but no significant difference between treatment groups (18). Beyond the obvious benefit of lower doses conferring a lower likelihood of side effects, there remains doubt about the role of SCIg in severe exacerbations and crises given the infusion volumes and infusion times required (5), which could potentially be ameliorated by lower dosing requirements.

The regression analyses we performed did not demonstrate significant correlations between clinical scores and the change in IgG level, although there was a trend toward significance for negative correlations between improvement in MG-ADL and change in IgG level as well as improvement in MGC and change in IgG level over the entire study period. There was also a trend toward significance for a positive relationship between

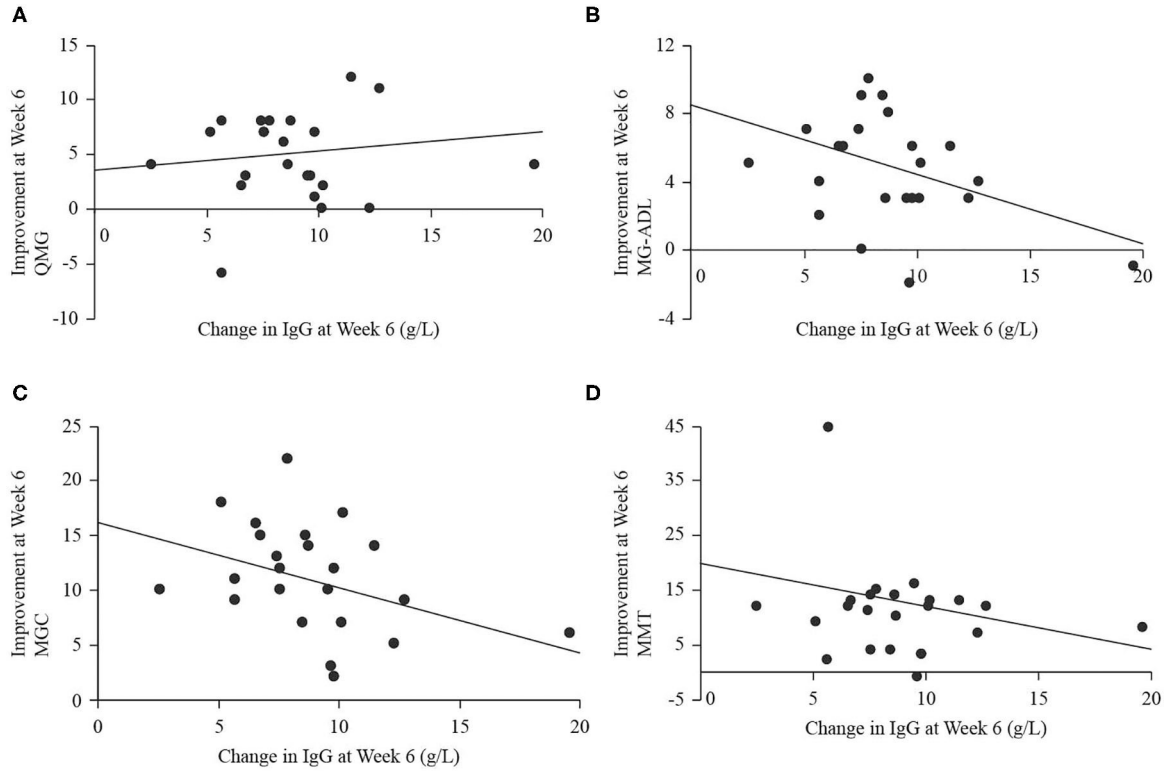

FIGURE 5 | Scatter plots with regression lines for improvement in QMG **(A)**, MG-ADL **(B)**, MGC **(C)**, and MMT **(D)** from baseline to week 6 (end of study; dependent variables) plotted on change in IgG level from baseline to week 6 (independent variable).

improvement in QMG and change in IgG level over the interval from weeks 2 to 4. Without a larger data set to explore whether these relationships would achieve significance, their meaning should not be overstated. In view of previous reports of stable IgG titers in patients treated with SCIg without the peak and trough changes expected from IVIg (5), it is possible that a steady state IgG level, manifested in minimal change from baseline, indicates effective immunomodulation and a resultant improvement in measures of disease burden. Indeed, we showed that peak IgG levels occurred before study end (3), but did not have longer term follow up data to assess whether levels remained elevated near the peak level or declined to a lower steady state. The trend toward significance for the positive relationship between QMG and IgG over the interval from weeks 2 to 4, during which change in IgG level was maximal, may represent attenuation of autoimmunity during rapid IgG rise. Taken together, a rapid rise in IgG may be required to blunt the immune response, then a steady state may be required to maintain relative quiescence. Adding to the complexity of using IgG levels as biomarker is the evident relationship between IgG levels and outcomes in acute demyelinating neuropathies, and lack of evidence for the same relationship in chronic demyelinating neuropathies (19). Clearly, more research is required to define the physiologic changes associated with SCIg infusion in MG, and subsequently to assess whether serum IgG titers have a role as a biomarker of immunomodulation in this condition. Finally, therapeutic mechanisms of SCIg are not fully understood, though evidence exists to support a pleotropic immunomodulatory role that extends beyond IgG levels and into other components of humoral as well as cellular immunity (20).

Considering that we demonstrated early and continued clinically meaningful improvement in all the disease scores evaluated, there is rationale for a strategy that employs sustained dosing in order to provide patients an adequate therapeutic trial before discontinuing SCIg. While our trial did not include long-term follow-up data, others have shown SCIg is a viable maintenance therapy in MG (2, 5–8). Adding to this, it is clear that SCIg is safe and tolerable, which lends further support to providing an adequate therapeutic trial for a given patient.

Our initial report indicated that mild adverse reactions occurred, but no serious adverse reactions, including hemolysis or acute kidney injury, were observed (3).

LIMITATIONS

The primary limitation of our study is that it was an open-label trial without a control arm wherein all participants followed a dose-escalation protocol. The analyses we presented regarding

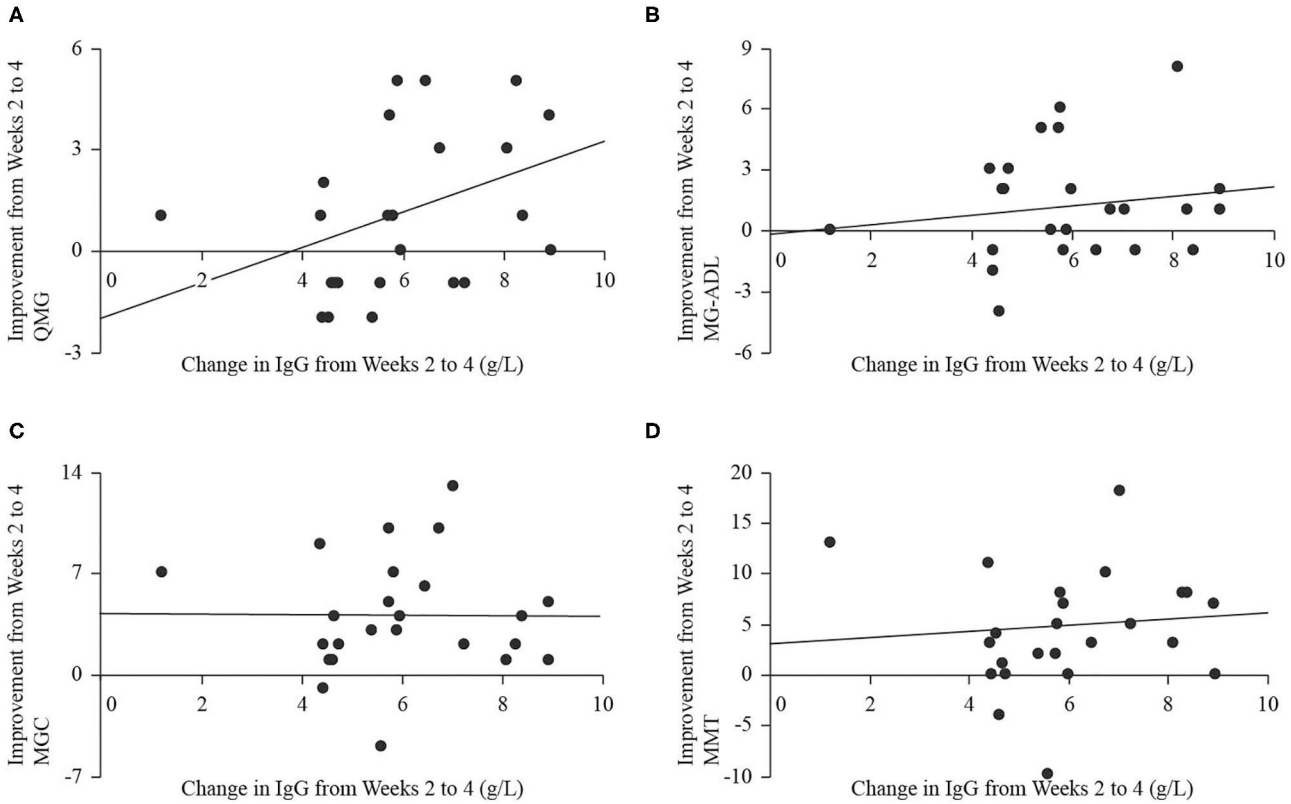

FIGURE 6 | Scatter plots with regression lines for improvement in QMG **(A)**, MG-ADL **(B)**, MGC **(C)**, and MMT **(D)** from weeks 2 to 4 (dependent variables) plotted on change in IgG level from weeks 2 to 4 (independent variable).

dosage and clinical improvement in **Figures 2**, **3** were made possible by the variability in per interval doses that occurred as a result of differences in individual tolerances and the interface of dosage timing and assessment days. As such, a secondary limitation is that the *post-hoc* analyses we present support the need for further investigation, but the trial was not specifically designed to answer a question regarding different dosing regimens.

CONCLUSIONS

Although the dose of SCIg varied over our study period, the odds of improvement were not significantly correlated with this, which suggests that lower doses of SCIg may be sufficient to both initiate and maintain a clinically meaningful response in patients with mild to moderate MG exacerbations. Head-to-head comparisons of different, possibly lower, dosing regimens for SCIg are warranted. The change in clinical scores was also not significantly associated with IgG levels, but there was a trend toward a negative relationship over the entire study period whereby less improvement in MG-ADL and MGC was seen with larger changes in IgG, but there was also a trend toward a positive relationship over weeks 2 to 4 for QMG and IgG level. These findings require further investigation; however, they indicate the complex nature of the effects of SCIg administration.

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 human participants were reviewed and approved by HREB, University of Alberta. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

BP performed the statistical analyses and drafted the manuscript. GB performed initial data collection and revised the manuscript. ZS designed and supervised the original study, conceptualized the *post-hoc* analyses, and revised the manuscript. All authors contributed to the article and approved the submitted version.

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frontiers in Neurology

REVIEW
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Reappraisal of Oral Steroid Therapy for Myasthenia Gravis

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Treatment with oral corticosteroids at high doses with an escalation and de-escalation schedule is effective against myasthena gravis (MG). In fact, the use of corticosteroids has led to a reduction in mortality to below 10% after the 1960s. However, long-term use of oral steroids above a certain dosage level is known to cause a number of problems. In 2014, the Japanese clinical guidelines for MG proposed that the first goal in MG treatment (treatment target) should be set at minimal manifestations (MM) with oral prednisolone (PSL) 5 mg/day or below, and that treatment strategies should strive to attain this level as rapidly as possible. In 2015, a multicenter, cross-sectional study revealed that higher PSL dose and longer PSL treatment do not ensure better outcome. In the absence of good response, the PSL dose should be decreased by combining with modalities such as plasma exchange/plasmapheresis and intravenous immunoglobulin (fast-acting treatments). In 2018, we conducted a multicenter, cross-sectional study in a large population of Japanese patients with generalized MG, aiming to elucidate the correlation between oral PSL regimens and achievement of treatment goals. The ORs for low vs. high dose to achieve treatment goals at 1, 2, and 3 years were 10.4, 2.75, and 1.86, respectively, whereas the corresponding ORs for low vs. medium dose were 13.4, 3.99, and 4.92. Early combination with fast-acting therapy (OR 2.19 at 2 years, 2.11 at 3 years) or combination with calcineurin inhibitors (OR 2.09 at 2 years, 2.36 at 3 years) were also positively associated with achieving treatment goals. These results indicate that early combination of low-dose PSL regimens with other therapies is the key for early achievement of treatment goals in generalized MG. However, even with this regimen, ∼35% of patients did not achieve the treatment target after 3 years. These results suggest the limitation of the current oral corticosteroid therapy. We need to develop new treatment options to increase the rate of satisfactory outcome.

Keywords: myasthenia gravis, oral corticosteroids, treatment strategies, cross-sectional study, logistic regression analysis

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INTRODUCTION

Oral corticosteroids remain the primary treatment for generalized myasthenia gravis (MG), although various other disease-modifying therapies have emerged (1). Primary disease-modifying therapies for MG include immunosuppression therapy using oral prednisolone (PSL), azathioprine, cyclosporine, mycophenolate mofetil, and tacrolimus (2–6). Methotrexate,

another immunosuppressant, is an effective steroid-sparing agent having similar efficacy and tolerability to azathioprine (7). On the other hand, additional immunomodulatory therapies may be required for aggressive exacerbations of MG, such as plasma exchange/plasmapheresis (PE/PP) and intravenous immunoglobulin (IVIg) (8–13). For patients receiving low-dose prednisolone, treatment goal is usually set at minimal symptoms (MM) according to the Myasthenia Gravis Foundation of America (MGFA) postintervention status (14). To achieve the treatment goal, various immunosuppressive agents have been added to corticosteroids as steroid-sparing agents at the start of treatment (5, 15–18).

This short review will provide an overview of corticosteroid treatment for generalized MG, and introduce a favorable regimen of oral corticosteroids for generalized MG based on a nationwide survey in Japan.

HISTORY OF CORTICOSTEROID TREATMENT FOR MG

In 1935, Simon (19) reported the effects of treating MG with anterior pituitary extract. This was probably the first description of the therapeutic effect of corticosteroid-related agents on MG. Subsequently, many reports of small-scale studies in the 1950s and 1960s described favorable effects of adrenocorticotropic hormone and corticosteroids on MG. Grob et al. (20) underscored the fact that the use of corticosteroids led to a reduction in mortality to below 10% after 1966.

Prednisone and prednisolone are the oral corticosteroids commonly used for MG treatment. Both are synthetic corticosteroids sharing similar pharmacological properties such as effectiveness, adverse side effects, dosing schedules, and drug interactions. Prednisone is a biologically inactive compound which must be converted by liver enzymes to prednisolone before it can act. Therefore, it is prudent to use prednisolone that do not require enzymatic activation in clinical settings in which liver enzymatic activity is impaired (such as severe hepatic failure) (21).

In 1970, Warmolts et al. (22) reported the beneficial effect of alternate-day prednisone in a patient with MG. In the 1970s and 1980s, many clinicians preferred to start prednisone at a low dose (10–25 mg) gradually increasing to 60–100 mg on alternate days, maintain the dose until maximum improvement is reached, and then taper the dose ("dose escalation and de-escalation"). Pascuzzi et al. (23) retrospectively analyzed 116 MG patients treated with prednisone 60–80 mg daily until the onset of improvement, followed by lower-dose alternate-day therapy. They reported that sustained improvement was achieved after a mean of 13.2 days (range, 12 h to 60 days; SD, 11.5 days) of high-dose oral prednisone, and that the duration of high-dose oral prednisone to the time of maximal improvement ranged from 2 weeks to 6 years (mean, 9.4 months; SD, 8.8 months). Finally, they found 80.2% of the patients achieved either remission (27.6%) or marked improvement (52.6%). Sghirlanzoni et al. (24) evaluated the effects of oral corticosteroids in 60 MG patients by long-term observation, and noted improvement in 72% of the patients. In addition, they found the best results in those whose symptoms started after the age of 40 years, and a correlation between the starting dose of prednisone and the rate of improvement. On the other hand, Bae et al. (25) reported that a high daily dosage of prednisone relative to body weight was neither a predictor of exacerbation nor a predictor of early improvement in bivariate correlation analysis. They noted the possibility of steroid-induced exacerbation when prescribing prednisone for MG, especially when treating elderly patients and patients with bulbar dominant or severe disease. Although there are few randomized trials of oral corticosteroids alone, a Cochrane systematic review on corticosteroids for MG published in 2005 concluded that limited evidence from randomized controlled trials does not show any difference in efficacy between corticosteroids and either azathioprine or intravenous immunoglobulin (26).

Dose escalation and de-escalation was also performed traditionally in Japan. Oral steroids were often given using a dose escalation schedule until the symptoms improved sufficiently or until a maximum dose of 50–60 mg/day was reached. Treatment was continued at the highest dose followed by gradual tapering, although the oral steroids usually had to be given chronically with significant risk of adverse events. To address the difficulty of achieving complete remission in adult-onset generalized MG cases, the Japanese clinical guidelines for MG published in 2014 recommend that treatment strategies should aim to maintain health-related quality of life and mental health, considering the possibility of prolonged treatment (27). The guidelines also recommend to reconsider the use of high-dose steroids with escalation and de-escalation, in view of the problems associated with long-term use and the availability of other treatment options.

DOSE-DEPENDENT EFFECTS OF CORTICOSTEROIDS

The expected pharmacologic actions of corticosteroids for treating MG may be divided into an anti-inflammatory action and an immunosuppressive action. Corticosteroids target the postsynaptic membrane to suppress inflammatory reactions including complement-mediated reactions at the endplates. The corticosteroids also inhibit the immune system at multiple sites, including sequestration and decrease of lymphoid cells (28). The anti-inflammatory and immunosuppressive actions of corticosteroids are inextricably linked, perhaps because they both involve inhibition of leukocyte functions (29).

In pharmacokinetics, glucocorticoids (GC), a class of corticosteroids, diffuse across cell membrane and bind to cytoplasmic GC receptor (GR). This binding leads to dissociation of heat shock protein 90, and induces transport of the GC-GR complex across nuclear membrane to the nucleus. In the nucleus, the GC-GR complex binds with various genetic promoters and enhancers of genomic DNA according to the GC responsive elements to regulate the transcription of the target genes (21). These mechanisms would suggest that higher doses of corticosteroids are effective to activate more GRs to obtain favorable anti-inflammatory/immunosuppressive

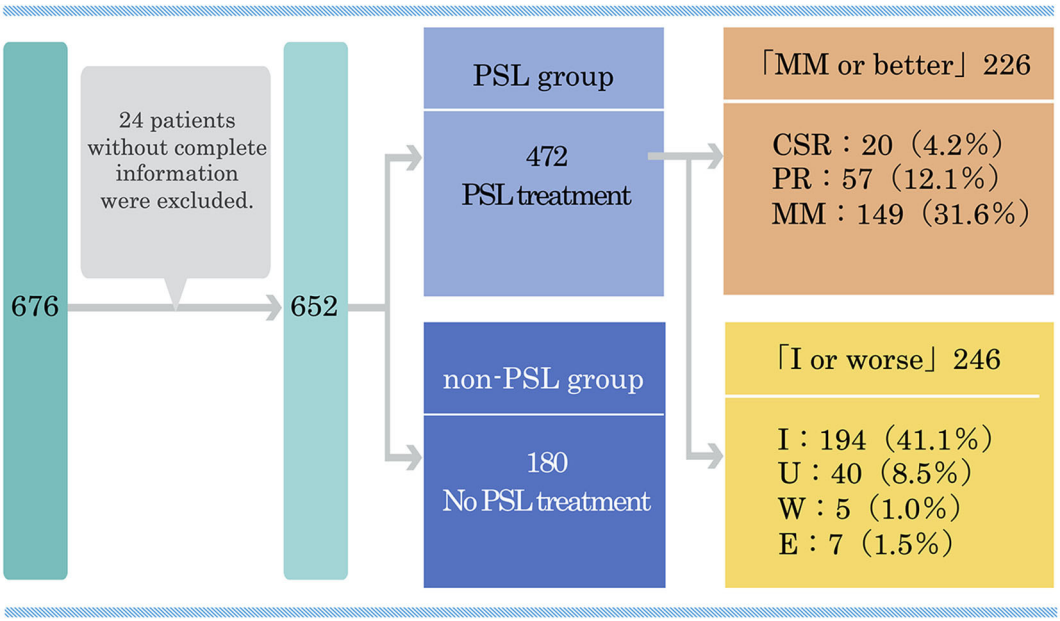

FIGURE 1 | Classification of 472 MG patients treated with prednisolone according to the present disease status in a multicenter, cross-sectional study in 2015. MG, myasthenia gravis; PSL, prednisolone; CSR, complete stable remission; PR, pharmacological remission; MM, minimal manifestations; I, improved; U, unchanged; W, worse; E, exacerbation. This figure is drawn from data published in (32).

effects. Indeed, it is known that high doses of GCs inhibit immunoglobulin synthesis, kill B cells (30), and decrease production of components of the complement system (31).

Then, the clinical question is: Does higher doses of corticosteroids ensure better outcome in MG treatment?

IS HIGH-DOSE CORTICOSTEROID SUPERIOR TO LOW-DOSE IN MG TREATMENT?

Oral Corticosteroid Therapy and Present Disease Status in MG

As described in the history of MG therapy, oral corticosteroids are traditionally used at high doses with escalation and de-escalation schedules. High-dose oral steroids may not always provide sufficient improvement and may induce long-term steroid-related side effects that impair the quality of life (QOL) of many patients (5, 23).

We studied 472 MG patients in 2015 to investigate the relationship between oral prednisolone (PSL) dosage and the status of disease at the time of study (current status) (32). These patients were divided by current status into a group of MM or better (complete stable remission, pharmacological remission, MM) ($n = 226$) and a group of improved or worsening status (improvement, unchanged, worse, or exacerbation) ($n = 246$) (**Figure 1**). There was no significant difference in baseline severity based on clinical classification of MGFA between the MM or better group and the improved or worse group by Pearson χ^2 test. The treatment duration with PSL was also similar in the two groups (6.5 ± 6.4 vs. 7.1 ± 7.0 years, $p = 0.56$). Patients taking <5 mg/day of oral PSL were more likely to be classified in the MM or better than in the improved or worse group (75.2 vs. 48.8%, $p < 0.0001$). The daily dose of PSL was significantly lower in the MM or better group than in the improved or worse group (4.7 ± 5.3 vs. 7.3 ± 6.5 mg, $p < 0.0001$). The duration of taking PSL ≥10 mg/day was significantly shorter in the MM or better group than in the improved or worse group (10–20 mg/day: 1.9 ± 4.0 vs. 2.1 ± 3.9 years, $p = 0.01$; 20 mg/day or more: 0.6 ± 1.2 vs. 1.4 ± 3.5 years, $p = 0.0002$). In addition, cumulative PSL doses received in the past year was smaller in the MM or better group than in the improved or worse group (1705.9 ± 1791.2 vs. 2460.2 ± 2009.8 mg, $p < 0.0001$).

Independent Predictors for MM or Better Status From Multivariate Logistic Regression Modeling

Multivariate logistic regression identified MM or better status at peak dose of PSL ($p < 0.0001$) and treatment with PE/PP and/or IVIg ($p = 0.04$) as significant independent positive predictors of achieving MM or better status, and total PSL dose in the past year as the only independent negative predictor ($p = 0.03$). OR was the highest for MM or better status at peak dose of PSL (12.25; 95% CI 7.22–21.43), followed by treatment with PE/PP and/or IVIg (1.92; 95% CI 1.03–3.66) and total dose of PSL in the past year (0.17; 95% CI 0.03–0.88) (**Table 1**). Other significant variables identified in univariate analyses and entered into the logistic regression model, including the worst QMG score, PSL dose and duration, and use of calcineurin inhibitors (CNI), were

TABLE 1 | Positive and negative predictors for MM or better status from multivariate logistic regression modeling.

Parameters		Odds ratio (95% CI)	*p*-value
Positive predictors	MM or better at peak dose	12.25 (7.22–21.43)	<0.0001
	PE/PP and/or IVIg	1.92 (1.03–3.66)	0.04
Negative predictor	Total PSL dose during past 1 year	0.17 (0.03–0.88)	0.03

Modified from (32). MM, minimal manifestations; PSL, prednisolone; PE, plasma exchange; PP, plasmapheresis; IVIg, intravenous immunoglobulin.

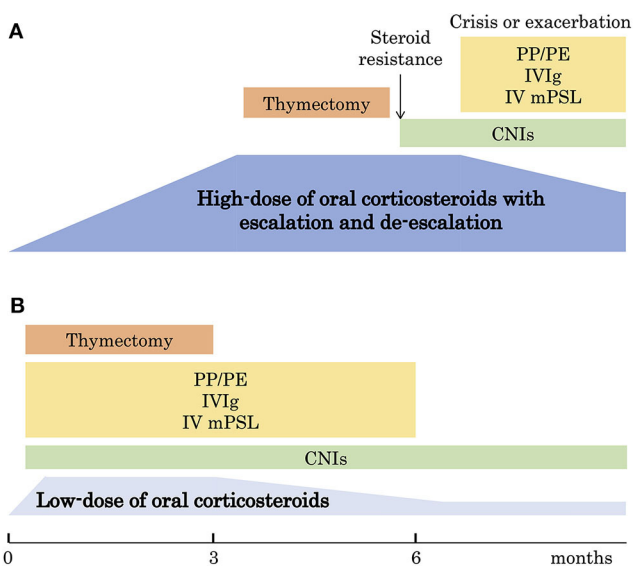

FIGURE 2 | Changes of therapeutic strategy. **(A)** The traditional strategy with high-dose oral corticosteroids with escalation and de-escalation schedule. **(B)** The new strategy with low-dose oral corticosteroids. PE, plasma exchange; PP, plasmapheresis; IVIg, intravenous immunoglobulin; mPSL, methylprednisolone; CNI, calcineurin inhibitor. This figure is drawn from data published in (32).

not significant independent predictors for the achievement of current status of MM or better.

Changes of Therapeutic Strategy

These findings lead to the conclusion that higher doses of PSL and longer duration of PSL treatment are not associated with improvement of current condition and that response to PSL treatment is independent of baseline disease severity based on MGFA classification. In other words, MG patients do not possess specific clinical factors associated with poor response to oral corticosteroids, but they are composed of patients who respond well and others who response poorly to oral corticosteroids. Our results also suggest the need for fast-acting combination therapies such as PE/PP and/or IVIg to achieve MM or better in patients who respond poorly to oral corticosteroids. PE/PP, which uses filtration to remove pathological antibodies through three to seven repeated plasma exchanges, has been used in patients with crisis or aggravated MG (8–10). In addition, IVIg is more frequently used as a promising alternative to PE/PP during exacerbations of MG (11–13). However, according to our results, even in the absence of a crisis or exacerbation, fast-acting treatment may be recommended to induce MM or better status at peak doses of oral PSL.

Many patients and physicians prefer to taper corticosteroid doses by combining with other immunosuppressive agents to reduce the side effects of long-term monotherapy with high-dose oral corticosteroids, including mood symptoms and cosmetic problems (33–38). We found that in Japan, percentage of CNI use was high in both the MM or better group and the improved or worse group (51.3 vs. 70.7%). CNIs such as cyclosporine and tacrolimus are recognized as potent corticosteroid-sparing agents, especially in patients receiving high-dose oral corticosteroids for extended periods of time (4, 36–46). If the patients in this study had not been taking CNIs, they may have had to take higher doses of corticosteroids.

We proposed a low-dose regimen of oral corticosteroid treatment in MG based on the results of our nationwide survey in 2015 (32) (**Figure 2**). The low-dose regimen includes low dose of oral corticosteroids, early combination of CNIs, and fast-acting treatments to improve remaining symptoms quickly. The next clinical question is: Is the low-dose regimen superior to the high-dose regimen for long-term prognosis of MG?

FAVORABLE REGIMEN OF CORTICOSTEROIDS FOR MG TREATMENT

Oral Corticosteroid Dosing Regimen and Long-Term Outcome in MG

Even the international consensus guidance does not include an internationally accepted standard dosing regimen for oral corticosteroids (14). We conducted a multicenter cross-sectional study to examine the correlation between oral PSL administration method and actual achievement of treatment goals (47). A total of 590 patients with generalized MG were classified into three groups according to the dose level of oral PSL during the treatment period: high dose ($n = 237$), intermediate dose ($n = 187$), and low dose ($n = 166$) (**Figure 3**). Clinical characteristics, history of non-PSL treatment, and prognosis were compared among the three groups. The effect of oral PSL regimen on the achievement of treatment goals was followed over a 3-year treatment period.

Independent Predictors for MM-or-Better-5 mg Identified by Multivariate Logistic Regression Modeling

Our group also suggests that MM status or better with PSL 5 mg/day or less (MM-or-better-5 mg) is a more realistic treatment goal than CSR, and is achievable by more patients (48).

Multivariate logistic regression analysis identified low-dose regimen, early combination with fast-acting treatment (high-dose methylprednisolone or PE/PP or IVIg), and early use of CNI as predictors of achieving the treatment goal of MM-or-better-5 mg over 6 months (47). ORs for low-dose (vs. high-dose)

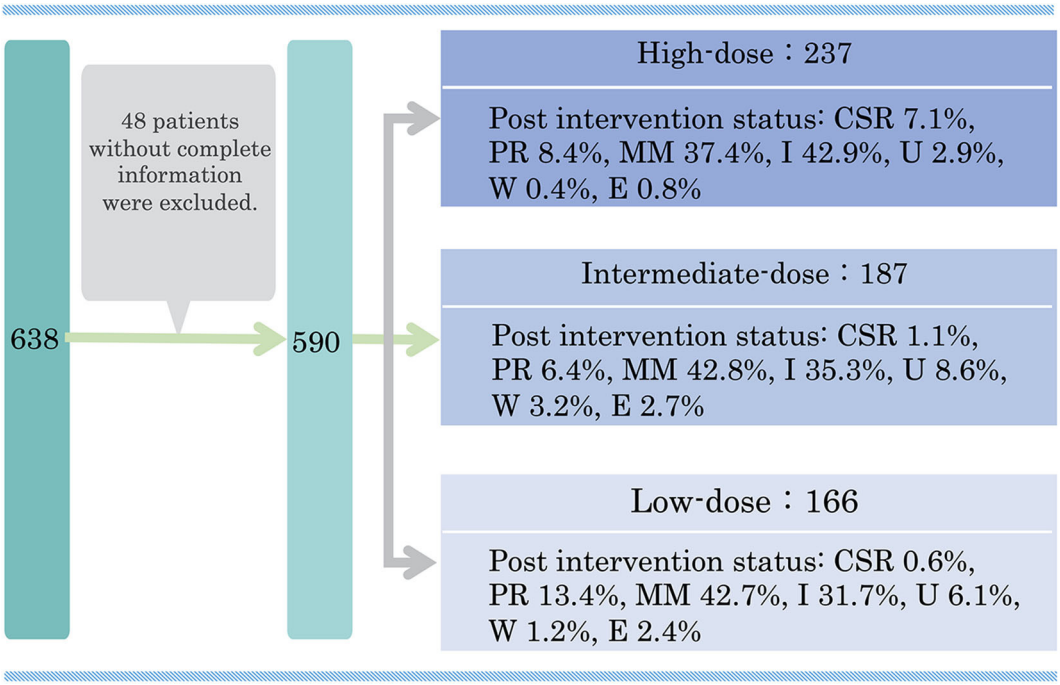

FIGURE 3 | Classification of 590 prednisolone-treated generalized MG patients according to the present disease status in a multicenter, cross-sectional study in 2018. MG, myasthenia gravis; PSL, prednisolone; CSR, complete stable remission; PR, pharmacological remission; MM, minimal manifestations; I, improved; U, unchanged; W, worse; E, exacerbation. This figure is drawn from data published in (47).

TABLE 2 | Independent predictors of MM-or-better-5 mg for ≥6 months identified by multivariate logistic modeling.

Parameter	Odds ratio (95% CI), *p*-value		
	1 year	2 years	3 years
Low-dose regimen (vs. high-dose regimen)	10.4 (4.54–25.2), <0.0001*	2.75 (1.31–5.88), 0.007*	1.86 (0.79–4.49), 0.15
Early HMP/PP/IVIg	2.04 (0.89–4.78), 0.09	2.19 (1.11–4.42), 0.02*	2.11 (1.03–4.44), 0.04*
Early use of CNIs	1.59 (0.78–3.24), 0.20	2.09 (1.09–4.06), 0.03*	2.36 (1.13–5.09), 0.02*

Modified from (47).
HMP, high-dose intravenous methylprednisolone; PE, plasma exchange; PP, plasmapheresis; IVIg, intravenous immunoglobulin; CNI, calcineurin inhibitor.

TABLE 3 | Achievement of MM-or-better-5 mg for ≥6 months classified by oral PSL dosing regimen.

Duration	High-dose regimen (*n* = 237)	Low-dose regimen (*n* = 166)
1 year	9.6%	52.1%*
2 years	29.9%	61.2%*
3 years	44.1%	64.1%*

Compiled from data published in (47).
**p < 0.0001 using ANOVA followed by Tukey–Kramer test.*

regimen were 10.4 ($p < 0.0001$) after 1 year, 2.75 ($p = 0.007$) after 2 years, and 1.86 ($p = 0.15$) after 3 years of treatment. ORs for early combination of high-dose methylprednisolone or PE/PP or IVIg were 2.19 at 2 years ($p = 0.02$) and 2.11 at 3 years ($p = 0.04$), and ORs for CNI were 2.09 at 2 years ($p = 0.03$) and 2.36 at 3 years ($p = 0.02$) (**Table 2**). These results suggest that early combination of low-dose PSL regimens with other therapies is useful for early achievement of treatment goals in patients with generalized MG. However, only 64.1% of patients who received low-dose PSL therapy were able to achieve the treatment goal until 3 years (**Table 3**). Approximately 35% of patients did not achieve satisfactory outcomes with the new treatment strategy. These results suggest the limitations of current oral corticosteroid therapy and the need to improve the safety and efficacy of corticosteroid therapy.

FUTURE CONSIDERATIONS

Oral corticosteroids may be effective for good responders regardless of dosage. MG patients who respond well for various reasons may be able to reduce the dosage of steroids with less difficulty because dose reduction may follow the achievement of good outcome but not cause the outcome. Moreover, it

is not necessary to use high dosage of oral corticosteroids because a number of new treatment options are now available to achieve good outcome. It is time to reconsider high-dose steroid treatment for MG and seek a novel strategy based on patients' QOL. On the other hand, fast-acting treatment for generalized MG is not suitable for all patients from different countries, especially for patients in developing countries. In this case, further development of steroid drugs is required.

Over the past few decades, considerable efforts have been devoted to increase the potency of corticosteroids while minimizing their side effects by modifying the chemical structure of natural GCs (49). Alternative splicing, alternative translation initiation of mature mRNAs, and post-translational modifications have generated multiple GR isoforms with unique expression, gene regulation, and functional profiles, which have advanced our understanding of the molecular basis of GC susceptibility diversity. Genome-wide GR recruitment studies have shown significant difference of tissue-specific chromatin landscape in GC susceptibility (50).

An important challenge in the clinical application of GC is the heterogeneity of GC response between individuals. Advances in our understanding of GC expression patterns may reveal important mechanisms of poor response in MG treatment. The breakthrough may accelerate not only the design of novel therapeutic strategies for poor responders but also the prediction of enhanced response to corticosteroids for good responders. The understanding of the heterogeneity of GR signaling will permit the development of safer and more effective corticosteroid therapies with improved benefit/risk ratios for MG patients.

AUTHOR CONTRIBUTIONS

All authors were involved in conception and design of the work, and in acquisition of data. TI was involved in analysis, interpretation of data, and drafted the article. All other co-authors revised it critically for important intellectual content.

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REVIEW
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From Traditional to Targeted Immunotherapy in Myasthenia Gravis: Prospects for Research

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Treatment of Myasthenia Gravis (MG) is still based on non-specific immunosuppression. Long-term high dose corticosteroids is still a major cause of side effects, in young as well as in elderly patients in whom comorbidities further increase the burden of chronic immunosuppression. Moreover, awareness of the limits of traditional therapies has led to the concept of "refractory MG." The therapeutic approach to MG is therefore progressively evolving from the classic combination of corticosteroids and immunosuppressive drugs to new biological compounds targeting different immunopathological steps. Killing of B cells with Rituximab has been proposed and tested with positive results, particularly in patients with MuSK-associated MG. Therapeutic monoclonals against B cells at different stages of their maturation, or against molecules involved in B cell activation and function, represent a new area for further investigation. A differently targeted approach involved Eculizumab, a monoclonal antibody preventing the formation of C59b-induced MAC causing destruction of the neuromuscular junction. Data from clinical trials led to the approval of Eculizumab in the United States and Europe for MG. Since Eculizumab is a complement-targeted therapy, its use is limited to anti-acetylcholine receptor-associated MG, since anti-MuSK antibodies belong to IgG4 subclass and do not fix complement. Several anti-complement compounds are under investigation. An even more recent approach is the interference with the neonatal Fc receptor leading to a rapid reduction of circulating IgGs and hence of specific autoantibodies, an approach suitable for both anti-acetylcholine- and MuSK-associated MG. The investigation of compounds that selectively target the immune system will stimulate the search for specific biomarkers of disease activity and response to treatment, setting the basis for personalized medicine in MG.

Keywords: myasthenia gravis, autoimmunity, monoclonal antibodies, complement, clinical trials, Rituximab, Eculizumab, Fc receptor

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INTRODUCTION

Myasthenia gravis (MG) is an autoimmune disease of the neuromuscular junction (NMJ) characterized by weakness and fatigability of voluntary muscles (1). MG is a prototypical model of organ-specific autoimmunity in which target antigens and specific autoantibodies have been identified. The disease has been linked first to antibodies against the acetylcholine receptor (AChR), detectable in about 85% of patients, and more recently to the muscle-specific kinase (MuSK) or the

lipoprotein-related peptide 4 (LRP4). MuSK and LRP4, together with agrin, are involved in NMJ formation and clustering of AChRs on the postsynaptic membrane. Specific autoantibodies impair neuromuscular transmission according to different mechanisms. Anti-AChR antibodies block the acetylcholine binding site of the AChR, increase internalization and degradation of AChRs and, since they belong to the IgG1 subclass, fix complement ultimately leading to destruction of the NMJ (2). Anti-MuSK antibodies belong mainly to the IgG 4 subclass and therefore do not activate complement, but impair neuromuscular transmission by interfering with agrin-related AChR clustering. Anti-LRP4 antibodies belong to the IgG1 subclass, activate complement, and interfere with the LRP4-agrin interaction pathway (2). Whatever the mechanism and antibody specificity involved, the final outcome is the impairment of neuromuscular transmission leading to the typical muscle weakness and fatigability complained by MG patients.

Therapy of MG, regardless of antibody specificity, is still based on symptomatic treatment and non-specific immunosuppression (3, 4). Cholinesterase inhibitors are the first-line treatment and maybe sufficient for mild MG at least at the beginning of the disease, but in the majority of patients variable degrees of immunosuppression are required and corticosteroids still represent the mainstay. Evidence of the efficacy of corticosteroids comes from retrospective studies spanning several decades showing that they are effective usually within a few weeks in generalized MG. The superiority of prednisone over placebo has been demonstrated by a randomized study in ocular MG; however the effect of corticosteroids in preventing generalization in ocular MG has not been demonstrated (5). Notwithstanding the proven rapid effectiveness of corticosteroids, the burden of long-term toxicity has been evident for many years, promoting the use of immunosuppressive drugs as add-on therapy with a steroid-sparing effect. Azathioprine and mycophenolate mofetil remain the most frequently used compounds, and demonstration of their clinical efficacy derives almost exclusively from retrospective studies. Indeed, end points of efficacy for mycophenolate mofetil were not reached in a randomized study, likely due to protocol design, and the drug is prescribed according to clinical experience (6, 7). Even the steroid-sparing effect attributed to non-biological immunosuppressive drugs has not been demonstrated in a controlled fashion except for azathioprine (8). A comprehensive review on immunosuppression in MG has been recently published (9). Cyclosporine and Tacrolimus, another inhibitor of calcineurin activity, but more potent than cyclosporine, are used as second-line therapy in MG patients, particularly in Eastern countries (10).

Immunomodulating therapies, i.e., those directly interfering with autoantibody activity such as intravenous immunoglobulin (IVIg) and plasmaexchange (PLEX), are used to obtain a rapid clinical response in patients with severe clinical compromise or in case of myasthenic crisis. IVIg and PLEX are considered equally effective according to results from randomized studies (11–13). The fast and short-term effect of PLEX is considered undisputable even though not investigated in a controlled fashion due to ethical reasons.

The occurrence of thymic abnormalities, particularly thymic hyperplasia reported in up to 70% of patients with early-onset MG, represents the immunopathological rationale for thymectomy as a therapeutic strategy to modify the natural course of the disease, with the idea of removing a site of autosensitization or perpetuation of the autoimmune attack (14). After four decades during which thymectomy was generally recommended for young-onset MG, a meta-analysis of the literature considered the procedure potentially capable of facilitating remission or improvement of MG, but still lacking a definitive demonstration (15). A controlled study published in 2016 showed that thymectomy improved the clinical outcome at 3 years and reduced the need for corticosteroids (16); remission was not recorded, but remission was not considered as an outcome in the study. Extension of the clinical observation up to 5 years still showed benefit from thymectomy and prednisone in non-thymomatous MG, albeit the patients' sample was small (17). At present, thymectomy is recommended for antiAChR-positive MG, increasingly performed with non-invasive techniques (18, 19). A further observation emerging from the above studies is that, even after thymectomy, MG still requires corticosteroids and immunosuppressive drugs for several years.

Our clinical experience in a very large series of MG patients treated according with traditional guidelines showed that complete stable remission was observed in 22% of AChR-positive MG patients, and about 30% were still symptomatic with various degrees of impairment at the end of the clinical follow-up (20). A shared clinical observation is that a subgroup of patients with MG can be affected with an unstable, poorly controlled form of the disease for a considerable time, leading to the concept of "refractory MG" (21–23). The definition of refractory MG is not a unique concept. The current definition includes patients failing to respond to adequate immunosuppression, or developing severe side effects or have comorbidities hindering the use of conventional therapies, patients needing frequent rescue treatment with IVIg or PLEX, or with frequent myasthenic crisis (24). Younger age at onset, female sex, history of thymoma, and positive MuSK antibodies have been associated with refractory MG in a series of patients (21). However, the burden of refractoriness goes far beyond the clinical features to which it has been associated and is likely to be considerably underestimated (25). Indeed, the impact on physical and mental functioning, ability to work and employment, and on activity of daily living need further investigation in order to be adequately weighed in the definition and assessment of refractoriness (24, 26). Moreover, we lack biomarkers correlated with response to treatments as well as guidelines on the optimal sequence of therapeutic interventions to adopt in refractory MG.

Despite the availability of several therapeutic options, the need to avoid the use of corticosteroids, or at least reduce their use as much as possible, is still unmet, and such a need is not limited to refractory patients but should concern all patients. Interestingly, RCTs in which the primary end-point was the reduction up to withdrawal of prednisone failed, though caveats in the protocols might have influenced the results (7, 27). Moreover, the effect of immunosuppressive drugs is usually too slow to justify their

use as a single drug in the majority of patients, particularly in those with bulbar impairment. The duration of corticosteroid therapy in MG is not predictable, and in most patients spans from several months to years, not to mention patients who become steroid-dependent. A systematic analysis on the socio-economic impact of corticosteroids in MG is not available but the risk of health concerns including osteoporosis, metabolic, endocrine, ophthalmologic, and cardiovascular complications is considerable, even when corticosteroids are used in combination with immunosuppressive drugs. Another variable in the therapeutic decision is the increasing unwillingness to accept the iatrogenic burden of traditional treatments.

The introduction of new biological compounds directed specifically against different steps of the autoimmune process at the basis of MG has opened a new era in the field of its treatment. New classes of drugs, mainly biological, have entered clinical experimentation, and eventually reached Drug Agencies authorization; they belong to three major groups: a. Complement inhibitors; b. Neonatal Fc Receptor (nFcR) antagonists; and c. anti-B cell therapies (**Figure 1**).

Complement Inhibitors

Among complement inhibitors, Eculizumab (ECU), a humanized monoclonal antibody, was the first drug tested due to its effect on complement-fixing anti-AChR antibodies, thus matching the concept of "Precision Medicine." ECU targets C5 and prevents the formation of C5b which leads to the formation of the C5b-9 complex and thus prevents the effect of micro-destruction of the post-synaptic membrane, a crucial mechanism for the derangement of neuromuscular transmission (28). Clinical trials on ECU indicated that the drug was clinically effective, also in consideration that they included patients with refractory MG (29). Furthermore, ECU had a good safety profile as observed both in the Phase 3 study and the open-label extension (30). Notably, ECU safety in MG was similar to that observed in neuromyelitis optica (31) as well as in the long-term use for paroxysmal nocturnal hemoglobinuria (32). The risk of meningitis was negligible due to vaccination to Neisseria Meningitidis as to date only one non-fatal case was observed in a generalized MG patient concomitantly treated with two immunosuppressive drugs. Zilucoplan and Ravulizumab are other complement inhibitors currently tested in MG. Zilucoplan is a subcutaneously self-administered peptide of 15 aminoacids that binds specifically to C5 and prevents the cleavage of C5 into C5a and C5b; Zilucoplan gave positive results in a phase 2 study recently reported (33). Ravulizumab has been developed by re-engineering ECU to create a novel longer-acting antibody allowing administration every 8 weeks (34). Interestingly, Ravulizumab offers the opportunity of a subcutaneous administration hence allowing patients to be treated at home.

Neonatal Fc Receptor Antagonists

Neonatal Fc Receptor (nFcR) antagonists is a new class of drugs used for the first time in MG. The capacity of these drugs to rapidly reduce circulating Igs offers a new therapeutic option for antibody-mediated disorders; if proven effective, nFcR will be an alternative to intravenous immunoglobulins or plasmaexchange, overcoming the increasing need of human plasma or the feasibility of apheresis when vascular access is poor.

nFcR antagonists include three groups of compounds: a) Recombinant Fc multimers, with multiple effects including FcRn targeting and inhibition of complement activation; b) Neonatal Fc receptor antagonists, including both IgG-derived Fc fragments, monoclonal antibodies or peptide mimetics; and c) antiFcgR antagonists. A comprehensive updated review on Fc-receptor targeting has been recently published (35).

Compounds under investigation in clinical trials in MG belong to nFcR antagonists, among these Efgartigimod, Rozanolixizumab, Nipocalimab (M281) and RVT-1401. The mechanism of action operates through the binding of the "antagonist" with the nFcR, a molecule responsible for IgG recycling at the endothelial level, and the binding results in a rapid and significant degradation (and reduction) of overall plasma IgG levels and hence pathogenic autoantibodies (36, 37). nFcR antagonists are very selective as they reduce IgG but not the other Ig isotypes or other plasma proteins, such as albumin. The clinical relevance of Efgartigimod, an engineered IgG1-derived Fc fragment, was given by the rapid (as early as 1 week) titer reduction of IgG associated with clinical improvement in MG-ADL, QMG, and MGQoL-15 scales (38); interestingly, the clinical improvement outlasted the recovery of IgG titer. Furthermore, the mechanism of action of nFcR antagonists enables treatment of both AChR- and MuSK-positive MG patients, since their mechanism of action is unrelated to complement activation. Rozanolixizumab, a humanized, high-affinity anti-nFcR monoclonal antibody administered subcutaneously provided promising results in a Phase 2 study (NCT03052751) on moderate to severe MG patients and is now tested in a Phase 3 double-blind, placebo-controlled, dose-selective (adaptive design) study. Nipocalimab, a fully humanized deglycosylated monoclonal antibody to nFcR is currently used in a Phase 2 study (NCT03896295) on moderate to severe MG patients. RVT-1401 (formerly IMVT-1401) is a human recombinant anti-nFcR monoclonal antibody under investigation in a phase 2 study in MG (NCT03863080).

Safety and tolerability of nFcR antagonists have been acceptable and different compounds share headache as the most frequent adverse event; infections were not different from those observed in the control groups considering severity and codification.

Anti-B Cell Therapies

B cells are crucial elements in the immune pathogenesis of MG, hence drugs targeting selectively these cells are likely to be relevant for treatment. The relevance of B cells is intrinsic to the multiple roles played in immune responses, among them: (i) B cells act as antigen-presenting cells; (ii) B cells interact with follicular helper T cells to generate memory B cells; (iii) B cell maturation leads to plasmablasts and plasmacells which generate immunoglobulins, including autoantibodies [reviewed in (39)]. B cell-targeted therapies can be performed by molecules that attack B cells both directly and indirectly, or via cytokine blockade.

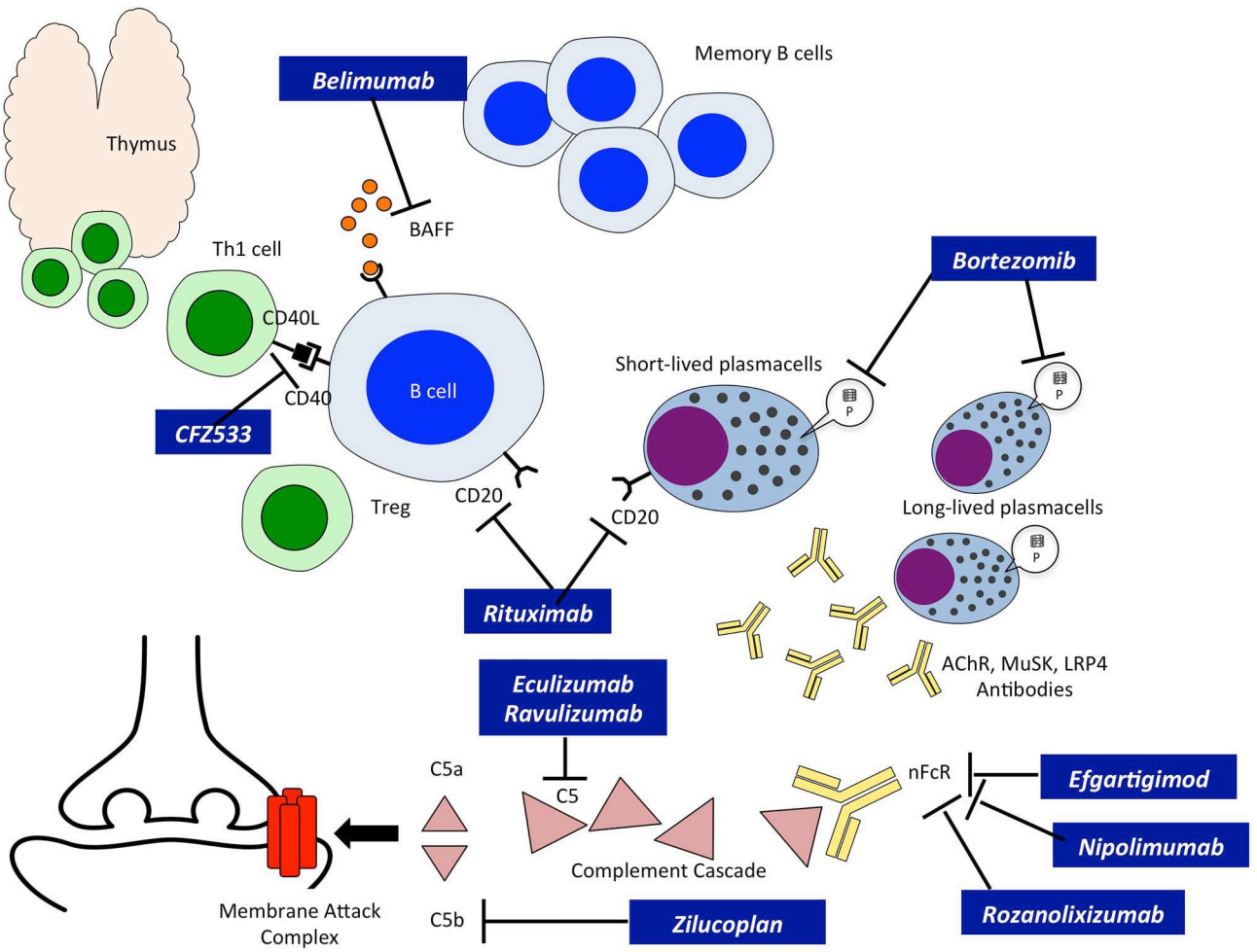

FIGURE 1 | Innovative therapies in Myasthenia Gravis and their site of action. A schematic drawing of autoreactive B cells, T cells and Plasmablasts/Plasmacells leading to the production of autoreactive antibodies. The site of action of the new therapies, indicated in black boxes, is also indicated. BAFF, B cell activating factor; CD20, B-lymphocyte antigen CD20; CFZ533, monoclonal antibody to CD40; Th1, T helper cell type 1; Treg, regulatory T cell; P, Proteasome; AChR, Acetylcholine Receptor; MuSK, Muscle Specific Kinase; LRP4, low density lipoprotein receptor-related protein 4; nFcR, immunoglobulin neonatal Fc Receptor; C5, complement component C5; C5a and b, fragments of C5.

Direct B Cell-Targeting

Rituximab, a monoclonal antibody developed for the treatment of lymphoma, has attracted much attention in the treatment of MG as it targets CD20, a molecule expressed on B cells from the stage of pre-B cells to that of mature/memory B cells. Case series and non-controlled studies have reported a beneficial effect of Rituximab in MG, showing a class IV evidence, with a particular emphasis on MuSK MG patients (40, 41). A recent phase 2 RCT (NCT02110706) performed on MG patients receiving Rituximab as a steroid-sparing agent did not meet the primary end-point (a prednisone reduction of at least 30%) and the *in fieri* phase 3 study was halted because of futility. Another study (NCT02950155) is ongoing to evaluate, as primary end point, the percentage of patients with a QMG score ≤ 4 and a daily Prednisolone dose of ≤ 10 mg at 16 weeks after randomization to Rituximab or placebo. Interesting clinical data emerged from a systematic retrospective review of the literature with collection of information regarding 169 MG patients from different centers (42). The authors reported a greater proportion of positive outcomes for MuSK- as compared with AChR-positive patients, as well as a significant reduction in the number of patients who experienced a relapse. Univariate analysis showed that MuSK antibody status was the only factor associated with improvement after Rituximab treatment. Multivariate analysis confirmed the importance of MuSK antibody status; moreover, mild to moderate severity of MG and median age lower than 45 years at the time of treatment were predictive of a positive outcome. Reduction in antibody titer did not predict a positive response to Rituximab. A retrospective cohort study reported recently showed that patients treated early in the course of the disease showed a greater benefit in non-MuSK MG compared with conventional immunotherapies (43).

The use of Rituximab in randomized controlled trials and the post-marketing surveillance highlighted a number of adverse

events with a wide range of severity. Rituximab appears to be well-tolerated with fewer side effects compared with those observed in more conventional therapies and chemotherapeutic regimens (42, 44). Rituximab use at present, however, should be carefully evaluated in the context of the benefit/risk ratio and the prospect of a chronic administration in the case of MG.

Several anti CD20 monoclonal antibodies are under investigation in several oncological diseases and Rheumatoid Arthritis and, hopefully potentially available for investigation in MG in the future (45, 46). Other anti-CD20 compounds include Ocrelizumab a recombinant, humanized anti-CD20 mAb that is approved for the treatment of primary progressive and relapsing multiple sclerosis, and ofatumumab, a cytolytic IgG1k fully human monoclonal antibody approved for the treatment of Chronic Lymphocytic Leukemia (47). Studies with these compounds in MG have not yet been proposed.

However, a limitation of Rituximab and similar compounds is that CD20 is not expressed on plasma cells and plasmablasts, the B cell subtypes responsible for antibody production. A new approach has been designed to target the B-cell maturation antigen (BCMA), a cell surface protein expressed only by antibody producing B cells, by means of CAR (chimeric antigen receptor) T cell technology. A phase Ib/IIa study to assess safety, tolerability and preliminary efficacy is ongoing in MG (Descartes-08, NCT04146051).

Another approach involved targeting of the CD40 signaling pathway, an approach that does not cause depletion of B cells but prevents their activation. Indeed, CD40 is expressed not only on B cells, but also on T cells and on antigen presenting cells. The binding of CD40L on T cell with CD40 on B cell leads to B cell activation and a cascade of events leading to differentiation into plasma cells and production of specific antibodies. CFZ533, a humanized monoclonal antibody against CD40, has been investigated in a RCT in MG; the results of the study are not yet available (NCT02565576) (48).

Indirect B Cell Targeting

Bortezomib is a dipeptide that, by binding the catalytic site of the 26S proteasome acts as a proteasome-inhibitor; it is registered for refractory or heavily treated multiple myeloma, and due to its pharmacological activity targets short and long lived plasmacells and, hence, could be potentially useful in MG. Bortezomib was effective in the treatment of EAMG (the experimental model of MG) and prevented the production of anti AChR antibodies by cultured thymic tissue (49, 50). A clinical study (NCT02102594) has been performed on antibody mediated autoimmune diseases, including MG, but no results have been posted yet. However, Bortezomib is associated with severe adverse events, e.g., 30% of treated patients showed a painful peripheral neuropathy, thus limiting their use.

Another interesting drug is Belimumab, a human monoclonal antibody that neutralizes BAFF, a B cell activating cytokine. Belimumab has been registered for treatment of systemic lupus erythematosus, an autoimmune disease with significant similarities with MG. Furthermore, elevated levels of BAFF

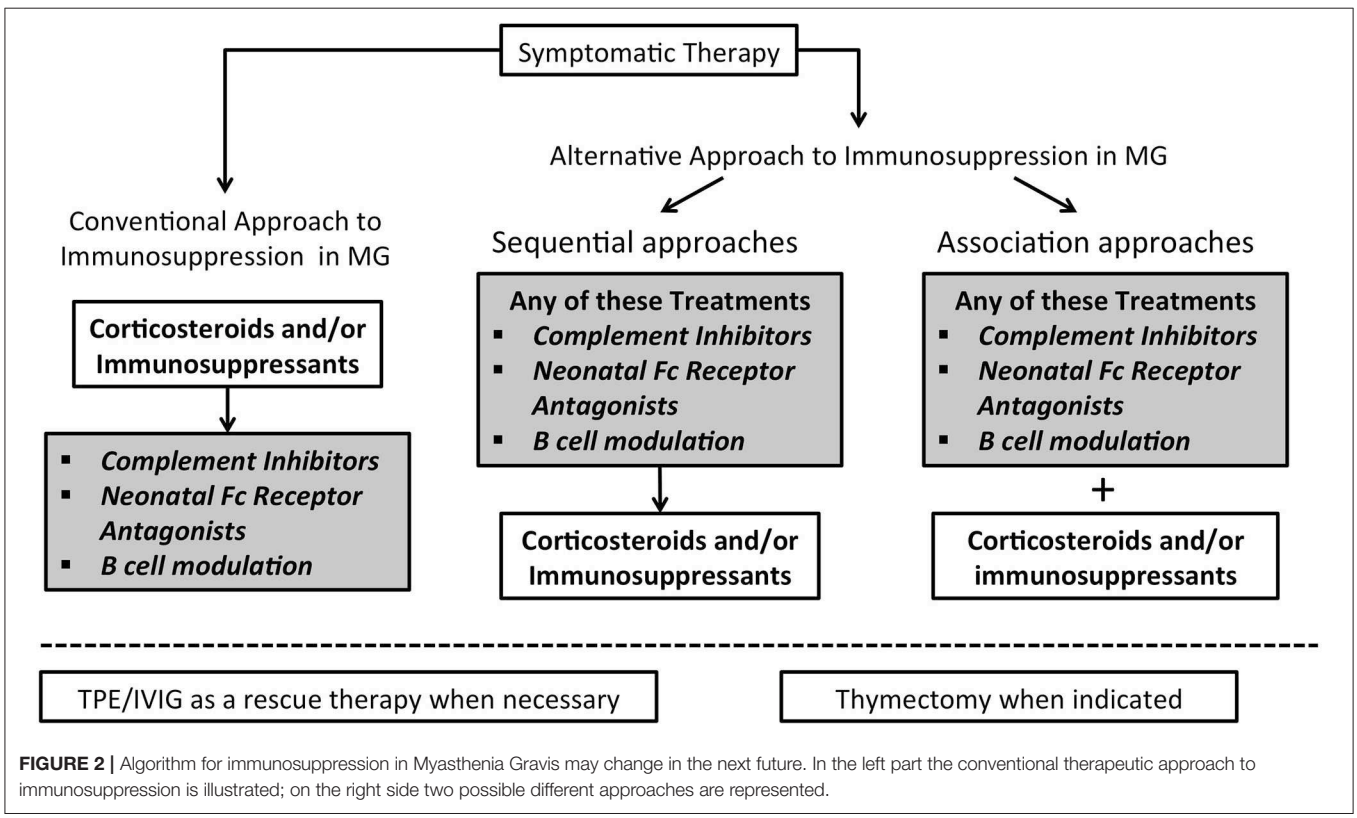

FIGURE 2 | Algorithm for immunosuppression in Myasthenia Gravis may change in the next future. In the left part the conventional therapeutic approach to immunosuppression is illustrated; on the right side two possible different approaches are represented.

were observed in MG patients (51). In the past years a Phase 2 RCT was conducted to evaluate clinical efficacy and safety of Belimumab: the primary endpoint was not met, but the study suffered several methodological flaws that prevented the assessment of a still potentially useful compound (NCT01480596) (27).

B Cell-Targeting via Cytokine Blockade

Interleukin 6 (IL-6) is a cytokine produced by several cell types including B cells, and is thought to be in involved in autoantibody production, making it a potential candidate for investigation in MG. Tocilizumab is an anti-IL-6-receptor humanized monoclonal antibody that binds to cell-surface and soluble IL-6 receptor and prevents the proinflammatory activity of IL-6. Indeed, Tocilizumab has been approved for treatment of Rheumatoid Arthritis. The drug has been investigated in Neuromyelitis Optica with promising results in preventing relapses (52). Anti-IL6 treatment reduced specific antibodies and improved signs of the disease in experimental MG (53). No studies have been performed yet, but preliminary evidence of its efficacy in two patients with refractory MG has been reported (54).

UNANSWERED MEDICAL QUESTIONS?

Will these drugs modify our current treatment strategies? Treatment of MG is a step-by-step approach in which decisions are based on the degree of clinical disability, taking into account comorbidities and the need to limit side effects. Such innovative therapeutics may significantly change our current approach to the treatment of MG and offer the opportunity to avoid, reduce or at least delay the use corticosteroids (45) (**Figure 2**). Most MG patients start with symptomatic treatment, but in a considerable proportion corticosteroids and/or immunosuppressants become necessary; IVIG and PLEX are used as rescue therapy in case of clinical deterioration. Indeed, whatever immunosuppressive drugs are employed, they are used on a chronic schedule that enhances the rate of adverse events, this being particularly true for corticosteroids. With the emergence of new therapeutic possibilities and rising reluctance of patients to accept the iatrogenic burden of traditional treatments, it remains to be seen whether patient compliance will improve.

The likelihood that doctors will prescribe innovative drugs will depend on: a. the ascertainment of their effectiveness as a first-line therapy and its ability to modify the course of the disease; b. the sustainability of the drug in clinical practice, particularly in the universalistic health systems; and c. the need to know the cost/effectiveness ratio for the disease treatment.

What data is still needed? At present, innovative drugs have been employed as add-on therapies and for most of them evidence of clinical benefit has been obtained. To date, a considerable time of follow-up (more than 3 years) is available only for Eculizumab. The length of follow-up and knowledge of long-term efficacy will be essential also for the other investigational products. We will need to know the ability of these drugs to work as immunosuppressants and how rapidly they can exert this effect; in this regard, we need to perform controlled clinical studies on MG patients naïve to immunosuppression. Such an approach is feasible with complement inhibitors and nFcR antagonist since they are fast in inducing clinical improvement (between 7 and 15 days), and the availability of rescue therapies should overcome ethical problems. Indeed, the time to obtain significant clinical benefit with both steroids and conventional immunosuppressants can be longer than that reported so far for complement inhibitors or nFcR antagonists.

FUTURE DIRECTIONS

The introduction of compounds that target selectively the immune system will also offer a new opportunity to investigate immunological markers of disease activity and response to treatment. The topic of biomarkers in MG and other autoimmune disorders is not new but data available from series of patients treated with conventional therapies are, not surprisingly, still far from being conclusive and suitable for clinical application since too many immunological variables related to the disease and ongoing therapy are at stake simultaneously. The investigation of targeted therapies, due to their specificity, is likely to be more informative in the future, resetting the basis for personalized medicine in MG (45, 55).

In the past 3 years the horizon for improvement in immunosuppression has included different focused approaches which will hopefully address the unmet clinical needs of MG patients, as well as of patients affected with other autoantibody mediated diseases. If the expectations mentioned above will be met, a new era for the treatment of autoimmune diseases will be at hand and, prospectively, we could end up with a substantial modification on how to immunosuppress our MG patients, possibly with better results and improved quality of life.

AUTHOR CONTRIBUTIONS

RM and CA have equally conceived, drafted, and revised the manuscript. All authors contributed to the article and approved the submitted version.

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Myasthenia Gravis Coexisting With Primary Sjögren's Syndrome: Report of Three Cases and Literature Review

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Objective: The coexistence of myasthenia gravis (MG) and primary Sjögren's syndrome (pSS) is rarely reported. This study aims to describe the clinical features, treatment and outcome of MG coexisting with pSS.

Materials and Methods: Herein we reported three cases with the two coexisting diseases, and also searched the PubMed, Medline databases, and China Wanfang databases for the relevant case reports written in English, Chinese, or Japanese with detailed data.

Results: We reviewed a total of 17 patients with both diseases. Fifteen patients were female. The median age at onset was 48 years (range 28–78 years). MG was the initial disease in nine of 17 cases. The median interval between the onsets of the two diseases was 30 months (range 7 months to 20 years). The symptoms of MG included fatigable ptosis (64.7%), bulbar symptoms (58.8%), muscle fatigability (64.7%), diplopia (64.7%), dyspnea (23.5%), and facial paralysis (5.9%). Anti-acetylcholine receptor antibody was positive in 70.6% patients. All the patients had sicca symptoms. Manifestations of pSS also included swollen exocrine glands (23.5%), joint pain (23.5%), hair loss (11.8%), leukopenia (11.8%), recurrent oral ulcers (5.9%), Raynaud phenomenon (5.9%), and fever (5.9%). ANA positivity was present in 70.6% patients, anti-SSA positivity in 47.1%, and double positivity of anti-SSA and anti-SSB in 17.6%. There were 12 patients (70.6%) with two autoimmune diseases (pSS and MG), and five patients with more than two autoimmune diseases. Cholinesterase inhibitors were the most commonly prescribed drugs (82.4%). Seven patients received thymectomy and one patient improved after the operation. Two patients were given intravenous methylprednisolone pulse therapy, and four patients oral steroids combined with immunosuppressants initially. Intravenous immunoglobulin and plasma exchange were used in two patients, respectively, for the respiratory failure. All the patients improved following treatment except one patient who died of MG crisis due to medication withdrawal.

Conclusion: The coexistence of SS with MG is quite rare. The onset of MG may occur before or after the diagnosis of SS. Co-morbidity with MG does not seem to adversely affect the course of SS. Thus, controlling the progress of MG is the critical aspect of treatment.

Keywords: myasthenia gravis, primary Sjögren's syndrome, autoimmune diseases, coexistence, outcome

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INTRODUCTION

Myasthenia gravis (MG) is a chronic autoimmune neuromuscular disease in which antibodies bind to acetylcholine receptors or to functionally related molecules in the post-synaptic membrane and cause weakness in the skeletal muscles resulting in difficulty in respiration and swallowing, diplopia, and ptosis (1). The weakness typically worsens with exercise and sustained muscle use and fluctuates over the course of a day. A few papers have noted that MG can be accompanied by concomitant autoimmune diseases (ADs), including thyroiditis, chronic inflammatory demyelinating polyradiculoneuropathy, neuromyelitis optica spectrum disease (NMOSD), and connective tissue diseases (CTD) (2). Among the CTDs, systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) are the most frequently mentioned (1). However, the association of MG with primary Sjögren's syndrome (SS) is unusual.

SS is a common multisystem autoimmune disease characterized by lymphocytic infiltration of exocrine glands. Patients often present with dry mouth and dry eyes due to hypofunction of salivary and lacrimal glands (3). It shows a female predominance of 9:1 and a peak incidence at the age of ∼50 years (4). SS may occur in isolation or coexist with organ-specific autoimmune diseases (called as primary SS, pSS), such as thyroiditis and NMOSD (5). It also can be secondary to other systemic ADs, such as RA, SLE, or systemic sclerosis (SSc) (4). The nervous system is one of the targets of systemic damage in pSS patients (6). Neurologic manifestations of pSS are diverse, and may involve the peripheral nervous system and/or central nervous system (7). The coexistence of MG and pSS is limited to reports of one to two cases (8–21). Herein we report three cases of MG with pSS diagnosed at our hospital and review the relevant literature.

MATERIALS AND METHODS

Case Presentation

Case 1

A 32-years-old Chinese woman presented with a 3-years history of bilateral fatigable ptosis and dysarthria and 1-year diplopia. She was admitted to the Department of Neurology of our hospital on June 12th, 2019, because of aggravation of her symptoms. She did not have difficulty in swallowing, shortness of breath, or muscle fatigability. The personal and family history was unremarkable. She had a positive response to the neostigmine test and the serum level of anti-acetylcholine receptor (AchR) antibody was >20 nmol/L (normal range 0–0.4 nmol/L). Computed tomography (CT) scan of the chest revealed a thymic remnant in the anterior mediastinum. She was diagnosed with MG according to definitions of MG (22). Meanwhile, the serum immunological examination was done routinely in order to screen for possible coexisting ADs. Surprisingly, the result of anti-nuclear antibody (ANA) spectrum showed that ANA was positive, with a titer of 1:3,200 and speckled pattern, and anti-SSA positive (++). Then the patient was transferred to our department to identify the rheumatic diseases. With regard to her illness history, the patient had suffered from dry mouth and dry eyes for half a year. Her dry mouth did not affect solid food intake. She denied having a rash, photosensitivity, oral ulcers, joint pain or Raynaud phenomenon. Blood routine tests showed a moderate leukopenia (white blood cells 2.59×10^9/L). Urinary analysis was normal. Liver, renal functions and levels of creatine kinase (CK) were within normal range. Thyroid function normal. Erythrocyte sedimentation rate (ESR) and C reactive protein (CRP) normal. Immunoglobulin (Ig) G slightly high (17 g/L) and complements slightly low (C3 0.64 g/L, C4 0.14 g/L). Other autoantibodies including rheumatoid factor (RF), anti-cyclic citrullinated peptide (CCP) antibody, anti-cardiolipin antibody, anti-neutrophil cytoplasmic antibodies, the myositis-associated autoantibodies (MAA), and myositis-specific autoantibodies (MSA) including 16 different antigens (Mi-2α, Mi-2β, TIF1γ, MDA5, NXP2, SAE1, Ku, PMScl100, PM-Scl75, Jo-1, SRP, PL-7, PL-12, EJ, OJ, Ro-52) were all negative. The anti-thyroid peroxidase (TPO) antibody and anti-thyroglobulin (TG) antibody were also negative. Oral unstimulated salivary flow rate was 0.8 ml/min. Tests for dry eyes done by an ophthalmologist showed tear film break-up time (BUT) of left/right eye was 3.44/10.77 s, respectively; Schirmer test: left 2 mm/5 min, right 3 mm/5 min; Corneal fluorescence staining (–). Biopsy of the labial gland revealed focal lymphocytic sialadenitis with a focus score ≥1. Eventually, the patient was diagnosed with MG and pSS. She was given pyridostigmine bromide 60 mg three times per day, prednisone 30 mg per day, tacrolimus 1 mg twice per day. In a follow-up of 8 months, her symptoms improved obviously.

Case 2

A 55-years-old Chinese woman was admitted to our department due to dry mouth and dry eyes on October 11th, 2019. She suffered from dry mouth for 1 year but without decay of teeth or swelling of parotid glands. Two months before her admission, she began to have dry eyes and asymmetrical fatiguable ptosis. She also developed diplopia, dizziness and proximal muscle fatigability of lower limbs. She was identified as an asymptomatic hepatitis B virus carrier for 8 years. On admission, physical examination showed bilateral ptosis, more serious on the right side. Bilateral limitation of eye movement. No oral saliva pool. Muscle strength of proximal muscles of lower limbs was grade IV.

She was given a comprehensive laboratory examination involving blood cells, acute phase response markers, serum biochemistry, CK, immunoglobulins, autoantibodies, and hepatitis B virus markers. The clinically significant results showed as follows: hepatitis B surface antigen (HBsAg), hepatitis B e antibody (anti-HBe), and hepatitis B core antibody (anti-HBc) positive. The levels of CK was normal. RF was 76 IU/ml. The level of anti-TPO antibody was 78.6 IU/ml (0–9), anti-TG antibody 10.6 IU/ml (0–4), but thyroid function normal. The MAA and MSA were all negative. ANA was positive with a titer of 1:320 (homogeneous pattern). Ig G/A/M, complement C3/C4, ESR, and CRP were all normal.

She had a positive response to the neostigmine test and the serum level of anti-AchR antibody was 20 nmol/L. Thyroid ultrasound revealed a diffusely uneven echo pattern and a 0.8 × 0.4 cm hypoechoic nodule in the left lobe. Contrast-enhanced

chest CT detected an anterior mediastinal mass indicating a thymoma (**Figure 1**). Oral unstimulated salivary flow rate was 0 ml/min. The presence of dry eyes was confirmed based on the ophthalmic examination. Biopsy of the labial gland revealed focal lymphocytic sialadenitis with a focus score ≥1. As a result, she was diagnosed with pSS, MG and Hashimoto's thyroiditis (HT). Thymectomy was performed and the post-operative pathology indicated Type B1 thymoma according to World Health Organization (WHO) classification (**Figure 1**). She was given pyridostigmine bromide 60 mg three times per day. In a follow-up of 6 months, her sicca and myasthenia symptoms were relieved.

Case 3

A 47-years-old Chinese woman was admitted to our department on November 7th, 2019. She suffered from dry mouth and dry eyes for 2 years. She also had hair loss, pain in knee and proximal interphalangeal joints without swelling. She developed fatigue and proximal muscle fatigability of lower limbs 1 year ago and diplopia 5 months ago. One month before admission, she began to have dysarthria, dysphagia, fatigability in chewing and facial paralysis. She had a 3-years history of Hashimoto's thyroiditis and hypothyroidism, and euthyrox was administered. Physical examination showed muscle strength of proximal muscles of lower limbs was grade IV. Laboratory tests showed as follows: white blood cells (WBC) 3.49 × 10^9/L, IgG 25.6 g/L, ESR 30 mm/h, and CRP normal. RF was 21 IU/ml and anti-CCP antibody 104.07 U/ml. Thyroid function was normal, but the level of anti-TPO antibody was 10.4 IU/ml (0–9), anti-TG antibody negative. Serum level of anti-AchR antibody was 43.89 nmol/L. The levels of CK was normal. The MAA and MSA were all negative. ANA was 1:320 (+) (speckled pattern), anti-SSA (+++). Oral unstimulated salivary flow rate was 2.4 ml/min and dry eyes were confirmed by an ophthalmologist. Biopsy of the labial gland revealed focal lymphocytic sialadenitis with a focus score ≥1. Chest CT detected no thymus abnormalities.

The patient was diagnosed with pSS, MG, and HT. She was given pyridostigmine bromide 60 mg three times per day, prednisone 30 mg per day, and tacrolimus 3 mg per day. Her muscle fatigability, dysphonia and dysphagia soon alleviated. But 2 months later, she developed dyspnea when prednisone was tapered to 25 mg per day. Then intravenous immunoglobulin (IVIG) 20 g per day was administered for 5 days. Her dyspnea was relieved soon. Followed up for 3 months, her condition was stable.

METHODS

All analyses were based on a review of medical records that had been obtained for clinical purposes or for previous published studies, thus ethical approval was waived. To describe the clinical features, treatment regimens and outcome about patients with pSS and MG, we searched the PubMed, Medline databases and China Wanfang databases for reports of cases by using the keywords "Sjögren's syndrome," "sicca syndrome," "autoimmune diseases," and "myasthenia gravis" in different combinations. Twenty-one cases with the two coexisting diseases were identified in total, and we reviewed the 14 cases written in English, Chinese,

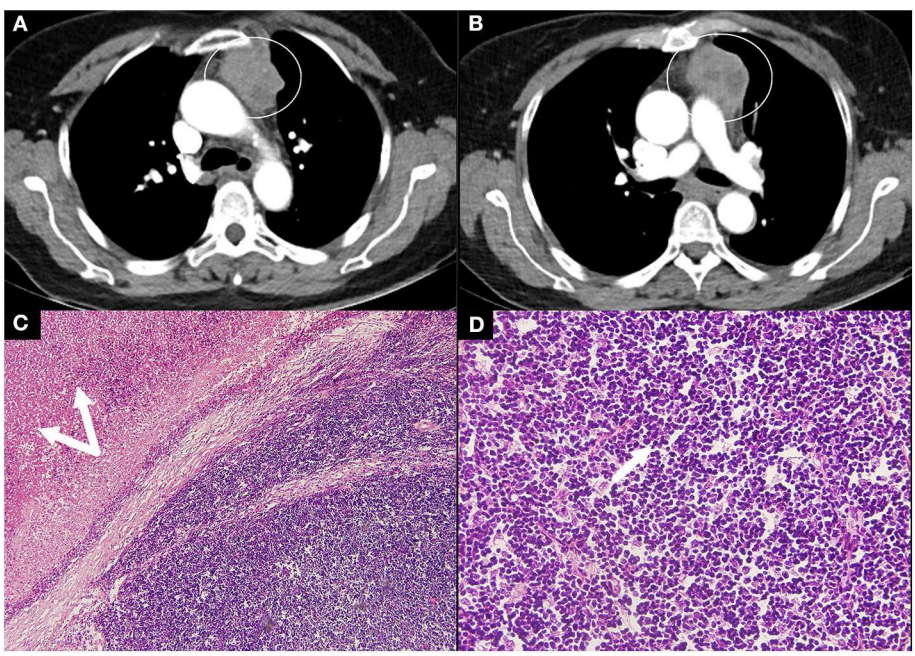

FIGURE 1 | Contrast CT scan and thymoma pathology in case 2. **(A,B)** Contrast CT scan of the chest showed a large rounded mass (circle) with multiple low-density areas reflecting necrosis. **(C,D)** Pathology revealed type B1 thymoma according to the WHO classification with massive necrosis (double arrows, magnification, H&E ×10) and a predominance of lymphocytes (arrow, magnification, H&E ×40). CT, computed tomography; WHO, World Health Organization; H&E, hematoxylin and eosin.

or Japanese with detailed data. The clinical features of MG patients with pSS were analyzed with descriptive statistics.

RESULTS

Through a comprehensive search of literature, we finally identified 14 other cases with MG and pSS (8–21). The details of the 17 patients (including three cases in the present study) are listed in **Table 1**.

The demographic features, precedence of disease development, common manifestations, autoimmune complications, treatment regimens, and outcome of all 17 patients are reviewed in **Table 2**. Notably, anti-AcR antibody were positive in 12 out of 17 cases (unclear in the other five patients). Most patients (16 out of 17 cases) were classified as generalized MG presenting with limb muscle fatigability or bulbar symptoms while only one patient was ocular MG. Of seven patients who underwent thymectomy, five had pathologic results: three had thymoma (two with type B1, one with type A according to the WHO classification), and two thymic hyperplasia. Additionally, there were six patients who had lip biopsy and all the results revealed focal lymphocytic infiltration. Interestingly, there were five patients having more than two ADs besides MG and pSS. With regard to treatment, thymectomy relieved the symptoms obviously in the patient with thymoma. Cholinesterase inhibitors were the most commonly used drugs, and three patients were treated efficiently with the drug alone. Other therapeutic methods, including intravenous methylprednisolone pulse, oral steroids, immunosuppressants, plasma exchange (PLEX), and IVIG were also used. Overall, all the patients improved following the treatment except one patient who died of MG crisis due to medication withdrawal by herself.

DISCUSSION

MG is a B-cell mediated organ-specific autoimmune disease with antibodies against the acetylcholine receptor, muscle-specific kinase (MUSK), lipoprotein-related protein 4 (LRP4), or agrin in the post-synaptic membrane at the neuromuscular junction (23). About 10% MG patients may have a thymoma, and conversely, one third of patients with thymoma can develop MG (1). Similarly, pSS is also a B-cell mediated systemic autoimmune disease with multiple antibodies including ANA, anti-SSA (Ro) antibody and anti-SSB (La) antibody. Thereafter, there might be a similar immunologic mechanism involving different targets shared by these two diseases. The overall prevalence of neurologic involvement in pSS is ∼20% (24). The neurologic manifestations include peripheral neuropathy, aseptic meningitis, NMOSD, and multiple sclerosis-like manifestations. But the coexistence of pSS and MG is really rare and limited to case reports.

Herein we present three cases of MG coexisting with pSS and offer a review based on the published literature. We notice that MG has rarely been reported to coexist with pSS. But its incidence may have been underestimated, because the sicca symptoms are easily overlooked by a neurologist. The coexistence of MG and pSS show a female predominance and a median age of 48 years at onset, which are consistent with the features of pSS and the age of early onset MG (EOMG). MG occurred before pSS in more than half of the patients. Thus, it is of great importance for a neurologist to screen patients with MG for the presence of other autoimmune rheumatic disorders including pSS.

The frequency of coexistence with other ADs in MG patients has been reported between 11.6 and 32% (2). Data from a large population-based survey showed that 214 ADs were diagnosed in 185 of 984 MG patients (18.8%). And 26 of these subjects had two or more ADs (25). Autoimmune thyroid diseases (AITD) is the most common coexisting condition, followed by SLE and RA. Other autoimmune disorders including chronic inflammatory demyelinating polyradiculoneuropathy and NMOSD were also reported. Furthermore, inflammatory myopathy is another rare AD which can coexist with MG. Garibaldi et al. observed that 13 out of 441 (2.9%) MG patients developed myositis and 10/13 patients occurred simultaneously (26). So the authors recommended myositis should be considered when MG patients had the features of elevated serum CK levels or stable muscle weakness unresponsive to acetylcholinesterase inhibitors (26), in particular for MG patients with thymoma (27). As for the three patients we reported, they all had normal serum CK levels and responded well to the acetylcholinesterase inhibitors. As a result, myositis was ruled out.

Actually, the term "polyautoimmunity" has been used for decades and defined as the presence of two or more ADs in an individual. In this paper, there are 12 patients (70.6%) having two ADs (pSS and MG), and five patients having more than two ADs. Moreover, one patient suffered from four ADs simultaneously. On the other hand, pSS often occurs with organ-specific ADs and the most common coexisting AD is also AITD (28). The prevalence of AITD was 11.1–15.7% in patients with pSS (29, 30). Moreover, a study by Lazarus reported that 7.9% of patients with pSS had two or more ADs (31).

Since the coexistence of MG and pSS is quite rare, the pathogenesis remains elusive. Berrih-Aknin summarized the common mechanisms between MG and SS (32). The frequency of human leukocyte antigen (HLA)-DR3 in the whole group of MG patients was increased compared with the control population, and the same HLA haplotype was one of the loci for susceptibility for SS. The EOMG patients were predominantly women (female to male 9:1) with thymic hyperplasia, including the development of ectopic germinal centers in the thymus and high levels of anti-AChR antibody. Sex hormones which may affect both innate and adaptive immune systems are mainly thought to be responsible for this bias. And the formation of ectopic germinal centers in salivary glands was also commonly found in SS patients (32). Serum levels of both B cell activating factor (BAFF) and a proliferation-inducing ligand (APRIL) increased in SS and MG patients (32). In addition, increased expression of interleukin-17 was found in the sera and the thymus of anti-AcR positive MG patients as well as in the saliva of SS (32). These findings highlight the key role of the target organ in the initiation and development of the disease.

It is noticed that although all 17 pSS patients with MG presented with sicca symptoms, no one developed severe systemic damage as far as pSS is concerned (four patients with

TABLE 1 | Review of present and previous reported cases with MG and pSS.

Country	Sex/age	MG features	pSS features	Laboratory tests	Treatment	Outcome
1966, United Kingdom (8)	F/51	Diplopia, dysphagia, muscle fatigability and dyspnea for 23 years; anti-AcR NA	Joint pain for 3 years, dry mouth for 3 months; Raynaud's phenomenon; keratoconjunctivitis sicca(+);	ESR elevated; ANA(+)	Cholinesterase inhibitor; thymectomy; analgesics	Improved
1973, Japan (9)	M/53	Dysphagia, muscle fatigability, and ptosis for 13 years; neostigmine test(+); anti-AcR NA; RNS(+); CT: thymic hypertrophy	Dry mouth for 1 year; Schirmer(+); salivary flow rate decreased; Sialography (+); Lip biopsy(+)	γ-globulin elevated; ESR normal, CRP elevated; RF(+); ANA(–); anti-SMA(+)	Cholinesterase inhibitor	Improved
1973, Scotland (10)	F/66	Ptosis, diplopia and generalized muscle weakness for 3 years; neostigmine test(+); anti-AcR NA	Left parotid gland enlargement, dry eyes for 2 years; Sialography (+); keratoconjunctivitis(+);	RF(+); ANA(+)	Cholinesterase inhibitor	Improved
1990, Sweden (11)	F/40	Muscle fatigability, dysarthria for 9 months; anti-AcR(+); tensilon test(+)	Transient dry mouth and a swelling below the mandible appeared after pyridostigmine bromide treatment; Schirmer normal; FL(+); salivary flow rate 1.8 ml/min; Lip biopsy(+)	WBC decreased; ESR elevated; RF(+); ANA(–)	Pyridostigmine bromide; thymectomy (thymic hyperplasia)	Improved; recurrent swollen exocrine glands
1999, Japan (12)	F/28	Exhaustion, ptosis and diplopia for 2.5 years; severe muscle fatigability and dyspnea for 1 year; anti-AcR (+); tensilon test(+); No thymic tumors	Sicca symptoms for 3 years; Swelling of bilateral parotid glands; Schirmer(+); salivary flow rate 0.5 ml/min; Sialography (+)	ANA, anti-SSA, anti-SSB(+)	Steroid pulse therapy; Pred 2 mg/kg daily; pyridostigmine bromide; 5 plasma exchanges	Improved
2003, China (13)	F/48	Muscle fatigability for 8 years; ptosis, mild diplopia and fatigue for 3 years; anti-AcR elevated; RNS(+)	Dry mouth, dry eyes, dental caries for 3 years; Schirmer (+); FL (+); salivary flow rate 0.5 ml/min	ESR 23 mm/h; CRP 1.61 mg/dl; anti-SSA, anti-SSB (–)	Cholinesterase inhibitor	Improved
2004, Japan (14)	F/36	Nausea, cough and high fever; chest CT: thymoma; 2 months post-thymectomy, bilateral ptosis, dysphagia and generalized fatigability developed; anti-AcR(+); tensilon test(+); RNS(+)	Dry mouth and dry eyes developed 1 month before surgery; Schirmer (+); salivary flow rate 0.15 g/2 min; lip biopsy (+); 11 months after surgery, PRCA occurred	γ-globulin elevated; ANA (+); anti-SSA, anti-SSB (-);	Thymectomy(type-B1 thymoma); local radiotherapy; prednisolone; PRCA: cyclosporin	Improved
2006, China (15)	F/28	Dysphagia for 1 year; fatigable ptosis for 3 weeks; neostigmine test(+); anti-AcR NA; fatigue test(+); RNS(+)	Dry mouth for 2 years; Schirmer (+)	RF (+); ANA: NA; anti-SSA (+)	Pred 1 mg/Kg/d	Improved
2006, China (16)	F/71	Muscle fatigability and mild diplopia for 4 years; neostigmine test(+); anti-AcR NA; fatigue test(+); RNS(+); pyridostigmine bromide 60 mg tid, improved; 3 days of dysphagia, worsening muscle fatigability, dyspnea, MG crisis	Dry mouth, dry eyes and joint pain for 5 years; FL (–); parotid ECT (+)	ESR elevated; γ-globulin elevated; ANA, anti-SSA, anti-SSB (–)	Cholinesterase inhibitor; tracheal intubation and ventilator assisted breathing	Die
2008, China (17)	F/41	Muscle fatigability and mild diplopia for 10 months; dysphagia for 3 months; anti-AcR(+); fatigue test(+); RNS(–)	Dry eyes and hair loss for 3 months; Schirmer (+); FL (+); parotid ECT (+)	IgG/A/M elevated; RF (+); ANA, anti-SSA (+)	Thymectomy(thymic hyperplasia); steroid + pyridostigmine bromide	Improved
2009, China (18)	F/78	Fatigable ptosis, diplopia and dysphagia for 7 years; anti-AcR(+); fatigue test(+); RNS(+); treatment: pred 60 mg qd → 15 mg qd and pyridostigmine bromide; improved	Dry mouth, dry eyes, joint pain; swelling of parotid glands and low grade fever for 1 year; Schirmer (+); salivary flow rate 0.05 ml/min	RF (+); ANA, anti-SSA, anti-SSB (+)	Pred 15 mg qd; pyridostigmine bromide	Improved
2013, China (19)	M/63	Productive cough, fatigability and ptosis for 3 months; anti-AcR(+); RNS(+); chest CT: thymoma	Dry eyes for 1 year; Schirmer (+); parotid ECT (+)	ANA (+)	Thymectomy(type-A thymoma)	Improved
2013, China (20)	F/46	Ptosis and mild diplopia for 2 months; neostigmine test(+); anti-AcR(+); fatigue test(+); RNS(+)	Dry mouth, dry eyes, oral ulcers and muscle pain of lower limbs for 2 years; Schirmer (+); FL (+); parotid ECT (+)	ESR 38 mm/h; RF (+); ANA, anti-SSA, anti-SSB (+)	Pred 50 mg qd; pyridostigmine bromide	Improved

(Continued)

TABLE 1 | Continued

Country	Sex/age	MG features	pSS features	Laboratory tests	Treatment	Outcome
2018, China (21)	F/52	Fatigable muscle fatigability for 11 years, 10 years ago, dyspnea developed; anti-AcR(+); fatigue test(+); pred and pyridostigmine bromide relieve her symptoms. improvement of muscle fatigability post-thymectomy	Dry mouth and dry eyes for 9 years; parotid ECT (+)	RF (+); ANA, anti-SSA (+); Anti-TPO, anti-TG (+); Anti-AQP4-IgG (+)	MP pulse → pred; MMF	Improved
Case 1	F/32	Ptosis and dysarthria for 3 years, diplopia for 1 year; neostigmine test(+); anti-AcR(+); RNS(+)	6 months of dry mouth and dry eyes; Schirmer:(+); FL(-); salivary flow rate 0.8 ml/min; lip biopsy (+)	WBC decreased; IgG elevated; ANA, anti-SSA(+)	Pyridostigmine bromide; pred; tacrolimus	Improved
Case 2	F/55	Ptosis, diplopia and muscle fatigability for 1 month; neostigmine test(+); anti-AcR(+); RNS(+); chest CT: anterior mediastinal mass	Dry mouth for 1 year; dry eyes for 2 months; Schirmer:(+); FL(+); salivary flow rate 0 ml/5 min; lip biopsy(+)	RF(+); ANA(+); Anti-SSA, anti-SSB(-)	Thymectomy (type-B1 thymoma); pyridostigmine bromide	Improved
Case 3	F/47	Muscle fatigability for 1 year, diplopia for 5 months; dysarthria, dysphagia, fatiguable chewing and facial paralysis for 1 month; anti-AcR(+); fatigue test(+); RNS(+)	Dry mouth, dry eyes, hair loss and joint pain for 2 years; Schirmer(+); salivary flow rate 2.4 ml/min; lip biopsy(+)	WBC decreased; IgG elevated; ESR 30 mm/h; RF, anti-CCP(+); ANA, anti-SSA(+)	Pyridostigmine bromide; pred; tacrolimus; MG crisis: IVIG	Improved

MG, myasthenia gravis; pSS, primary Sjögren's syndrome; F, female; M, male; anti-AcR, anti-acetylcholine receptor; FL, corneal fluorescence stain; RF, rheumatoid factor; ANA, anti-nuclear antibody; pred, prednisone; RNS, repetitive nerve stimulation test; ESR, erythrocyte sedimentation rate; CRP, C reactive protein; PRCA, pure red cell aplasia; NA, not available; ECT, emission computed tomography; Ig, immunoglobulin; anti-TPO, anti-thyroid peroxidase; anti-TG, anti-thyroglobulin; MP, methylprednisolone; MMF, mycophenolate mofetil; anti-AQP4, anti- aquaporin 4; WBC, white blood cells; anti-CCP, anti-cyclic citrullinated peptide; IVIG, intravenous immunoglobulin.

TABLE 2 | Features of patients with MG and pSS.

Items	Features
Median age at onset (years, y)	48 (28∼78) y
Gender ratio, female:male	15:2
Initial onset of MG	9/17 (52.9%)
Median duration	
MG to pSS (months, m)	30 m (7 m to 20 y)
pSS to MG (months, m)	11.5 m (3 m to 22 m)
MG manifestations	
Fatigable ptosis	11/17 (64.7%)
Bulbar symptoms	10/17 (58.8%)
Muscle fatigability	11/17 (64.7%)
Diplopia	11/17 (64.7%)
Dyspnea	4/17 (23.5%)
Facial paralysis	1/17 (5.9%)
Anti-AcR antibody (+)	12/17 (70.6%)
pSS manifestations	
Sicca symptoms	17/17 (100%)
Swollen exocrine glands	4/17 (23.5%)
Joint pain	4/17 (23.5%)
Hair loss	2/17 (11.8%)
Recurrent oral ulcers	1/17 (5.9%)
Raynaud phenomenon	1/17 (5.9%)
Fever	1/17 (5.9%)
Decreased WBC	2/17 (11.8%)
ANA (+)	12/17 (70.6%)
Anti-SSA (+)	8/17 (47.1%)
Anti-SSA and anti-SSB (+)	3/17 (17.6%)
Rheumatoid factor (+)	9/17 (52.9%)
Anti-CCP antibody (+)	1/17 (5.9%)
Lip biopsy (+)	6/6 (100%)
Polyautoimmuty	
pSS, MG, and HT	3/17 (17.6%)
pSS, MG, and thymoma	3/17 (17.6%)
pSS, MG, and NMOSD	1/17 (5.9%)
pSS, MG, and PRCA	1/17 (5.9%)
Treatment	
Cholinesterase inhibitor	14/17 (82.4%)
Steroids	9/17 (52.9%)
Immunosuppressants	4/17 (23.5%)
IVIG	1/17 (5.9%)
Plasma exchange	1/17 (5.9%)
Thymectomy	7/17 (41.2%)
Outcome	
Improved	16/17 (94.1%)
Died	1/17 (5.9%)

MG, myasthenia gravis; pSS, primary Sjögren's syndrome; anti-AcR, anti-acetylcholine receptor; WBC, white blood cells; ANA, anti-nuclear antibody; anti-CCP, anti-cyclic citrullinated peptide; HT, Hashimoto's thyroiditis; NMOSD, neuromyelitis optica spectrum disorder; PRCA, pure red cell aplasia; IVIG, intravenous immunoglobulin.

swollen exocrine glands, four with joint pain, two with slightly decreased WBC). Therefore, no steroids or immunosuppressants were given for treating pSS. Topically symptomatic treatments

were used as the first-line therapy for oral and ocular dryness based on the European League Against Rheumatism (EULAR) recommendations for the management of pSS (33). The treatment regimens in these coexisting conditions were mainly aimed at MG. Cholinesterase inhibitors were the most common drugs for alleviating the symptoms of MG with affirmative effect (34). For patients with MG who did not respond to an adequate trial of pyridostigmine, corticosteroids could be recommended. Attention should be paid to the precaution that the dose of corticosteroids should be increased gradually to avoid an initial deterioration (35). Immunosuppressive agents were recommended to be used alone or used as corticosteroid-sparing agents, when corticosteroids were contraindicated or refused (35). Among the 17 patients, there were two patients given intravenous methylprednisolone pulse therapy with subsequent oral steroids. And four patients took oral steroids combined with immunosuppressants initially. They all had a good response. IVIG and PLEX could be used in pSS patients with severe systemic involvements, such as severe thrombocytopenia or in MG patients with life-threatening signs, such as respiratory insufficiency or dysphagia (33, 34). The effect of IVIG and PLEX treatment was seen in two patients who developed MG crisis with respiratory difficulty (one treated with IVIG, and the other with PLEX). In addition, thymectomy is a special option, not only for the patients with a thymoma, but also for the non-thymomatous anti-AChR antibody positive MG (36). Thymectomy should be considered early in treatment decisions to improve clinical status as the thymus is thought to be a major trigger of autoantibody production (36). We observed that there were seven patients with MG and pSS who received thymectomy, and one patient improved after thymoma resection without any medication.

A single-center retrospective study showed rituximab was effective in patients with MG, supporting the role of B cell depletion in the management of MG (37). It has been reported that rituximab may be a useful treatment for pSS (33). Thus, it is reasonable to speculate that rituximab may be effective for the co-morbidity of MG and pSS. However, no such patient has been treated with rituximab, to date.

Another question is whether hydroxychloroquine (HCQ) can be used in patients with pSS and MG. HCQ is an essential drug for patients with CTDs including SLE and SS, but special caution should be taken when used in MG patients. Although data from the Spanish society of Rheumatology Lupus Registry showed that HCQ protected against polyautoimmunity for patients with SLE (38), Varan et al. reported a case of SLE in which MG developed with the use of HCQ, and regressed with its withdrawal (39). In a series of 17 patients with SLE and MG, Jallouli et al. found that eight patients (47%) developed MG after initiation of HCQ, but only one patient who received HCQ had an exacerbation of myasthenic symptoms (40). It is worth mentioning that MG has long been recognized as one of the 19 neuropsychiatric manifestations of SLE (41). Therefore, whether it is caused by HCQ or associated with SLE is worth further research. As for the effect of HCQ on the patients with both pSS and MG, there has been no such report to date.

CONCLUSION

The coexistence of MG with pSS is quite rare according to the reported cases. The onset of MG may occur before or after the diagnosis of pSS. It is of great importance to screen for ANA during the clinical course of MG, and to screen for MG when pSS patients complain of muscle fatigability or fatigable ptosis. Severe morbidity due to pSS is uncommon in patients with both diseases. Thus, controlling the progress of MG is the critical aspect of treatment. Therapeutic decisions should be made following a multidisciplinary approach. Multicenter prospective studies of larger sample sizes are needed to achieve a better understanding of this co-morbidity.

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 written informed consent was obtained from the participants for the publication of this paper.

AUTHOR CONTRIBUTIONS

XL and YZ collected the patients' data and designed the study. XL wrote the manuscript. YZ, QL, and YD critically revised the manuscript. All authors read and approved the submitted version.

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Determinants of Quality of Life in Myasthenia Gravis Patients

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Background: Although approximately half of myasthenia gravis (MG) patents achieve remission, for the remaining group MG is often a life-long disease. Better understanding of the determinants of Quality of Life (QoL) in MG is needed to optimize treatment goals in chronic cases.

Materials and Methods: We performed a single center cross-sectional study in 339 MG adult patients (64.9% women), with ocular or generalized disease. SF-36 and a structured questionnaire was administered, including information on previous and current MG severity, medications, comorbidities, education, occupation and BMI of the patient. Mean disease duration was 7.5 + 9.3 years. Current age was 51.6 + 18.3 years, 55% had Early-Onset (<50 years) MG.

Results: There were no statistically significant differences in mean SF-36 subscores between women and men. Worse MGFA class was related to lower QoL in physical (PCS) and mental (MCS) subscore ($p = 0.000$ for both). Patients with MGFA I-II class had significantly better QoL in physical and mental subscores than patients with more severe MG ($p < 0.005$). Late-onset MG patients had worse QoL than EOMG in physical score domain PCS ($p = 0.049$). Overweight and obese patients had lower PCS ($p = 0.002$) and MCS ($p = 0.038$) than patients with normal BMI. University education was related to statistically higher PCS ($p = 0.015$) and MCS ($p = 0.006$). QoL in currently employed was better in PCS and MCS ($p = 0.000$), with white collar workers reporting higher PCS ($p = 0.049$) than the remaining group. Patients living with family evaluated their MCS ($p = 0.015$) better than living alone. Moderate physical activity (twice a week) improved PCS ($p = 0.045$).

Conclusion: Our study confirmed that greater severity of symptoms, age, age of onset but also BMI, type of work, education status and physical activity affect QoL in MG.

Keywords: myasthenia gravis, quality of life, SF-36, obesity, employment, MGFA

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INTRODUCTION

Myasthenia gravis (MG) is a rare autoimmune disease of neuromuscular junction causing muscle weakness and fatigability. The incidence of MG is around 30/1,000,000/year (1). Eighty five percentage of patients have specific autoantibodies against acetylcholine receptor (AChRAb), minority have autoantibodies against muscle-specific kinase (MuSKAb) or low density lipoprotein receptor-related protein 4 (2–4). Myasthenic symptoms range from ocular to generalized muscle

weakness that can result in respiratory failure. Treatment of MG is often lifelong, the patients may require acetylcholinesterase inhibitors, immunosuppressants, plasma exchange, immunoglobulins and/or thymectomy, depending on the severity of symptoms and thymic pathology (5–8). MG affects many aspects of patient's life including mental and social level (9). Quality of life (QoL) of myasthenic patients was studied using different scales such as general or MG-specific MG-QoL (10–13) or just simple one question scale (14). The aim of our study was to assess factors influencing QoL in patients with MG.

MATERIALS AND METHODS

A single-center cross-sectional study was conducted in 339 MG adult patients, with ocular or generalized disease after informed consent. Study was approved by local ethical committee in 2007 (IRB/KB/186/2007). Studied group consisted of patients with diagnosed and treated in Department of Neurology in Warsaw Medical University in years 2010–2015. MG diagnosis was based on clinical presentation, and results of AChRAb or MuSKAb assay and/or results of repetitive nerve stimulation or single-fiber electromyography studies. Clinical status (using Myasthenia Gravis Foundation of America scale—MGFA), intervention status (using MGFA Post-intervention Status) (15), medical history and medication dosage was obtained by medical personnel (co-authors). SF-36 and a structured questionnaire was administered, including information on education, occupation and body mass index (BMI) of the patient. Early-onset myasthenia gravis (EOMG) was defined as first symptoms before the age of 50, and late-onset myasthenia gravis (LOMG) as 50 years old and above, respectively.

Summary of patients' demographics, clinical and social status is listed in **Table 1**.

QoL was evaluated with Short-Form 36-item questionnaire for health survey, Polish version (SF-36) (16). SF-36 measures eight general health dimensions: physical functioning (PF)—which shows interference with physical activities, physical role functioning (RP)—which shows degree to which physical health changed activities in last 4 weeks, bodily pain (BP)—which represents the amount of pain experienced during the last 4 weeks, general health (GH)—shows overall perceived health, vitality (VT)—shows experienced energy during last 4 weeks, social functioning (SF)—shows interference with social activities, emotional role functioning (RE)—shows degree to which emotional health changed in the last 4 weeks and mental health (MH)—shows general mood in the last 4 weeks. Scores are shown in numerical scale from 0 to 100, lower score results in worse QoL. Two composite scores are available to summarize these results: Physical Composite Score (PCS) and Mental Composite Score (MCS) (17).

TABLE 1 | Demographics, social and clinical status and treatment of MG patients.

Variable	Value	Variable	Value
Gender (number of patients)		Glucocorticoids in the past (number of patients)	
Male	119 (35.1%)	Yes	194 (57.2%)
Female	220 (64.9%)	Never	141 (41.6%)
Current age (years)		No data	4 (1.2%)
Mean ± SD	51.63 ± 18.31	BMI interpretation (number of patients)	
Disease duration (years)		Women	
Mean ± SD	7.48 ± 9.30	Underweight or normal	102 (46.4%)
Type of MG (number of patients)		Overweight or obesity	112 (50.9%)
EOMG	186 (54.9%)	No data	6 (2.7%)
LOMG	135 (39.8%)	Men	
T-MG	18 (5.3%)	Underweight or normal	20 (16.8%)
Serological status (number of patients)		Overweight or obesity	95 (79.8%)
AChRAb +	260 (76.7%)	No data	4 (3.4%)
AChRAb -	44 (13.0%)	Education (number of patients)	
MuSK +	9 (2.7%)	Primary	49 (14.5%)
No data	26 (7.7%)	Secondary	159 (46.9%)
Current MGFA (number of patients)		University	126 (37.2%)
Remission	56 (16.5%)	No data	5 (1.5%)
I	55 (16.2%)	Occupation (number of patients)	
IIA	79 (23.3%)	Blue collar work	137 (40.4%)
IIB	101 (29.8%)	White collar work	140 (31.3%)
IIIA	7 (2.1%)	No data	62 (18.3%)
IIIB	32 (9.4%)	Current employment status (number of patients)	
IVB	9 (2.7%)	During education	37 (10.9%)
Myasthenic crisis in the past (number of patients)		Currently employed	92 (27.1%)
Yes	43 (12.7%)	Retirement	94 (27.7%)
No	272 (80.2%)	Disablement pension or benefits	97 (28.6%)
No data	24 (7.1%)	No data	19 (5.6%)

SD, standard deviation; T-MG, thymoma-associated myasthenia gravis; AChRAb+, antibodies against acetylcholine receptor's positive status; AChRAb-, antibodies against acetylcholine receptor's negative status; MuSK+, autoantibodies to muscle-specific tyrosine kinase's positive status; BMI, body mass index.

Statistical Analysis

All continuous data are expressed as means and standard deviations (SDs). To test distribution of continuous variables we used Kolmogorov-Smirnov or Shapiro-Wilk tests according to the size of different subgroups. The *t*-Student test and Mann-Whitney test were used to compare continuous variables between two groups as appropriate. Differences between more than two groups were tested using ANOVA with Bonferroni *post hoc* tests and Kruskal-Wallis test with *post hoc* multiple comparisons (all pairwise) as appropriate. Correlations were assessed using Pearson's correlation coefficients or Spearman's correlation coefficients according to the data distribution. To test interactions among variables, multivariate linear regression analysis was applied, including all variables from univariate models with the minimum significance level of 0.05. In linear

regression, MGFA Clinical Classification was implemented as numeric variable (0–4). In this study we did not subdivide into A or B, according to the localization of weakness. Patients with no symptoms were scored as 0. Similarly, Post-Intervention MGFA status was treated as numeric variable, coded "1" for remission up to "5" for worsening. For education level, we coded "1" for primary education, "2" for secondary and "3" for university education. For the statistical analysis, SPSS version 20.0 was used.

RESULTS

The mean scores of the SF-36 scale are provided in **Table 2**.

There were no statistically significant differences in mean SF-36 subscores between women and men. LOMG patients had worse QoL than EOMG in PF ($p = 0.002$), BP ($p = 0.041$) and PCS ($p = 0.049$). Antibody status had no influence on QoL in PCS, MCS, and GH, however MuSK-MG represented only 2.7% of the group. Higher MGFA score was related to worse QoL in GH ($p < 0.001$), PCS ($p < 0.001$), and MCS ($p < 0.001$) domains. These data are provided in **Figure 1**. Influence of MGFA score on assessment of QoL in PCS and MCS ($p < 0.001$ in both) is independent of age and sex.

Also, worse MGFA post intervention status was related to worse QoL in GH ($p = 0.001$) and PCS ($p = 0.002$). Significant differences in PCS were found between remission and worsening ($p = 0.023$), pharmacological remission and worsening ($p = 0.009$) and improvement and worsening ($p = 0.035$). Worsening of symptoms influenced negatively GH assessment as compared with group with improvement of symptoms ($p = 0.004$), or in pharmacological remission ($p = 0.001$). There is still a significant negative influence of worse Post Intervention status on assessment of QoL in PCS ($p < 0.001$) and MCS ($p = 0.012$) independent from age and sex.

Patients treated with GCS in the past evaluated their QoL significantly worse in GH ($p = 0.037$) than these who have never required such treatment. We have found no differences in QoL depending on thymectomy status. The negative impact of BMI on QoL of MG patients is provided in **Figure 2**. Overweight and obese woman had worse PF ($p < 0.001$), VT ($p < 0.001$), PCS ($p = 0.002$), and MCS ($p = 0.038$) than those with normal BMI. There is still a significant negative influence of BMI score on assessment of QoL in PCS ($p = 0.046$) but not on MCS independently of age and sex.

University education was related to higher PF ($p < 0.001$), MH ($p = 0.006$), PCS ($p = 0.047$), and MCS ($p = 0.049$), than primary education. University education was also related to higher evaluation of PCS ($p = 0.043$) and PF ($p = 0.002$) as compared

TABLE 2 | The mean scores of the SF-36 scale.

SF-36 domains	Mean	Standard deviation
Physical functioning	48.79	25.27
Role limitations due to physical health	37.98	42.32
Role limitations due to emotional problems	56.64	44.83
Vitality	41.54	20.35
Mental Health	54.87	20.34
Social functioning	52.17	25.12
Bodily Pain	48.71	28.73
General health	39.73	12.04
Physical Component Summary Measures	44.57	19.79
Mental Component Summary Measures	51.05	20.53

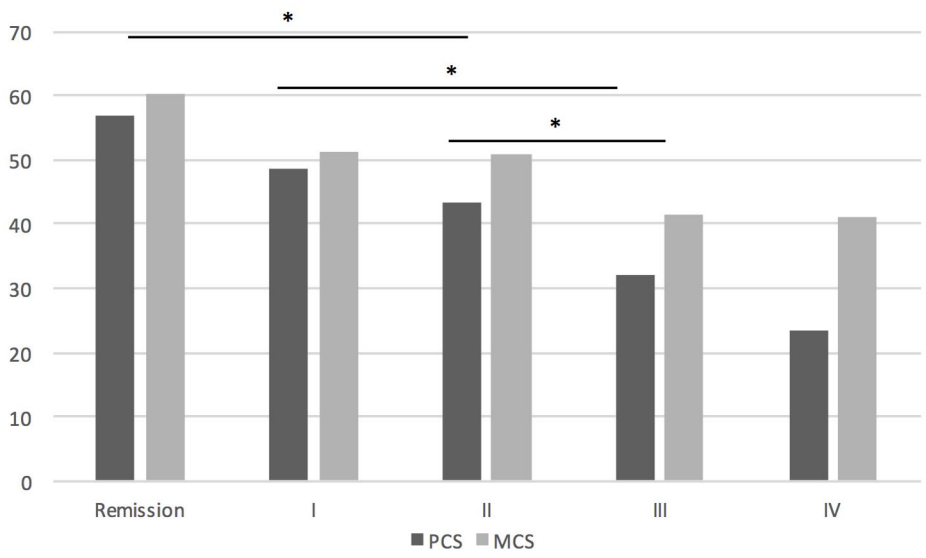

FIGURE 1 | The influence of MGFA score on QoL assessment. *$P < 0.05$. In PCS there were significant differences between patients in remission and MGFA II or more, between MGFA I and III or more, MGFA II and III or more; in MCS significant differences were found between patients in remission and MGFA II and more.

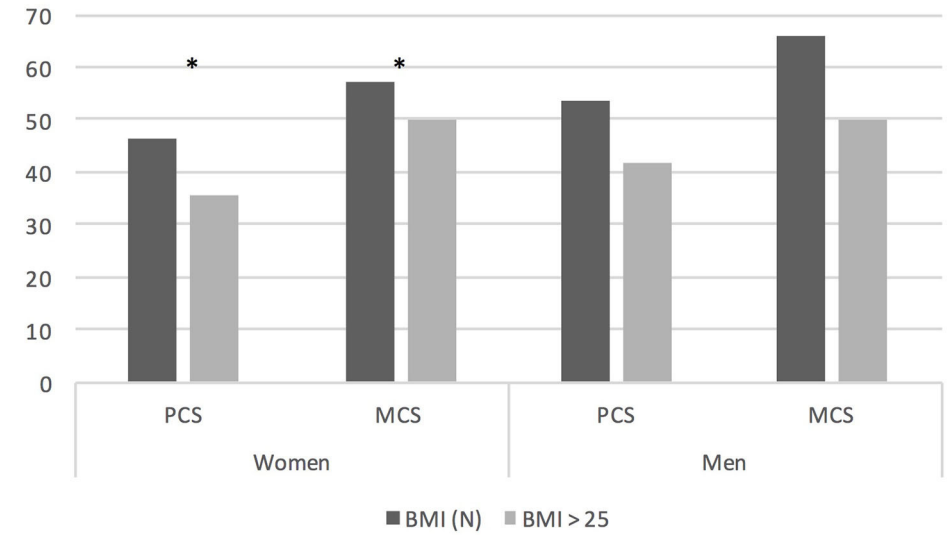

FIGURE 2 | QoL assessment in PCS and MCS domain depending on BMI grouped by gender; *$P < 0.05$. BMI, body mass index; BMI (N), BMI up to 25 kg/m^2.

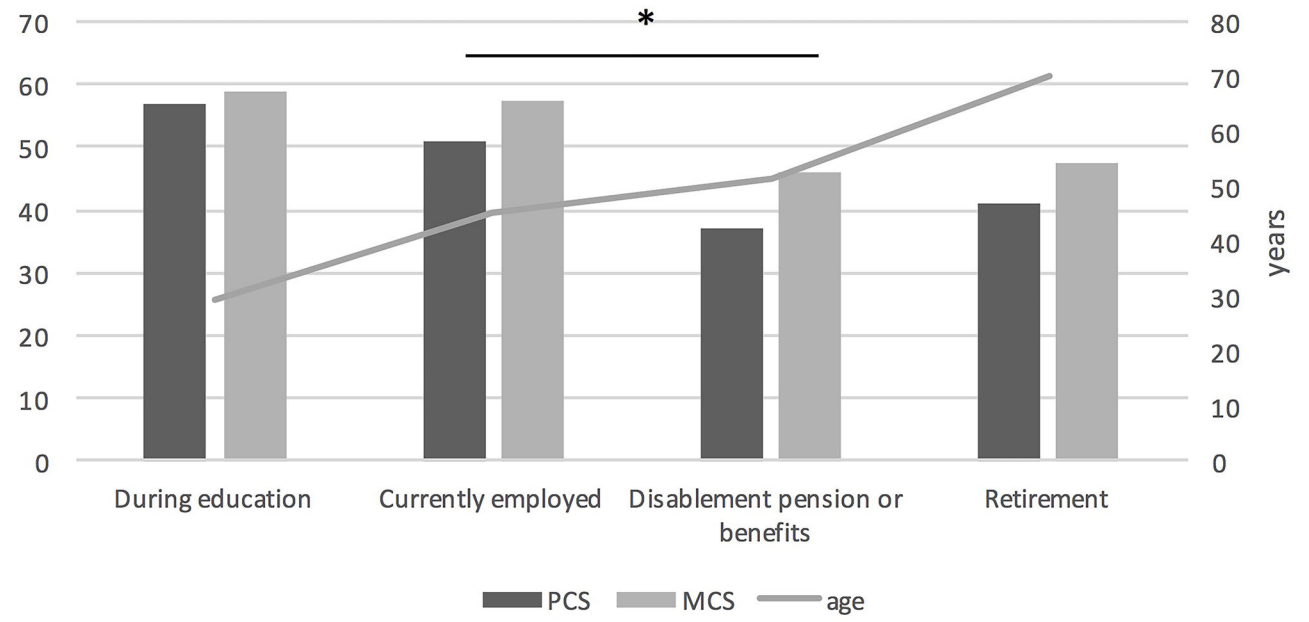

FIGURE 3 | QoL assessment in PCS and MCS domain depending on employment status; *$P < 0.05$.

with group with secondary education. We found no differences in QoL between patients with primary and secondary education.

We found statistically significant differences in PCS, MCS, and GH assessments depending on employment status is shown in **Figure 3**. Patients who were still during education assessed their PCS, GH, and MCS significantly better than patients on retirement or disablement pension ($p < 0.001$), but this difference was age-dependent. There was no difference in QoL assessment between patients during education and currently employed, despite significant difference of age in those two groups ($p < 0.001$). Patients who were currently employed assessed their PCS ($p < 0.001$), GH ($p = 0.007$) and MCS ($p = 0.001$) significantly better than patients on disablement pension and these two groups did not differ depending on age. Currently employed patients assessed their QoL significantly better than patients retired, but this difference was age-dependent. There was no significant difference in QoL assessment between retired and patients on disablement pension, even though the second group was significantly younger ($p < 0.001$). There is still a significant positive influence of current employment on PCS ($p = 0.021$)

TABLE 3 | Multivariate linear regression model. Predictors of Physical health.

	Unstandarized coefficients		Standarized coefficients	*t*	Significance
	B	Std. Error	Beta		
(Constant)	80.245	8.661		9.265	0.000
Age	−0.303	0.076	−0.278	−3.974	0.000
BMI	−0.402	0.207	−0.101	−1.944	0.053
Post Intervention Status (numeric)	0.707	1.061	0.038	0.667	0.506
Education level (numeric)	0.321	1.434	0.011	0.224	0.823
Gender (male)	8.735	2.169	0.21	4.027	0.000
Prednison usage in the past	−2.398	1.975	−0.06	−1.214	0.226
MGFA scale (numeric)	−7.642	1.195	−0.375	−6.396	0.000
During education	3.533	5.358	0.056	0.659	0.510
Currently employed	2.127	4.784	0.048	0.445	0.657
Pension	−1.318	4.906	−0.03	−0.269	0.788
Disablement pension or benefits	−5.706	4.69	−0.13	−1.217	0.225

$R = 0.558$. $R^2 = 0.312$. Adjusted $R^2 = 0.288$. $p = 0.000$.

and MCS ($p = 0.013$) and a negative influence of being on disablement pension or benefits on PCS ($p = 0.000$) and MCS ($p = 0.016$) independent of age and sex.

White collar workers had better PF ($p = 0.017$), BP ($p = 0.025$), and PCS ($p = 0.032$) than the hard physical workers. We found no differences in QoL between hard and light physical work. Patients living alone evaluated their MCS ($p = 0.015$) worse than those living with family. Moderate physical activity (at least 2 × week) was related with higher PCS ($p = 0.045$).

We ran multiple linear regression analysis to identify independent MCS and PCS predictors for the whole group as well as for men and women separately. Results of the analysis are presented in **Table 3** (Additional analysis are presented in **Supplementary Tables 1**–**3**). Despite age and sex, MGFA score appears to be the strongest predictor of QoL in PCS and MCS in MG patients. For females MGFA score, patient's age and BMI had strongest influence on QoL in PCS, for men only MGFA score significantly influenced PCS assessment. Multivariate linear regression model of PCS predictors explained nearly 30% of the variance in QoL among MG patients. For MCS the strongest model explained only 11% of the variance in QoL and showed significant impact of MGFA score and age on MCS assessment.

DISCUSSION

There is clear evidence showing lower quality of life in patients with MG compared to the healthy population (9) or to other diseases (17), therefore in our work we focused only on which aspects of the disease affect QoL the most. Our work confirms that QoL is lower in patients with more severe symptoms (18, 19). Previous studies also showed lower QoL in patients with general vs. ocular MG (20). Our results confirm, that QoL is highest in MG patients who achieved remission. Interestingly, we found no difference between groups MGFA I and II, and between MGFA III and IV. It seems that interference of MG symptoms with the patients' activities in MGFA I-IV is not gradual, but step-wise, with the important worsening of QoL when the symptoms become at least moderate. Authors believe that this may be a useful information, when considering escalation of long-term immunosuppression in patients with mild generalized MG and defining treatment targets depending on severity of clinical symptoms.

MG affects quality of life on many levels, one of them is lack of employment or decrease in income (21, 22). Our results confirm that lack of employment is connected to lower QoL compared to patients who still work. There is some interesting data on this topic. Minority of patients with MG are able to work, numbers varies from 22% thru 30% to 33% (21, 23, 24) and 27% in our group. Our study showed like others (23, 24) that patients still working had higher QoL but we excluded influence of age and MG severity. In our work, we also found that patients with university level education have higher QoL than those with primary or secondary education. The type of work also influenced QoL, patients who do hard labor had a lower QoL. This results are comparable with previous studies showing higher QoL in patients with higher vs. elementary education, white collar work vs. retirement (19, 25). Our study and previous studies provide solid evidence that myasthenia is still a disabling disease, especially for patients who do hard physical work and have a lower level of education. We demonstrated that not only employment status is important for MG patients but also family support. Our patients living alone had worse QoL compared to ones living with family, this finding was also supported by others (19).

It has already been proven in general population that obesity lowers quality of life (26). Obesity is a frequent problem in our MG patients. BMI>25 had 50.9% of women and 79.8% of men in our study. This may be due to a number of reasons, including reduced physical activity or long-term use of steroids. Our results showed that excessive weight and obesity have a significant negative impact on QoL in women with MG. BMI as predictor of low quality of life in MG was demonstrated by Winter et al. using EuroQol and in SF 36 in a physical composite score (27) but a large study using MG-QOL15-J on 640 MG patients from Japan showed that BMI was not a predictor of lower QoL (28). Authors are convinced that patients with MG should be carefully monitored for signs of obesity and should be advocated to lose weight not only for clear health-related issues but also for better QoL. Our study showed interesting results regarding physical exercise. Patients who exercised lightly at least 2x times a week had higher QoL. This finding may be important to routine practice. The patients should not be discouraged from light exercise, which is safe and may improve physical performance-based measures as well (29, 30).

Our study has some limitations. We used self-reported information, including BMI of the patients. SF-36 was used to allow comparison with previous MG studies, but no large

normative data was available for our population. Also, no patient-reported outcome measures were employed.

Identification of factors that have significant impact on the health-related quality of life is important and may guide some treatment choices in MG. Our study confirmed that greater severity of symptoms, age but also BMI, employment status and type of work, disablement pension, education status and physical activity affect QoL.

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 human participants were reviewed and approved by Bioethical committee, Medical University of Warsaw. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

PS: manuscript writing and data analysis. ES data collection and statistical analysis. BS, ML, and JK data collection and analysis. AK-P protocol development, data analysis, and critical manuscript review. All authors have made substantial, direct and intellectual contribution to the work, and approved it for publication.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2020.553626/full#supplementary-material

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Regional Features of MuSK Antibody-Positive Myasthenia Gravis in Northeast China

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Objective: To summarize the characteristics of muscle-specific receptor tyrosine kinase antibody-positive myasthenia gravis (MuSK-MG) in Northeast China.

Methods: We retrospectively collected 183 confirmed MG patients and divided them into three groups based on the type of serum antibodies: MuSK-MG (14 cases), acetylcholine receptor (AChR)-MG (130 cases), and double-seronegative (DSN)-MG (39 cases). The clinical, diagnostic, therapeutic, and prognosis data were analyzed.

Results: MuSK antibody was detected in 26.7% of seronegative MG. The mean age of onset in MuSK-MG was 53.2 ± 13.6 years. Fifty percent of MuSK-MG patients with an onset symptom of pure ocular muscle weakness. The time from onset to other muscle groups' involvement and the time from onset to myasthenic crisis had no significant difference among the three groups ($P > 0.05$). The proportion of Osserman classification I in MuSK-MG group was lower than that in DSN-MG group. The proportion of Osserman classification IV in MuSK-MG group was higher than that in the other two groups. The incidences of other coexisting autoimmune diseases in MuSK-MG group were higher. Prognosis after the treatment of steroid combined with tacrolimus for MuSK-MG was similar to AChR-MG treated with steroid combined with an immunosuppressant agent ($P > 0.05$).

Conclusion: Patients with MuSK-MG in Northeast China have a modestly later onset age and a proportion of patients may have a mild form of the disease with delayed disease progression. We confirmed the existence of a rare ocular MuSK-MG phenotype, a high proportion of coexisting with other autoimmune diseases, and a good response to steroids combined with tacrolimus for our MuSK-MG series.

Keywords: muscle specific receptors tyrosine kinase, acetylcholine receptor, myasthenia gravis, ocular muscle, tacrolimus

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INTRODUCTION

Myasthenia gravis (MG) is an autoimmune disorder caused by antibodies targeting proteins associated with neuromuscular junction (NMJ) transmission (1). The most common antibodies are against acetylcholine receptor (AChR). Such patients are called AChR-MG. However, 10–20% of MG patients, often termed seronegative MG (SNMG), do not have serum AChR antibodies (Ab). A proportion of those patients have relatively mild manifestations, whereas another subgroup of

patients have generally severe disease with severe respiratory and bulbar muscle weakness. In 2001, Hoch et al. confirmed the presence of muscle-specific receptor tyrosine kinase (MuSK) Ab in 70% of SNMG patients (2). MuSK Ab exerts a dose-dependent block of MuSK binding to ColQ, leading to the reduction of ColQ binding to NMJ. The lack of ColQ in NMJ can compromise agrin-mediated AChR clustering (3).

MuSK Ab-positive MG (MuSK-MG) has been defined as a distinct MG sub-group, which often leads to more severe muscle weakness (2). Some patients lack AChR Ab and MuSK Ab (double-seronegative MG, DSN-MG), and often have a mild manifestation, which seems to explain the phenomenon of polarized clinical features in SNMG. However, not all MuSK-MG patients have severe clinical features. Previous studies have found that the clinical presentation of MuSK-MG may vary by region and race. For example, the positivity for MuSK Ab in SNMG patients were 40% in Mediterranean countries (4), whereas in Asian countries it is 20–30% (5). In Europe and the USA, most MuSK-MG patients onset before 40 years (6). However, the onset age of MuSK-MG patients was slightly older ranging from 40 to 50 years in Japan (7), South Korea (8), and Taiwan (9). Initially, rare reports from western countries suggested that pure ocular muscle weakness at onset of MuSK-MG can occur (10, 11). On the contrary, it was the only onset symptom in Taiwan (9). Moreover, recent reports on ocular MuSK-MG are also increasing worldwide (12–18). In addition, previous studies have not reached consensus on immunotherapy option of MuSK-MG. Some scholars recommend the first choice of rituximab (19). Therefore, the specific clinical information and treatment of MuSK-MG patients still need further observation and investigation.

This study retrospectively analyzed the clinical data of MuSK-MG patients in Northeast China, and reviewed the previous literature to summarize the clinical feature and diagnosis as well as the therapeutic management of MuSK-MG in this area, so as to improve the clinicians' further understanding of the disease.

METHODS

Patient Information

We retrospectively collected 183 confirmed MG patients who were admitted to the First Hospital of Jilin University from January 1, 2015 to December 31, 2019. According to the type of serum antibodies, the patients were classified into three groups, MuSK-MG (14 cases), AChR-MG (130 cases), and DSN-MG (39 cases). The clinical, diagnostic, therapeutic, and prognosis data of these patients, including gender, age of onset, initial symptom, disease progression, clinical classification, disease severity, pharmacological, electrophysiological, and serological findings, results of thymus examination, comorbidities, therapeutic options, and prognosis, were analyzed.

Incomplete information was completed at outpatient clinic visits. Each patient gave informed consent. The study was approved by the ethics committee of the First Hospital of Jilin University.

Diagnosis Criteria

The diagnosis of MG was confirmed based on typical clinical features of fluctuating muscle weakness, and at least one of the following positive tests: a positive pharmacological response on intramuscular injection of neostigmine; decrement of >10% on repetitive nerve stimulation (RNS) test; AChR Ab or MuSK Ab positive.

Disease Severity Assessment

The clinical status and disease severity were evaluated according to the Osserman classification and quantitative MG score (QMGs), respectively (20).

Neostigmine Trial

First, the initial disease severity of a patient was evaluated by full QMGs. After a 10-min rest, appropriate dose (0.02–0.03 mg/kg body weight) of neostigmine was administered. To relieve the possible muscarinic side effect, atropine (0.5 mg) was administered before the neostigmine injection. Then, disease severity was evaluated by QMGs at 10-min intervals until 60 min after the neostigmine injection. The most significant improvement in QMGs was calculated: (QMGs before the injection – minimal QMGs)/QMGs before the injection × 100%. The results of the neostigmine trial were categorized into positive (>60%), probable positive (25–60%), or negative (<25%).

Repetitive Nerve Stimulation

The methods measured by RNS are as follows: First, routine nerve conduction studies were performed to exclude peripheral neuropathy. Next, repetitive stimulation was performed in selected muscles at a rate of 2–5 times and 10–50 times per second, respectively. A compound muscle action potential (CMAP) of the fourth to fifth wave decrement of >10% compared with the first wave was considered abnormal. In addition, a CMAP increment of >100% was also regarded as abnormal.

Antibody Testing

MG-related serum antibodies were tested using a commercial ELISA kit (Euroimmun, Lübeck, Germany). All patients were tested for AChR Ab titers before receiving immunosuppressive therapy. If the AChR Ab were negative, MuSK Ab is further tested. One hundred microliters of patient serum was diluted at 1:100 into the microtiter well-plate. The microtiter plate was incubated for 60 min at room temperature (RT) on an orbital shaker (500 rpm) and then washed three times with 250 μl of diluted wash buffer. One hundred microliters of antiserum was pipetted in each well and incubated in a microtiter plate for 60 min at RT on an orbital shaker (500 rpm). The incubation solution was discarded and the plate was washed three times with 250 μl of diluted wash buffer. One hundred microliters of *p*-nitrophenyl phosphate (PNPP) substrate solution was pipetted into each well and incubated in a microtiter plate for 30 min at RT. The substrate reaction was stopped by adding 100 μl of PNPP stop solution into each well, and then the optical density was measured with a photometer at 405 nm.

Therapy

Therapeutic strategies mainly consist of symptomatic treatment and immunosuppressive therapy. Symptomatic treatment with oral pyridostigmine bromide was performed to the patients who responded positive on the neostigmine trial. Corticosteroids were combined with an immunosuppressive agent (azathioprine, tacrolimus, mycophenolate mofetil, cyclophosphamide). All of the follow-up MuSK-MG patients were prescribed with tacrolimus as an immunosuppressant. During the treatment period, the dosage of steroid was gradually increased or decreased according to the patient's condition, and the patient was given corresponding supportive treatment to prevent the corticosteroid side effects. Intravenous immunoglobulin (IVIg) and plasma exchange was given according to the patient's condition and economic affordability.

Prognosis

The QMG score of a patient at the first visit was regarded as baseline. Follow-up clinic visits were performed at the first and third months after initial visit and 3-month intervals thereafter, and a QMG score was evaluated at each visit and compared with the baseline QMG score: (baseline QMG score – a follow-up QMG score)/baseline QMG score × 100%. A degree of improvement >95% was classified as cured, 80∼95% was basically cured, 50–79% was markedly effective, 25–49% was effective, and < 25% was ineffective, respectively. This method was originated from the clinical absolute and relative score system for MG proposed by Professor Xu Xianhao in 1993 (21) and has been largely used in China.

Statistical Analysis

SPSS 22.0 statistical software was used for data statistics. Normally distributed data analysis was performed by Student *t*-test. Non-normally distributed data analysis was performed by Mann–Whitney *U*-test. For categorical data, χ^2 test or Fisher exact test was used for analyzing the difference between groups. $P < 0.05$ was considered statistically significant.

RESULTS

Detailed clinical data of the 14 MuSK-MG patients are presented in **Tables 1A**,**B**. The mean time of patient follow-up was 11.8 ± 11.0 months, ranging 1–42 months.

Demographic Data of MuSK-MG Patients

Anti-MuSK antibodies were detected in 26.4% of SNMG. Females predominated in both MuSK-MG (11/14, 78.6%) and AChR-MG (82/130, 63.1%). On the contrary, DSN-MG showed a marked male predominance (24/39, 61.5%). The mean onset age of MuSK-MG was 53.2 ± 13.6, which is not different from AChR-MG group and DSN-MG group ($P = 0.876$, $P = 0.080$) (see **Table 2**).

Tables 3A–C summarizes the clinical features, diagnostic tests, and comorbidities or coexisting antibodies for the three groups of patients.

MuSK-MG With a High Rate of Ocular Muscle Weakness at Onset

Weakness of muscle involvement at onset for MuSK-MG patients was classified into ocular (7/14, 50.0%), bulbar (3/14, 21.4%), respiratory (1/14, 7.1%), limb (1/14, 7.1%), and oculobulbar muscles (2/14, 14.3%). Patients with MuSK-MG had a high rate pure bulbar muscle weakness at onset compared with those with AChR-MG ($P = 0.044$). There were no significant differences in other muscle involvement at onset among the three groups ($P > 0.05$) (**Table 3A**).

Atypical Clinical Feature and Tongue Muscle Atrophy in MuSK-MG

Compared with AChR-MG and DSN-MG groups, the positive rate of Jolly test in MuSK-MG group was lower ($P < 0.05$). Tongue muscle atrophy in patients with MuSK-MG was more frequent than AChR-MG and DSN-MG patients ($P < 0.05$) (**Table 3A**).

A Similar Disease Progression Between MuSK-MG and AChR-MG

The median time from onset to other muscles' involvement among the three groups had no significant difference (**Figure 1A**). In addition, the median time from onset to myasthenic crisis was 25.75 (5.9, 64.5) months in MuSK-MG group, which was not different from AChR-MG group ($P = 0.267$) (**Figure 1B**).

Ocular and Late Severe Phenotype MuSK-MG

As shown in **Table 3A**, the proportion of Osserman classification I between MuSK-MG group and AChR-MG group had no significant difference ($P = 0.147$); however, such proportions in both MuSK-MG and AChR-MG groups were much lower than that in DSN-MG group ($P = 0.001$, $P = 0.001$). Compared with the AChR-MG group and DSN-MG group, the proportion of Osserman classification IV in MuSK-MG group was higher ($P = 0.007$, $P = 0.016$).

Figure 2 shows the maximum QMGs of the patients in each group. The QMGs in the MuSK-MG group were more severe than that in AChR-MG group and DSN-MG group ($P = 0.023$, $P < 0.001$). In addition, the QMGs in the AChR-MG group was also significantly higher than that in DSN-MG group ($P = 0.001$).

Diagnostic Testing

The positive rate of neostigmine trial in MuSK-MG group was lower than that in AChR-MG group and DSN-MG group ($P < 0.001$, $P = 0.008$). The incidence of cholinergic side effects in MuSK-MG group was significantly higher than that in AChR-MG group and DSN-MG group ($P < 0.001$, $P < 0.001$) (**Table 3B**).

The positive rate of RNS decrement (3 Hz) among the three groups was not significantly different ($P = 0.238$) (**Table 3B**).

As shown in **Table 1**, the auxiliary examinations for the diagnosis of MuSK-MG included pharmacological tests, neurophysiological examinations, and serological tests. Among

TABLE 1A | Detailed clinical data of the 14 MuSK-MG patients.

Patient number	1	2	3	4	5	6	7
Sex	Female	Female	Female	Male	Female	Female	Female
Onset age (years)	53	62	71	70	63	34	64
MuSK Ab titer (U/ml)	4.45	>12.00	>12.00	3.63	5.48	5.47	1.01
Disease duration (months)	17	74	4	96	78	25	38.5
Onset symptom	Ptosis, slurred speech	Dysphagia, slurred speech	Dyspnea	Slurred speech, dysphagia	Slurred speech	Diplopia	Limb weakness
Tongue muscle atrophy	Yes	Yes	No	Yes	No	No	No
Jolly test	Positive	Negative	Positive	Positive	Positive	Positive	Positive
Time from onset to involvement of other muscle groups (months)	10.5	71	2	90	60	–	12
Most severe Osserman classification	IIb	IIb	III	IIb	IV	I	IV
Maximum QMG scores	13	21	22	10	24	4	24
Time from onset to the peak (months)	17	72	3.2	96	73.5	0.3	37.5
Number of myasthenic crisis	0	0	1	0	1	0	2
Neostigmine trial	Positive	Negative	Negative	Positive	Positive	Positive	Negative
Cholinergic side effect	Yes	Yes	Yes	No	Yes	No	Yes
Decrement on RNS (3 Hz)	Yes	Yes	Yes	Not done	Not done	No	No
Thymus CT scan/pathology	Normal	Normal	Normal	Normal	Normal	Normal	Normal
TG/TPO Ab	Normal	Increased	Increased	Normal	Increased	Normal	Increased
Other AD	Hyperthyroidism	No	Hashimoto thyroiditis	No	No	Behcet's disease	Hashimoto thyroiditis
Intravenous immunoglobulin	Yes	No	No	No	Yes	No	Yes
Glucocorticoid	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Tacrolimus	Yes	Yes	Yes	Yes	Yes	Yes	No
Pyridostigmine bromide	30 mg tid	No	No	60 mg tid	No	60 mg tid	No
Follow-up time (months)	12	12	21	3	1	42	0.25
QMG scores after treatment	5	4	5	4	15	0	–
Prognosis	Markedly effective	Basically cured	Markedly effective	Markedly effective	Effective	Cured	Died

them, four patients were positive for all three auxiliary examinations, nine patients were positive for two of the three auxiliary examinations, and one patient had merely MuSK antibody positive as the basis for diagnosis.

Complications

Thymic CT scan and/or thymic pathological examination was performed in all enrolled patients. Only one patient (#12) in MuSK-MG group had a small nodule in the thymus region (**Figure 3A**). The pathological report of thymus indicated that the lesion was thymic bronchogenic cyst (**Figure 3B**). In contrast, 42 patients had thymoma and 16 patients had thymic hyperplasia in AChR-MG group. Five out of 39 (12.8%) patients with DSN-MG had thymoma; another 5 out of 39 (12.8%) patients with DSN-MG had thymic hyperplasia. The proportion of thymus abnormalities in the MuSK-MG group was lower than that in AChR-MG group ($P = 0.007$), whereas it did not differ in the DSN-MG group ($P = 0.280$) (**Table 3C**).

The frequency other autoimmune disease (AD) in MuSK-MG was higher than that in AChR-MG group ($P = 0.023$), but not different from DSN-MG group ($P = 0.135$). In MuSK-MG group,

TABLE 1B | Detailed clinical data of the 14 MuSK-MG patients.

Patient number	8	9	10	11	12	13	14
Sex	Female	Male	Female	Female	Female	Male	Female
Onset age (years)	64	51	62	55	50	32	29
MuSK Ab titer (U/ml)	1.08	1.20	>12.00	5.90	5.18	1.40	>12.00
Disease duration (months)	4	2	54	135.6	3	70	1
Onset symptom	Ptosis, diplopia	Ptosis	Diplopia	Ptosis, diplopia	Diplopia	Ptosis, diplopia	Ptosis, dysphagia
Tongue muscle atrophy	No	No	No	No	No	No	No
Jolly test	Positive	Positive	Positive	Negative	Negative	Positive	Positive
Time from onset to involvement of other muscle groups (months)	3.5	1.5	3	132	7	6	1
Most severe Osserman classification	IIb	IIb	IV	IIb	IIa	IIb	IIb
Maximum QMG scores	14	11	22	12	4	20	13
Time from onset to the peak (months)	4	2	54	135.6	7	7	1
Number of myasthenic crisis	0	0	2	0	0	0	0
Neostigmine trial	Positive	Positive	Negative	Negative	Positive	Positive	Positive
Cholinergic side effect	No	No	Yes	Yes	No	No	No
Decrement on RNS (3 Hz)	Not done	No	Yes	Yes	Yes	Yes	Yes
Thymus CT scan/pathology	Normal	Normal	Normal	Normal	Bronchogenic cyst	Normal	Normal
TG/TPO Ab	Yes	Yes	Yes	Yes	No done	No done	No done
Other AD	Psoriasis	Allergic dermatitis	Without	Without	Without	Without	Without
Intravenous immunoglobulin	No	No	Yes	Yes	No	No	Yes
Glucocorticoid	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Tacrolimus	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Pyridostigmine bromide	60 mg tid	60 mg tid	No	No	60 mg tid	60 mg tid	60 mg tid
Follow-up time (months)	3	3	3	12	12	12	18
QMG scores after treatment	7	3	10	2	2	4	4
Prognosis	Markedly effective	Markedly effective	Markedly effective	Basically cured	Markedly effective	Basically cured	Markedly effective

MuSK-Ab (U/ml) (cut-off >0.40); QMG, quantitative myasthenia gravis; tid, three times a day; TG Ab, thyroglobulin antibody; TPO Ab, thyroid peroxidase antibody; AD, autoimmune disease; RNS, repetitive nerve stimulation.

TABLE 2 | Basic information of the three groups.

Groups	MuSK-MG ($n = 14$)	AChR-MG ($n = 130$)	DSN-MG ($n = 39$)
Age at onset (years)	53.2 ± 13.6	53.9 ± 16.7	45.0 ± 15.2
Gender (male/female)	3:11	48:82	24:15

patient 1 coexisted with hyperthyroidism, patient 3 and patient 7 coexisted with Hashimoto thyroiditis, patient 6 coexisted with Behcet's disease, patient 8 coexisted with psoriasis, and patient 9 coexisted with allergic dermatitis.

The positive rates of thyroglobulin (TG) and/or thyroid peroxidase (TPO) antibodies among the three groups were not statistically different ($P > 0.05$), although it was higher in the MuSK-MG group (**Table 3C**).

TABLE 3 | Clinical, diagnostic tests, complications, and laboratory information of three groups of patients.

Groups		MuSK-MG	AChR-MG	DSN-MG	*P* value
(A) Clinical features					
Onset distribution	Pure ocular	7/14 (50.0%)	98/130 (75.4%)	30/39 (76.9%)	0.107
	Pure limb	1/14 (7.1%)	10/130 (7.7%)	5/39 (12.8%)	>0.05
	Pure bulbar	3/14 (21.4%)	6/130(4.6%)	1/39(2.6%)	<0.05*
	Respiratory	1/14 (7.1%)	3/130 (2.3%)	-	0.339
	Oculobulbar	2/14 (14.3%)	13/130 (10.0%)	3/39 (7.7%)	>0.05
Positive rate of Jolly test		11/14 (78.6%)	130/130 (100%)	39/39 (100%)	<0.001***
Tongue muscle atrophy		3/14 (21.4%)	1/130 (0.8%)	-	0.003**
Most severe Osserman classification	I	1/14 (7.1%)	38/130 (29.2%)	23/39 (59.0%)	<0.001***
	IIa	1/14 (7.1%)	20/130 (15.4%)	4/39 (10.3%)	0.545
	IIb	8/14 (57.1%)	61/130 (46.9%)	11/39 (28.2%)	0.068
	III	1/14 (7.1%)	8/130 (6.2%)	1/39 (2.6%)	0.660
	IV	3/14 (21.4%)	3/130 (2.3%)	-	<0.05*
(B) Diagnostic tests					
Neostigmine trial		9/14 (64.3%)	108/109 (98.2%)	33/34 (97.1%)	<0.05*
Cholinergic side effect		7/14 (50.0%)	2/109 (1.8%)	-	<0.001***
Decrement on RNS (3 Hz)		8/11 (72.7%)	65/88 (73.9%)	16/28 (57.1%)	0.238
(C) Coexisting other AD/Abs					
Thymic abnormalities		1/14 (7.1%)	58/130 (44.6%)	10/39 (25.6%)	0.006**
Other AD		6/14 (42.9%)	19/130 (14.6%)	7/39 (17.9%)	0.030*
TG/TPO Ab		8/11 (72.7%)	35/93 (37.6%)	10/23 (43.5%)	>0.05

*Comparison of clinical, diagnostic tests, complications and laboratory examination data among the three groups was done by χ^2 test or Fisher exact χ^2 test. *indicates $P < 0.05$, **indicates $P < 0.01$, ***indicates $P < 0.001$. The data are shown as mean ± SD or ratio. AD, autoimmune disease; RNS, repetitive nerve stimulation; TG, thyroglobulin; TPO, thyroid peroxidase; Ab, antibody.*

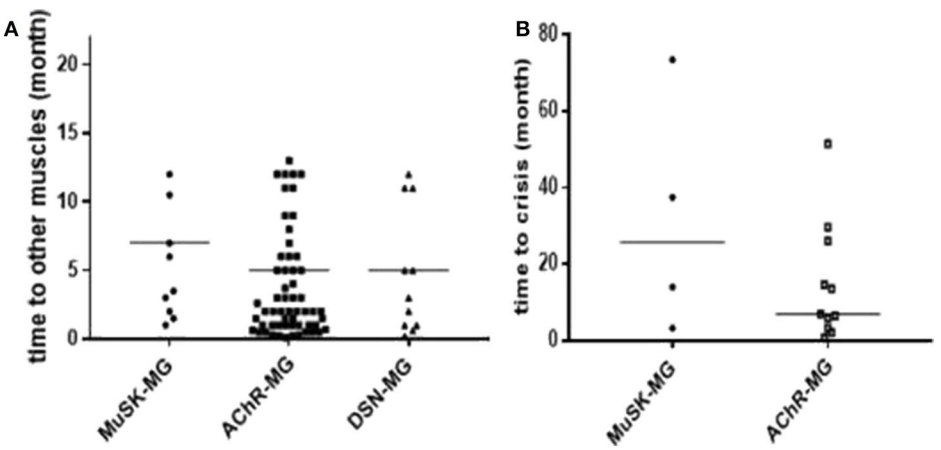

FIGURE 1 | Comparison of disease progression in each group. **(A)** There was no significant difference in the time from onset to other muscle group involvement among the three groups. **(B)** There was no significant difference in the time from onset to myasthenic crisis between MuSK-MG group and AChR-MG group. Comparison between groups was done by Mann-Whitney test.

Treatment and Prognosis

As shown in **Table 4**, 8 out of 14 MuSK-MG patients showed a good response to pyridostigmine bromide therapy, and this proportion was lower than that in AChR-MG group and DSN-MG group ($P < 0.001$, $P < 0.001$). The rates of treatment with glucocorticoid, immunosuppressants, and IVIg in MuSK-MG group were not different from those in the AChR-MG group ($P = 0.051$, $P = 0.099$, and $P = 0.356$), but higher than those in DSN-MG group ($P < 0.001$, $P < 0.001$, and $P = 0.046$). Plasma exchange was performed only in

two AChR-MG patients. No patients received rituximab in our study.

Two patients died from MG during the follow-up, including one MuSK-MG patient (patient 7 died in the first week after the initial visit) and one AChR-MG patient, and the mortality between the two groups had no statistical difference ($P = 0.186$).

Thirteen MuSK-MG patients, with an average follow-up time of 11.8 ± 11.0 (range 1–42) months, received treatment with prednisone (1 mg/kg body weight daily, tapered to 5 mg every 1–2 weeks) combined with tacrolimus (3 mg/day).

Treatment with prednisone plus an immunosuppressant (tacrolimus 3 mg/day, cyclophosphamide 100 mg/day, mycophenolate mofetil 1.0–2.0 g/day, or azathioprine 2–3 mg/kg body weight daily) was performed in 88 out of 130 AChR-MG patients.

The prognosis between MuSK-MG group and AChR-MG group (mean follow-up time was 26.9 ± 14.8 (range 3–58) months) was not significantly different (**Table 5**). Most patients in the DSN-MG group only received symptomatic treatment with pyridostigmine bromide; thus, the analysis of prognosis comparison was not included. In addition, the prognosis of MuSK-MG patients in several special situations were also compared in this study (see **Figure 4**). The prognosis (degree of QMGs improvement) in MuSK-MG patients with thyroid antibodies [73.0% (50.0%, 81.0%)] compared with that of MuSK-MG patients without thyroid antibodies (62.0% (61.0%, 81.0%)] had no significant difference ($P = 0.569$) (**Figure 4A**).

In addition, the prognosis of MuSK-MG patients in several special situations was also compared in this study (see **Figure 4**). The prognosis (degree of QMGs improvement) in MuSK-MG patients with thyroid antibodies [73.0% (50.0%, 81.0%)] compared with that of MuSK-MG patients without thyroid antibodies [62.0% (61.0%, 81.0%)] had no significant difference ($P = 0.569$) (**Figure 4A**). Moreover, the prognosis of MuSK-MG patients with co-existence of other AD [73.0% (56.0%, 88.5%)] compared with that of MuSK-MG patients without AD [64.5% (51.25%, 80.75%)] had no significant difference as well ($P = 0.628$) (**Figure 4B**). The prognosis between MuSK-MG patients treated with pyridostigmine bromide [62.0% (50.0%, 73.0%)] and those of patients without pyridostigmine bromide [73.5% (50.25%, 80.75%)] was also not different ($P = 0.567$) (**Figure 4C**).

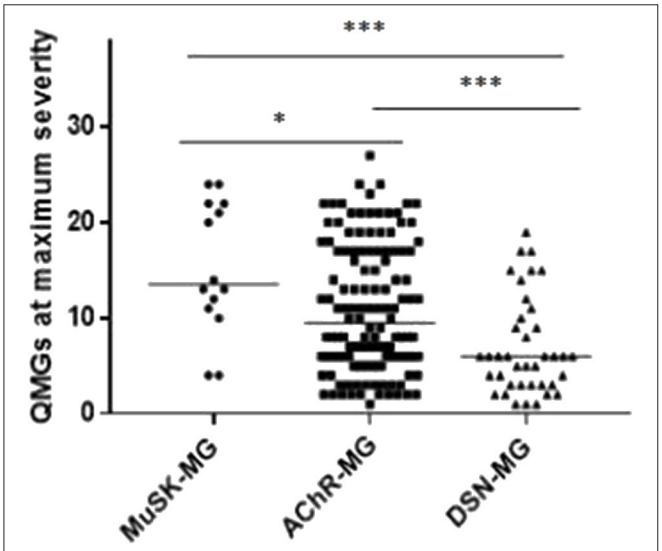

FIGURE 2 | Comparison of maximum QMGs in each group. The maximum QMGs in MuSK-MG group was more severe than that in AChR-MG group and DSN-MG group. Moreover, QMGs in AChR-MG group was significantly higher than that in DSN-MG group. Comparison between groups was done by Mann-Whitney test. * indicates $P < 0.05$, *** indicates $P < 0.001$.

DISCUSSION

Demographic Characteristics

We showed that the MuSK-MG patients represented 26.4% of SNMG patients. The prevalence of MuSK-Ab was similar to the frequency as found in Japan (7) and South Korea (8). Some studies reported that patients with MuSK-MG showed a more female predominance compared with the other two groups (22, 23). Another study reported that women predominated in all

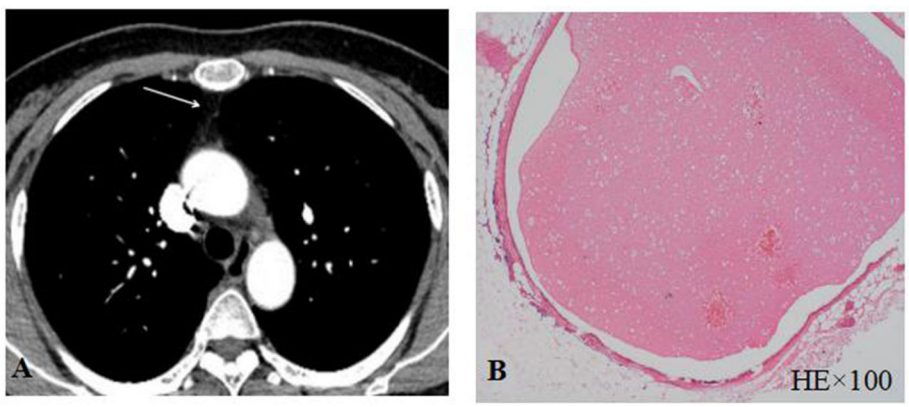

FIGURE 3 | (A) Thymus CT scan revealed a small nodule in the thymus area; **(B)** thymic bronchogenic cyst was shown by HE staining.

TABLE 4 | Therapeutic strategy among the three groups.

Groups	MuSK-MG	AChR-MG	DSN-MG
Pyridostigmine bromide	8/14 (57.1%)	130/130 (100%)	39/39 (100%)
Glucocorticoid	14/14 (100%)	94/130 (72.3%)	16/39 (33.3%)
Immunosuppressant	13/14 (92.9%)	86/130 (66.2%)	1/39 (2.6%)
Intravenous immunoglobulin	6/14 (42.9%)	37/130 (28.5%)	5/39 (12.8%)
Plasma exchange	–	2/130 (1.6%)	–

TABLE 5 | Comparison of the prognosis after steroid combined with immunosuppressive therapy between MuSK-MG and AChR-MG group.

Groups	MuSK-MG	AChR-MG	*P*-value
Cured	1/13 (7.7%)	5/88 (5.7%)	0.572
Basically cured	3/13 (23.1%)	24/88 (27.3%)	1.000
Markedly effective	7/13 (53.8%)	45/88 (51.1%)	1.000
Effective	2/13 (15.4%)	14/88 (15.9%)	1.000
Ineffective	0	0	–

Comparison of prognosis between MuSK-MG and AChR-MG was done by Fisher exact χ^2 test. Significance was set at $P < 0.05$. The data are shown as ratio.

groups (23). However, in our results, MuSK-MG and AChR-MG patients showed a similar female predominance, but DSN-MG patients were predominantly male. The onset age of MuSK-MG was not statistically different from the other two groups, which was consistent with previous studies (23, 24). Interestingly, however, the mean age at onset in our MuSK-MG series was 53.2 ± 13.6 years, which was different from most Western countries. The onset age of MuSK-MG patients is prominent in the fourth decade (6). However, the onset ages of MuSK-MG in Japan (7), South Korea (8), and China's Taiwan (9) are mostly between 45 and 50 years. Compared with the earlier results, the onset age of MuSK-MG patients was even later in Northeast China.

Clinical Features

In this study, ocular muscle weakness was the most common onset symptom in the three groups. Although the proportion of pure bulbar muscle weakness at onset in MuSK-MG (21.4%) was higher than that of AChR-MG, such a proportion is far lower than the proportion of 60.1% found by Baggi et al. (24). In addition, interestingly, our results showed no differences in the time from onset to the involvement of other muscle groups and the time from onset to myasthenic crisis among three groups, which was different from the previous reports of the rapid progress for MuSK-MG (10). Four MuSK-MG patients manifested mild symptoms for a long time. Patient 4 only showed mild dysarthria and remained stable for about 8 years. He then came to the hospital because of diplopia. The main manifestation of patient 5 was mild fluctuating slurred speech, and the symptoms lasted for more than 6 years. Then she came to the hospital due to limb weakness. Patient 11 initially showed ptosis and diplopia only, which lasted for 11 years, and she came to our hospital because of slurred speech. Patient 6 had purely ocular symptoms for more than 2 years; the Osserman classification of patient 6 was graded I. Therefore, the clinical progression in some of our MuSK-MG patients seems to be mild. In addition, ocular MG may be the fourth clinical phenotype for MuSK-MG, which differs from the typical three generalized phenotypes (18). Despite the subset of MuSK-MG patients with slow disease progression and relatively mild symptoms, the overall disease severity was higher in patients with MuSK-MG than that of the other two groups, consistent with previous studies (24).

Diagnostic Testing

In our cohort, MuSK-MG had a lower positivity rate to neostigmine trial and a higher prevalence of cholinergic side effects compared with the other two groups, which were in accord with previous studies (25). Moreover, Wolfe et al. found that the overall positive rates of RNS between MuSK-MG and AChR-MG were not significantly different, but higher than that of DSN-MG (25). In this study, DSN-MG did have a relatively low positive rate of RNS, but the difference was not statistically significant compared with the other two groups. In addition, interestingly, one of 14 patients (patient 7) in this study lacked positive evidence of both neostigmine test and low-frequency RNS test, but this patient had the typical fluctuation of muscle weakness. The antibody testing result of this patient indicated that the MuSK-Ab titer was 1.01 U/ml (cut-off >0.40). After the immunosuppressive experimental treatment, the condition of the patient improved, and the patient was diagnosed as definite MuSK-MG. However, a more sensitive test—single-fiber electromyography—was not performed in this study, which limits the diagnostic efficacy of neurophysiological tests.

Atypical Clinical Feature and Tongue Muscle Atrophy

Fluctuating skeletal muscle weakness is considered to be the typical clinical feature of MG. Interestingly, in this study, 21.4% (3/14) of MuSK-MG patients lacked the fluctuation of muscle weakness, which was different from the other two groups with a positive rate of 100% to Jolly test. Basic research has found that the presynaptic acetylcholine (ACh) release was increased in patients with AChR-MG, which is a compensation mechanism for failure of neuromuscular transmission caused by AChR loss (26). Therefore, most of the AChR-MG patients have fluctuating muscle weakness. However, such a compensation mechanism was not detected in MuSK-MG (27). The levels of presynaptic ACh release were low in MuSK-MG, and the miniature endplate potentials were small at the same time, which may explain why some MuSK-MG patients have no obvious fluctuation of muscle weakness.

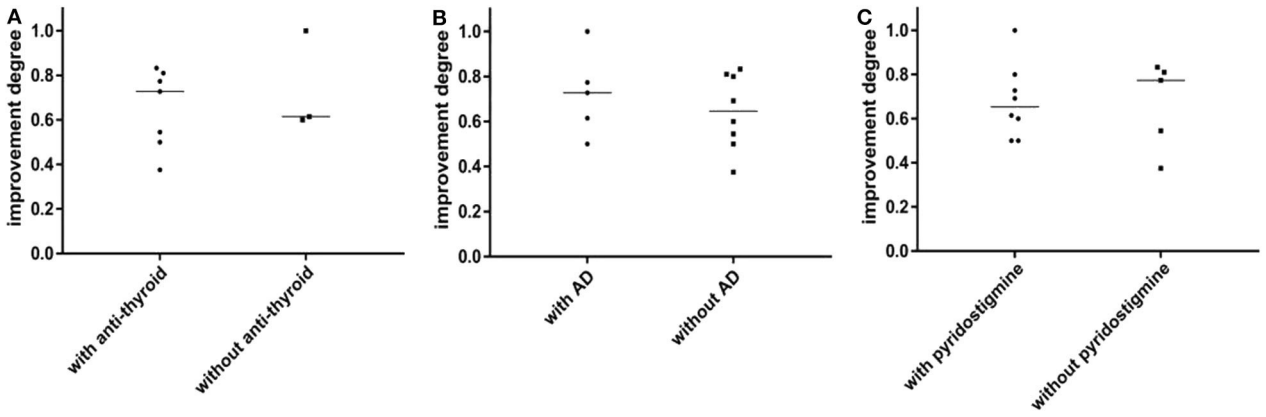

FIGURE 4 | (A) The prognosis between MuSK-MG with thyroid antibodies and without thyroid antibodies has no significant difference. **(B)** The prognosis between MuSK-MG patients coexist with other autoimmune disease and without other autoimmune disease has no statistical difference. **(C)** There was no statistical difference in the prognosis between MuSK-MG patients who were treated with pyridostigmine bromide and those who were not. Comparison between groups was done by Mann-Whitney test. $P < 0.05$ indicated a statistically significant difference.

However, not all MuSK-MG patients lack the typical fluctuating characteristics; hence, more research is still needed to explore for this issue.

In addition, we found that three MuSK-MG patients (1, 2, and 4) have tongue muscle atrophy, which showed a higher frequency of tongue muscle atrophy in MuSK-MG compared with AChR-MG and DSN-MG. In particular, the disease courses in those patients were insidious, which can lead to confusion with amyotrophic lateral sclerosis (ALS). Patient 2 had a 6-year duration of the disease, with no fluctuating muscle weakness, a negative neostigmine trial, and appeared to have fasciculations. This patient was easily misdiagnosed as ALS with bulbar-onset symptom. However, her neurological examination and neuroelectrophysiological studies revealed no typical changes in ALS, but a decrement response on low-frequency RNS was revealed in bilateral orbicularis oculi muscles. She then was prescribed with steroid experimental treatment and the QMG score was significantly reduced after the treatment. This response definitively ruled out the suspicion of ALS. Because of the degeneration of ALS motor axon branches and immature collateral regenerative nerves, some ALS can also lead to transmission failure on repetitive stimulation (28). Hence, steroid therapy and the MG-related antibody detection may be the break points to identify the two diseases.

Other Coexisting ADs and Autoantibodies

Coexisting ADs were detected in 14.6% of AChR-MG patients and in 17.9% of DSN-MG patients, which were consistent with the previous reported total frequency of MG (8–26%) (29, 30). However, the frequency of coexisting AD in MuSK-MG patients was much higher (42.9%). In addition, the frequency of coexisting thyroglobulin (TG) and/or thyroid peroxidase (TPO) antibodies in MuSK-MG patients was 72.7%, which was also higher than the previous reported frequency (15–40%) (31). A Danish study based on the MG population found that MG patients with coexisting AD had a lower rate of disease remission than MG patients without coexisting AD. The study believed that there is a more serious autoimmune reaction in these patients with AD (32). We explored the clinical prognosis between MuSK-MG patients with and without AD, and, specifically, the clinical prognosis between MuSK-MG patients with thyroid antibodies and those without thyroid antibodies. Our results revealed that there was no significant difference in prognosis between the two groups, respectively. In the future, it is still necessary to expand the MuSK-MG sample to verify the relationships between coexisting ADs/thyroid antibodies and prognosis.

Treatment and Prognosis

Several studies reported that rituximab is an effective immunosuppressant for MuSK-MG patients (19), although this expensive drug is not covered by health insurance in China; thus, no patients received rituximab in our study. Tacrolimus, another effective immunosuppressant drug, has been used for treating AChR-MG (33). In our study, glucocorticoid combined with tacrolimus was prescribed in 13 MuSK-MG patients; however, as the treatment time of some patients in this study was too short, even < year, it may not be appropriate to use the MGFA post-intervention status as the method to evaluate the prognosis. Therefore, we chose the clinical absolute and relative score system for myasthenia gravis as the method to evaluate the therapeutic efficacy, which calculated the percent change of QMG score at each visit from baseline (first visit). Also, no significant difference in the degree of QMG score improvement after treatment was found compared with AChR-MG patients treated with glucocorticoid and an immunosuppressant agent.

This study has several limitations. Comparing clinical outcomes in this case series with other previous reports is difficult because patients were managed differently. In particular, no patients in this study received rituximab. In addition, owing to the small number of MuSK-MG patients and the short follow-up time in a proportion of patients, this study describes the short-term treatment efficacy of MuSK-MG patients in our region. As the results of our study showed that the most severe clinical manifestations in a considerable proportion of MuSK-MG occurred many years after onset, a multi-center study with long-term follow-up is needed in the future. However, the outcomes of tacrolimus treatment in MuSK-MG patients seem to suggest that tacrolimus may not be a bad therapeutic option for those patients, at least in the short term.

In summary, compared with the previous reports, our results provide a distinct understanding of MuSK-MG in terms of age, clinical presentations, and treatment strategy. Patients with MuSK-MG in northeast China have a modestly later onset age and a proportion of patients may have a mild form of the disease with delayed disease progression. We confirmed the existence of a rare ocular MuSK-MG phenotype, a high proportion coexisting with other ADs, and a good response to steroids combined with tacrolimus for our MuSK-MG series.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study cannot be shared at this time as the data also forms part of an ongoing study. Requests to access the datasets should be directed to 763595105@qq.com.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethical committee of Jilin University First Hospital. The patients/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

ZZ and YG acquired the clinical data, reviewed the literature, and drafted the article. HD designed the study, supervised the initial drafting, and critically revised the article. JH, ML, and MS analyzed the clinical data and critically revised the article. All authors approved of the final version of the article.

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Favorable Effects of Tacrolimus Monotherapy on Myasthenia Gravis Patients

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Background and Purpose: Tacrolimus (TAC) has been proven to be a rapid-acting, steroid-sparing agent for myasthenia gravis (MG) therapy. However, evidence related to the effectiveness of TAC alone is rare. Therefore, this study was performed to investigate the effect of TAC monotherapy in MG patients.

Methods: Forty-four MG patients who received TAC monotherapy were retrospectively analyzed. A mixed effect model was used to analyze improvements in MG-specific activities of daily living scale (MG-ADL), quantitative MG score (QMG) and MG-ADL subscores. Kaplan-Meier analysis was used to estimate the cumulative probability of minimal manifestations (MM) or better. Adverse events (AEs) were recorded for safety analyses.

Results: Of the patients receiving TAC monotherapy, MG-ADL scores were remarkably improved at 3, 6 and 12 months compared with scores at baseline (mean difference and 95% CIs: −3.29 [−4.94, −1.64], −3.97 [−5.67, −2.27], and −4.67 [−6.48, −2.85], respectively). QMG scores significantly decreased at 6 and 12 months, with mean differences and 95% CIs of −4.67(−6.88, −2.45) and −5.77 (−7.55, −4.00), respectively. Estimated median period to achieve "MM or better" was 5.0 (95% CIs, 2.8, 7.2) months. Ocular MG (OMG) and generalized MG (GMG) showed similar therapeutic effects in cumulative probabilities of "MM or better" (P-value = 0.764). A better response was observed in MG-ADL subscores for ptosis and bulbar symptoms. AEs occurred in 37.5% of patients and were generally mild and reversible.

Conclusions: TAC monotherapy is a promising option to rapidly alleviate all symptoms of MG, especially for ptosis and bulbar symptoms.

Keywords: myasthenia gravis, tacrolimus, monotherapy, clinical effectiveness, adverse events

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INTRODUCTION

Immunosuppressive therapies are a major part of standard myasthenia gravis (MG) therapy. It is usually necessary for patients to maintain immunosuppression agent for many years, even for their whole life (1, 2). Corticosteroids are the most common immunosuppressive agents for MG patients. However, long-term therapy of corticosteroids is usually limited by severe adverse events (AEs), mood symptoms and cosmetic problems (3–5). In recent decades, non-steroidal

immunosuppressive agents, including azathioprine (AZA), methotrexate (MTX), mycophenolate mofetil (MMF), and cyclosporine A (CsA), have been successfully used in conjunction with corticosteroids to reduce the dose and side effects of corticosteroids (6). However, the relatively slow onset of action of AZA, MTX and MMF, and the severe nephrotoxicity of CsA limits their use in the treatment of MG (7–10).

Tacrolimus (TAC) acts in a manner similar to CsA and exhibits a similar effect to CsA at concentrations 100 times lower (11). Moreover, it has a lower incidence of nephrotoxicity than CsA (12). Several studies have proven that TAC co-administered with corticosteroids can rapidly improve myasthenic symptoms subjectively within 1 month and objectively at 2∼3 months (13–15). TAC is recommended to treat MG in different countries and the international MG treatment guidelines (2, 16, 17). Interestingly, four ocular MG (OMG) patients were reported to respond well to TAC alone (18). Meanwhile, CsA monotherapy could significantly improve MG symptoms in RCTs (19, 20). Therefore, we speculate that TAC monotherapy would be a promising option for patients who refuse or cannot tolerate corticosteroids and other immunosuppressive agents.

Herein, we investigated the effectiveness and safety of TAC monotherapy in MG patients. We also analyzed the differential sensitivity to TAC for MG symptoms.

MATERIALS AND METHODS

Participants

Data were collected from the Xuanwu Hospital Capital Medical University Myasthenia Gravis Trial Database from July 01, 2017, to June 01, 2020. A total of 185 MG patients who received TAC therapy were identified. The following exclusion criteria were applied. Patients who had a QMG or MG-ADL score of 0 at baseline were excluded. Any patient who received intravenous immunoglobulin or plasma exchange within 4 weeks prior to the start of TAC administration was excluded. Patients who had undergone thymectomy or received other immunosuppressive agents within 24 weeks prior to the start of TAC administration were excluded. Concurrent use of cholinesterase inhibitors within the usual dosage range was permitted. MG was diagnosed based on a combination of clinical pattern of myasthenia weakness (muscle weakness and fatigability), laboratory tests (positive for anti-AChR or anti-MuSK antibodies), neurophysiological tests (repetitive nerve stimulation) and positive response to acetylcholinesterase therapy.

Finally, we identified 48 patients with TAC monotherapy, for whom corticosteroids or other immunosuppressive agents were contraindicated or refused due to potential AEs. Four patients were excluded from effectiveness analyses who withdrew TAC within 1-month for AEs or patient decisions. Therefore, effectiveness analyses were evaluated in 44 patients, for whom TAC monotherapy was maintained for more than 1 month (**Figure 1**). Among them, 17 patients with OMG started TAC monotherapy due to inadequate or no response to pyridostigmine (2). The most common problem that limited the use of corticosteroids was contraindications of corticosteroids (28/48), including osteoporosis, poorly controlled hypertension, and diabetes. The remaining 20 patients refused corticosteroids or other immunosuppressive agents due to potential adverse events. The study was approved by the Ethics Committee of Xuanwu Hospital, Capital Medical University, China (No. 2017084) and was in accordance with the principles of the Declaration of Helsinki. Each participant provided written informed consent for participation.

Tacrolimus Therapeutic Regimens

All patients were treated with an initial daily dose of TAC 2 mg. TAC was increased or Wuzhi tablets were added to achieve adequate TAC concentrations (4.8–10 ng/ml) (21). The maintenance dose ranged from 2 to 4 mg and was adjusted depending on clinical efficacy, side effects, and TAC concentrations. TAC concentrations were commonly measured in whole blood by microparticle enzyme immunoassay.

Outcome Measurement and Follow-Up

The following characteristics of the patients were collected: sex, age at onset, disease course, serum antibodies, thymus histopathology, Myasthenia Gravis Foundation of America (MGFA) clinical classification, MG-specific activities of daily living scale (MG-ADL), quantitative MG score (QMG), and MGFA post-intervention status (PIS). The MG-ADL scores were assessed by follow-up at 1, 3, 6, and 12 months. A quantitative assessment of muscle strength with QMG scores provided further objective criteria for clinical improvement at 6-month and 12-month face-to-face clinic visits. In terms of PIS, the classification of "MM or better" included minimal manifestations, pharmacological remission, and complete stable remission. Clinical assessment was performed at a fixed interval from the last administration of cholinesterase inhibitor to avoid modification by pyridostigmine.

The therapeutic effects were first evaluated by the improvement in MG-ADL and QMG scores and the probability of achieving "MM or better" during the follow-up period. Next, the probability of achieving "MM or better" was compared between subgroups of OMG and generalized MG (GMG). Then, the differential sensitivity to TAC monotherapy for MG symptoms was investigated by the improvement in MG-ADL subsocres. Safety was assessed by the incidence and severity of AEs and the incidence of AEs leading to drug withdrawal. Renal and liver function injury were assessed by elevation above the upper normal limit of blood urea nitrogen (BUN)/serum creatinine (sCr) and liver enzymes.

Statistical Analysis

Data of categorical variables were represented as frequencies (%). Data of continuous variables were represented as mean ± standard deviation (SD) or median (interquartile range [IQR]).

Abbreviations: MG, myasthenia gravis; OMG, ocular myasthenia gravis; GMG, generalized myasthenia gravis; CsA, cyclosporine A; TAC, tacrolimus; AEs, adverse events; MGFA, Myasthenia Gravis Foundation of America; MG-ADL, MG-specific activities of daily living scale; QMG, quantitative MG score; MGFA-PIS, MGFA post-intervention status; IQR, interquartile range; 95%CI, 95 % confidence intervals; CIPS, calcineurin-inhibitor induced pain syndrome.

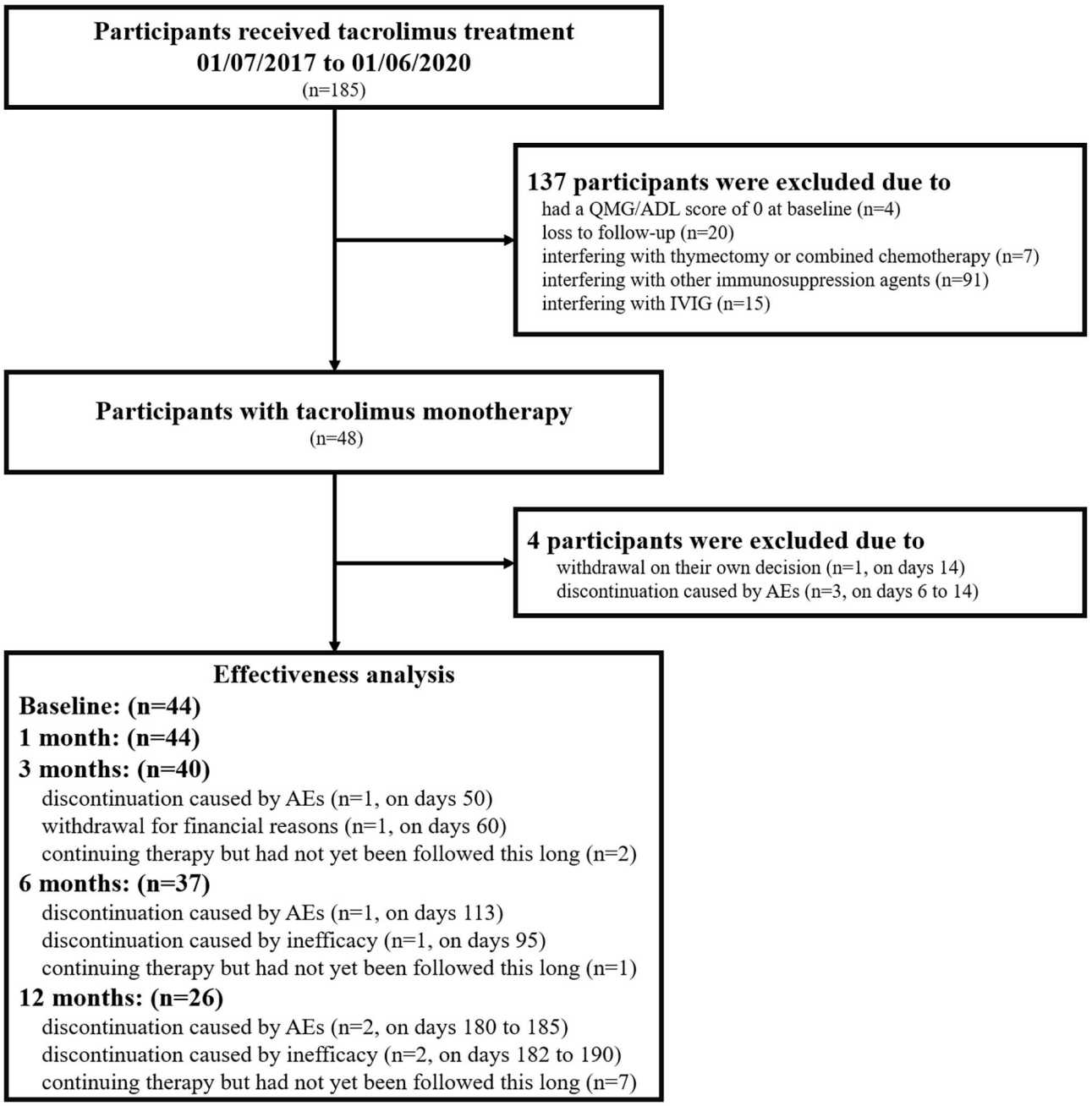

FIGURE 1 | Flowchart of the participants included in the current study. *n*, number of patients; QMG, quantitative myasthenia gravis score; MG-ADL, myasthenia gravis activities of daily living; IVIG, intravenous immunoglobulin; AEs, adverse events.

The linear mixed model for repeated measure analysis was used to compare MG-ADL, QMG scores or MG-ADL subscores among different follow-up periods. The estimates and 95% confidence intervals (CIs) of coefficients in the model were presented to describe changes in MG-ADL, QMG scores or MG-ADL subscores. The model included subjects as a random effect and follow-up period as a fixed effect. Kaplan-Meier analysis was used to estimate the cumulative probability of PIS status "MM or better." A log-rank test was used for the comparison of treatment outcome between subgroups of OMG and GMG. For patients who withdrew TAC due to ineffectiveness, the last collected data were used as records to be estimated during the remaining periods. No data were included in the effectiveness analyses at a particular time for patients who withdrew TAC due to AEs, failed to have a visit or had not yet been followed this long. Statistical analysis was performed using SPSS version 22.0.

TABLE 1 | Demographic features of 44 MG patients with tacrolimus monotherapy.

Demographic characteristics	
Age at onset (years) (mean ± SD)	54.1 ± 17.2
Early-onset MG[a]/Late-onset MG (*n*)	13/31
Sex(male/female) (*n*)	28/16
MGFA classification (*n*)	
I	17
II	20
III-IV	7
Serum antibodies positive (*n*)	
Anti-AchR	38
Anti-MuSK	1
Dual seronegative	5
Abnormal thymus gland (*n*)	
Thymoma	3
Thymic hyperplasia	4
Thymic cyst	2
Disease course (month) (median [IQR])	10.0 (3.2–20.8)
Tacrolimus dose (mg/day) (Mean ± SD)	2.70 ± 0.62
Tacrolimus trough concentration (ng/ml) (Mean ± SD)	6.21 ± 2.59

[a]*Onset age was younger than 50 years old.*
SD, standard deviation; IQR, interquartile range; n, number of patients; MG, myasthenia gravis; MGFA classification, Myasthenia Gravis Foundation of America clinical classification; AChR, acetylcholine receptor; MuSK, muscle-specific tyrosine kinase.

TABLE 2 | Scores of MG-ADL and QMG during follow-up periods.

	Scores, median (IQR)				
	Baseline	**1 month**	**3 months**	**6 months**	**12 months**
MG-ADL					
Overall	6 (4–7.75)	4 (2.75–6)	3 (1–4)	2 (0–3.25)	1 (0–2)
In OMG	5 (3–6)	3(2–5)	2.5 (1–3.75)	1.5 (0–3)	1 (0–3)
In GMG	7 (5–10)	5(4–7)	3 (1–4)	2 (0–4)	0.5 (0–2)
QMG					
Overall	7 (6–10)	-	-	3 (1–6)	3(1–3)
In OMG	6 (4–6)	-	-	1 (0.5–3.5)	1(0–3.75)
In GMG	10 (7–11.5)	-	-	5.5 (1–6.25)	3(1–3)

IQR, interquartile range; MG-ADL, myasthenia gravis-specific activities of daily living scale; QMG, quantitative myasthenia gravis score; MG-ADL, myasthenia gravis activities of daily living; OMG, ocular myasthenia gravis; GMG, generalized myasthenia gravis.

The Bonferroni correction was used to decrease the risk of a type I error by adjusting the probability *P*-values. An adjusted value of $P < 0.05$ was considered statistically significant.

RESULTS

Patient Characteristics

The demographic characteristics of 44 MG patients (28 males, 16 females) are summarized in **Table 1**. The mean age at onset was 54.1 ± 17.2 years old, and 29.5% (13/44) of patients had early onset (younger than 50 years old). Thymectomy was performed in eight patients (18.1%). Thirty-eight patients were anti-AChR antibody positive, and one patient was anti-MuSK antibody positive. According to the MGFA classification, there were 17 OMG and 27 GMG patients. The mean dose of TAC was 2.70 ± 0.62 mg, and the mean TAC trough concentration was 6.21 ± 2.59 ng/ml.

Therapeutic Effects of TAC Monotherapy

The median values of MG-ADL and QMG scores during follow-up periods are shown in **Table 2**. The linear mixed model for repeated measurements showed significant improvements in both MG-ADL and QMG scores. Scores of MG-ADL at 3, 6, and 12 months were significantly lower than those at baseline, with mean differences and 95% CIs of −3.29 ([−4.94]–[−1.64]), −3.97 ([−5.67]–[−2.27]), and −4.67 ([−6.48]–[−2.85]), respectively (**Figure 2A**). Scores of QMG at 6 and 12 months significantly decreased compared with scores at baseline, in which the mean difference was −4.67 (95% CIs, [−6.88]–[−2.45]) and −5.77 (95% CIs, [−7.55]–[−4.00]), respectively (**Figure 2B**). Remarkable improvements in MG-ADL and QMG scores were observed in both OMG and GMG patients, and were beginning at 3 months in MG-ADL scores (**Figures 2C,D**).

Up to 84.1% of patients reported subjective improvement within the first month. The cumulative probability of achieving "MM or better" in all patients showed a gradual increase and rose to 73.2% at 12 months (**Figure 3A**). The estimated median period to achieve "MM or better" was 5.0 (95% CIs, 2.8–7.2) months. Kaplan–Meier analysis showed no differences in cumulative probabilities between OMG and GMG (*P*-value = 0.764) (**Figure 3B**). The estimated median periods were 5.1 (95% CIs, 3.9–6.2) and 3.0 (95% CIs, 1.0–5.1) months for OMG and GMG, respectively.

The treatment was discontinued in 1 patient on days 95 and 2 patients on days 182 to 190 because it was judged to be ineffective by the treating physician (**Figure 1**). No patients experienced exacerbation or developed a crisis during the follow-up period.

Differential Sensitivity of TAC Monotherapy for MG Symptoms

A total of 68.2% of patients (30/44) reported subjective improvement of ptosis within the first month. The median values of MG-ADL subscores during follow-up periods are shown in **Table 3**. The linear mixed model showed significant improvements in MG-ADL subcores for ptosis and chewing from the first month and for talking and swallowing from 3 months (compared with baseline, *P*-value < 0.05) (**Figure 4**). For the symptoms of diplopia and limbs, subscores of MG-ADL showed no significant improvement until 6 months and 12 months, respectively. For breathing difficulty, no significant improvement was observed during the follow-up periods (**Figure 4**). More importantly, all symptoms in all patients were improved or stable, and there was no exacerbation within 12 months. An interesting finding in this study was that 25 patients (56.8%) complained of photophobia or light sensitivity along with the onset of MG symptoms. Among them, 44% of patients (11/25) achieved clinical improvement and 16% of patients (4/25) got remission

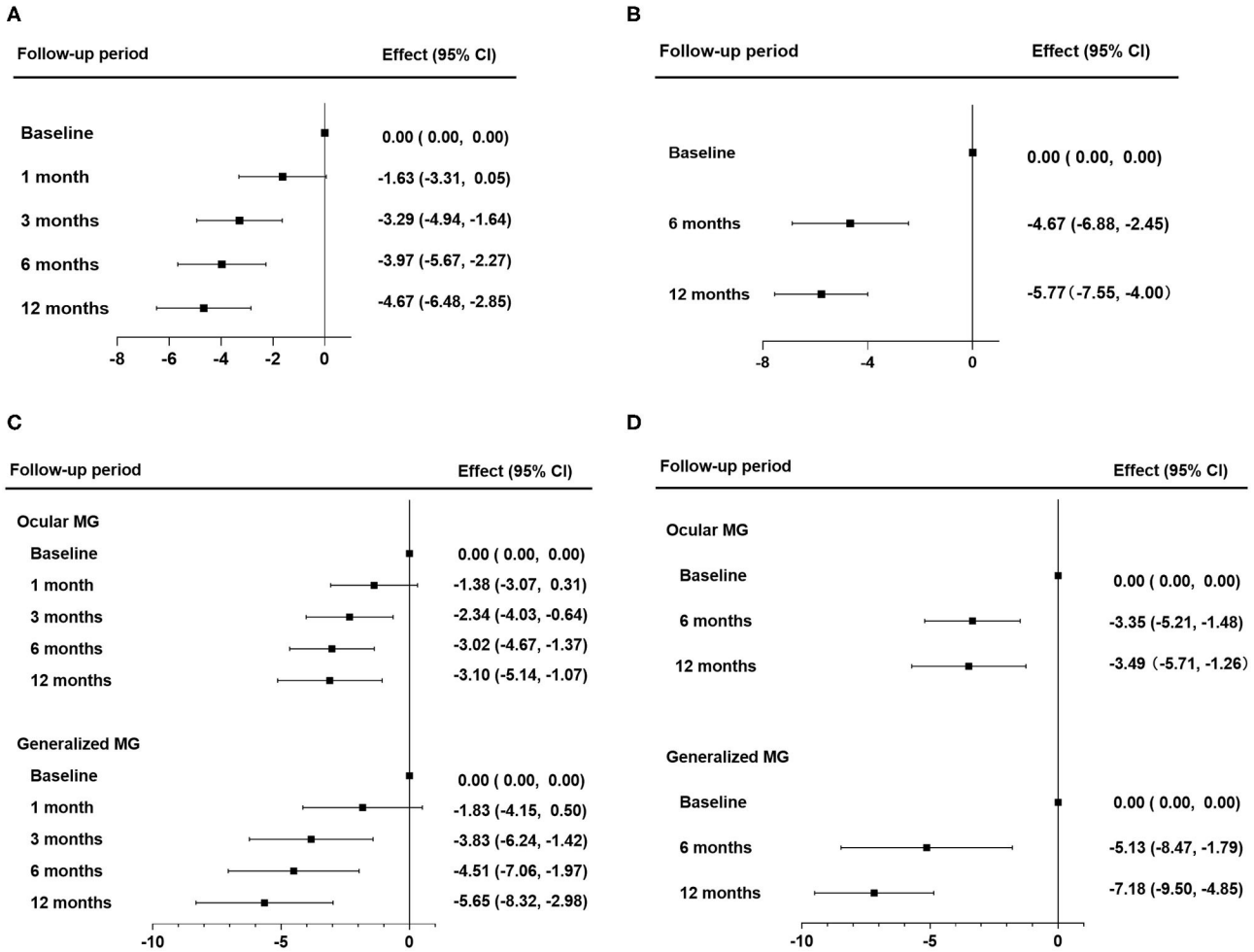

FIGURE 2 | Therapeutic effects of tacrolimus monotherapy. **(A,B)** Therapeutic effects in all patients were evaluated by changes in MG-ADL scores **(A)** and by changes in QMG scores **(B)**. **(C,D)** Therapeutic effects in subgroups of OMG and GMG were evaluated by changes in MG-ADL **(C)** and by changes in QMG scores **(D)**. The effect was the mean difference of MG-ADL or QMG scores during the follow-up periods. Statistical analysis was performed by linear mixed model for repeated measurements with Bonferroni correction. QMG, quantitative myasthenia gravis score; MG-ADL, myasthenia gravis activities of daily living; OMG, ocular myasthenia gravis; GMG, generalized myasthenia gravis.

in photophobia after 6 months treatment. Almost all of patients with photophobia had symptom of ptosis (24/25). Five of these patients (20.8%) reported clinical improvement of photophobia earlier than or along with the improvement of ptosis.

Safety of TAC Monotherapy

The incidence of AEs was 37.5% (18/48), and all observed AEs are shown in **Supplementary Table 1**. The most frequent AEs were BUN/sCr elevation (4/48, 8.33%) and liver enzyme elevation (3/48, 6.25%). Two patients experienced joint pain, which has never been reported in MG patients with TAC therapy before. Four patients who had BUN/sCr elevation were relatively old (the median age was 68.5 years old). All of them had a long history of hypertension (range 5–22 years), and two of them had diabetes mellitus. AEs that led to therapy discontinuation occurred in seven patients on days 6 to 185 (**Figure 1**). All AEs were mild and resolved after dose reduction or drug withdrawal. No deaths occurred during the follow-up period.

DISCUSSION

Our study showed that TAC monotherapy could significantly reduce MG-ADL and QMG scores, and induce remission of various symptoms in both OMG and GMG patients. A better prognosis and a more rapid onset of action were observed for ptosis and bulbar symptoms (talking, chewing, and swallowing) than for diplopia, dyspnea, and limb weakness. In addition, side effects were mild and reversible during the 12-month follow-up period. These results demonstrated that TAC monotherapy was an effective and safe therapeutic option for MG patients who refuse or have contraindications to corticosteroids and other immunosuppressive agents.

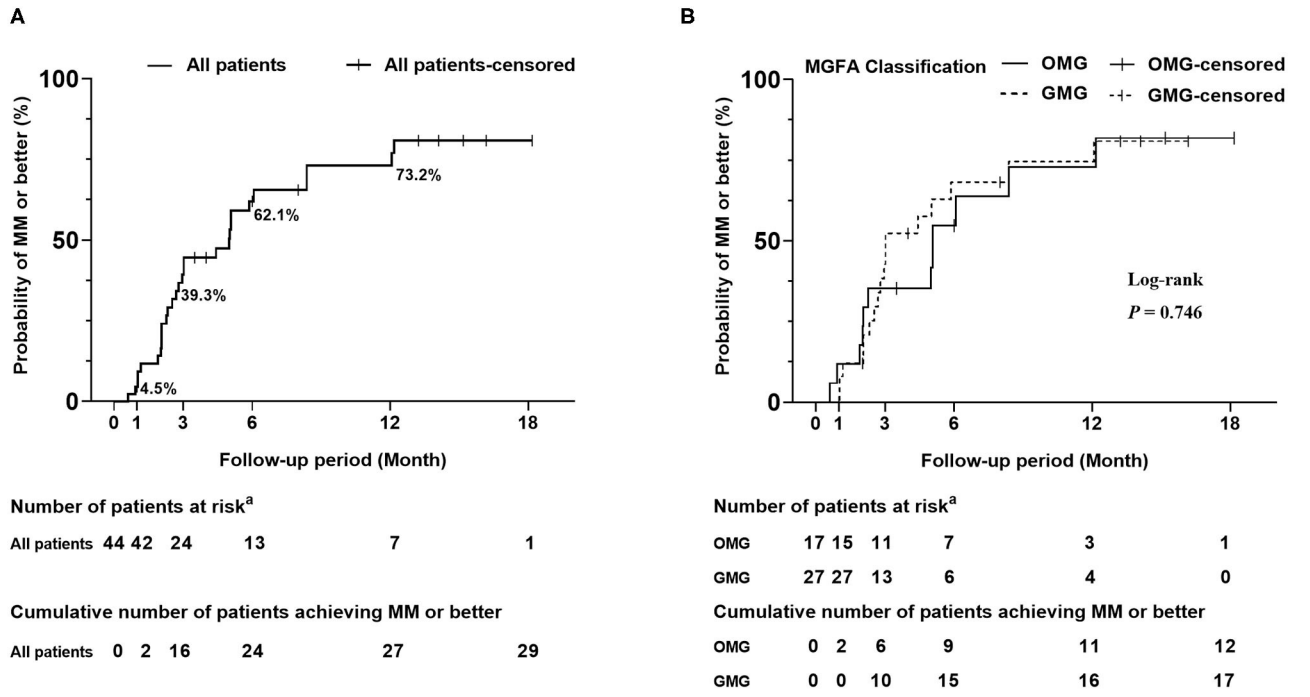

FIGURE 3 | Kaplan-Meier curves for the cumulative probability of achieving "MM or better". **(A)** Cumulative probability of achieving "MM or better" in all patients. The cumulative probability of achieving "MM or better" was 4.5, 39.3, and 62.1% at 1 month, 3 months, and 6 months, respectively. Finally, it rose to 73.2% at 12 months. **(B)** Cumulative probability of achieving "MM or better" in subgroups of OMG and GMG. A log-rank test was used for the comparison of treatment outcome between subgroups of OMG and GMG. Crossing marks indicated censoring time; "MM or better" included MGFA post-intervention status of minimal manifestations, pharmacological remission and complete stable remission. [a]Number of patients who were still in the study and did not achieve "MM or better" at the end of specified time. OMG, ocular myasthenia gravis; GMG, generalized myasthenia gravis.

TABLE 3 | MG-ADL subscores for MG symptoms during the follow-up periods.

Items of MG-ADL (*n*)	Subscores, median (IQR)				
	Baseline	1 month	3 months	6 months	12 months
Ptosis (39)	3 (2–3)	2 (1–3)	1 (0–2)	0 (0–2)	0 (0–1)
Diplopia (30)	2 (1–3)	1 (1–2.5)	1 (0–2.5)	1 (0–2)	0 (0–1.5)
Talking (15)	1 (1–2)	1 (1–1)	0 (0–1)	0 (0–1)	0 (0–0.5)
Chewing (20)	1 (1–2)	1 (0–1)	1 (0–1)	0 (0–1)	0 (0–0)
Swallowing (18)	1 (1–2)	1 (0–1.25)	0 (0–1)	0 (0–0)	0 (0–0)
Breathing (8)	1 (1–1.75)	1 (0.25–1)	1 (0–2)	1 (0–1)	0 (0–0.5)
Limbs[a] (7)	2 (2–4)	2 (1–4)	1.5 (0–2.25)	1.5 (0–2.25)	0 (0–1.25)

[a]*Limbs included upper and lower limbs.*

MG-ADL, myasthenia gravis-specific activities of daily living scale; n, number of patients with the symptom at baseline.

TAC monotherapy had favorable effects on the outcome of most patients in our study. TAC has been proven to be a steroid-sparing immunosuppressive agent (22) and showed significant improvement in MG-ADL and QMG scores in combination with corticosteroids (13, 23). In our study, among 44 patients who took TAC alone, 84.1% reported improvement within the first month. The MG-ADL scores improved significantly at 3 months in both the OMG and GMG. Six months later, more than 65% of individuals achieved "MM or better." Monotherapy with TAC in MG patients showed similar rapid onset of action, clinical effectiveness and stable remission to those in whom TAC was co-administered with corticosteroids (13–15). Our results suggested that TAC monotherapy is a reasonable option for patients who refuse or have contraindications for corticosteroids or other immunosuppressive agents.

The sensitivity to TAC monotherapy for various symptoms was differential. Wakata reported that the symptoms of lower extremities, grip strength, ptosis and swallowing responded well to TAC in combination with corticosteroids (24). The percentage improvement in the non-facial composite (arm and leg outstretch times, grip, forced vital capacity, and head lift) was less than that for vision and facial (ptosis, diplopia, swallowing, and chewing) with CsA alone or CsA co-administered with corticosteroids (19, 20). In our study, a better response and a more rapid onset of action were observed for ptosis and bulbar symptoms (talking, chewing, swallowing) than for diplopia, dyspnea, and limb weakness. Therefore, TAC monotherapy could be recommended as the initial treatment for GMG patients with bulbar symptoms and for OMG patients with ptosis who had an inadequate response to pyridostigmine. The clinical response for diplopia was insufficient until 6 months. Accordingly, for young patients

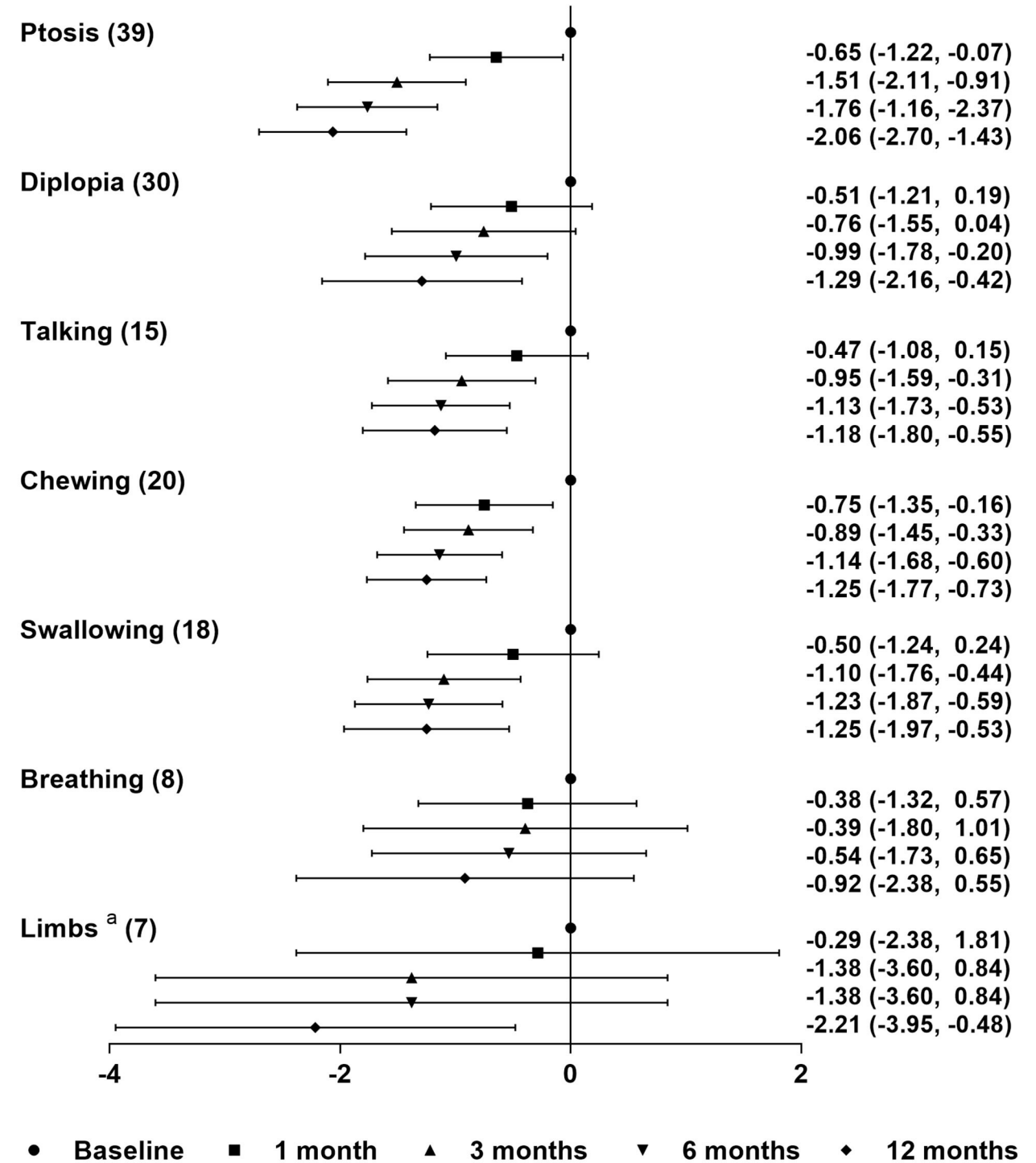

FIGURE 4 | Differential sensitivity of tacrolimus monotherapy for MG symptoms. The effect was the mean difference of MG-ADL subscores in each symptom during the follow-up periods. Statistical analysis was performed by linear mixed model for repeated measurements with Bonferroni correction. [a]Limbs included upper and lower limbs. MG-ADL, myasthenia gravis-specific activities of daily living scale; n, number of patients with the symptom at baseline.

with diplopia who refuse corticosteroids due to potential AEs or cosmetic problems, doctors are suggested to persuade them to accept corticosteroids or in conjunction with TAC to achieve adequate responses quickly. Several studies demonstrated that early stages of disease, thymoma and adequate TAC concentration were associated with responsiveness to TAC co-administered with corticosteroids (21, 25). Differential sensitivity for MG symptoms might be a potential predictive factor for effectiveness of TAC monotherapy. Therefore, multiple regression analysis with a larger sample size is required for further precision medicine.

The most interesting finding in our study was that 56.8% (25/44) of patients complained of photophobia or light sensitivity. They had to wear sunglasses, indicating that the intraocular muscles were involved in these patients. Slow pupillary responses and fatigability to light in MG have been reported in several studies (26, 27). The symptoms of photophobia or light sensitivity in our patients could be caused by dysfunction of the pupillary light reflex in bright sunlight. The pupil cycle time technique of Miller and Thompson (26), as a measurement of pupillary responses to light, would be needed to demonstrate the correlation between pupillary dysfunction and the symptom of light sensitivity in further studies. Two smooth muscles, the sphincter muscle, and the dilator muscle, comprise the iris and determine pupil dynamics (28). Lu reported pupillary dysfunction in MG not only involved the sphincter muscle but also the dilator muscle (27). Muscarinic and nicotinic AChR receptors are localized in the sphincter muscle and the dilator muscle, respectively (29). Most cases of MG were positive in antibodies against nicotinic AChR receptors. Antibodies against muscarinic AChR receptors have been detected in MG patients (30). Damaged muscarinic AChR receptors in sphincter muscle and nicotinic AChR receptors in dilator muscle might be the pathogenic mechanism of photophobia. However, the pathogenicity remains to be established. After MG treatments, symptoms of photophobia improved in 60% of patients, which indicated that photophobia in these patients was related to MG. Other common conditions associated photophobia, including ophthalmological pathology, neurological disorders, psychiatric disorders or drugs, should be considered in the remaining 40% of patients who had a poor prognosis of photophobia (31). Most of them only had mild weakness of the orbicular eye muscle instead of difficulties in closing eyes. Typical symptoms of conjunctival infections were absence during the follow-up visits. Unfortunately, none of these patients had records of periodic ophthalmological evaluation. It is unclear whether they had visual refraction defects or cataract in our retrospective study. Therefore, periodic ophthalmological evaluations should be concerned in patients with poor prognosis of photophobia. The improvement of ptosis may concern with photophobia in part of patients. However, the alleviation of photophobia in most patients was delayed than that of ptosis. Thus, ptosis and photophobia should be evaluated as two separated symptoms.

Monotherapy with TAC in 48 MG patients showed a favorable safety profile. Surprisingly, a different profile of AEs was found, contrasting with previous studies, in which TAC was usually co-administered with corticosteroids (22, 23, 32, 33). There were no reports of AEs linked to nephrotoxicity in most previous MG studies with TAC (22, 23, 33). BUN or sCr elevation was found in 8.33% of patients in our study, partly because physicians paid close attention to renal damage of TAC and reported AEs once the elevation was above the upper normal limits. It has been reported that TAC nephrotoxicity originates from a strong vasoconstrictive effect. A high TAC whole-blood concentration and a history of hypertension could increase the risk of renal damage in organ transplantation patients (34). All four patients with mildly elevated BUN/sCr in our study were of old age and had a long history of hypertension, although TAC whole-blood concentrations were much lower than those in organ transplantation. Thus, old MG patients with long-term hypertension need more frequent monitoring of subclinical renal damage and renal function when using TAC. Another AE, reversible joint pain, was found for the first time in two patients in our study. Joint pain is a rare but debilitating AE in organ transplantation patients with TAC treatment. It gradually receded after TAC withdrawal. This phenomenon was named calcineurin inhibitor-induced pain syndrome (CIPS) (35). The reason was suspected as a calcineurin inhibitor-induced vascular disturbance of bone perfusion and permeability causing bone marrow edema (35). The final diagnosis of CIPS requires further examinations, including bone mineral density tests, bone scintigraphy and magnetic resonance imaging (36).

There are several limitations in this study. First, the retrospective nature, a relatively small sample size and no controlled group for comparison weakened the evidences of our results. First, the limited sample size of patients who had dyspnea or limb weakness provided inadequate evidence of sensitivity for these symptoms. Second, loss to follow-up was reported in eight patients in effectiveness analysis. Among them, the therapeutic effects were estimated according to the last records in four patients who withdrew TAC due to ineffectiveness. It was plausible that attrition bias associated with loss to follow-up drove either overestimation or underestimation of therapeutic effect. Last, most patients evaluated had either OMG or mild GMG (MGFA clinical classification type II). There were only seven patients classified as MGFA type III or IV. More patients with MGFA type III or IV or high-quality RCT trials will be needed to further prove the efficacy of TAC monotherapy in MG patients.

CONCLUSIONS

TAC monotherapy is a fast-acting and efficacious regimen to alleviate all common symptoms of both OMG and GMG, especially for patients with ptosis and bulbar symptoms. Close monitoring of renal function is essential for older patients with hypertension.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Xuanwu Hospital, Capital Medical University, China (No. 2017084). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

ZF contributed with drafting and revising the manuscript, study concept and design, acquisition of data, and statistical analysis. ZL contributed with drafting and revising the manuscript, study concept and design. FS, XZ, LL, and SS contributed with acquisition of data. YL, LD, MW, and MX contributed with revising the manuscript and interpretation of the data. YD contributed with drafting and revising the manuscript, study concept and design, and interpretation of the data. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2020.594152/full#supplementary-material

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Concomitant Immunosuppressive Therapy Use in Eculizumab-Treated Adults With Generalized Myasthenia Gravis During the REGAIN Open-Label Extension Study

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Introduction: Chronic, broad-spectrum immunosuppressive therapy (IST) can be associated with side effects in many people with generalized myasthenia gravis (gMG), and treatment guidelines recommend that the IST dose be tapered once patients achieve a stable treatment response. We therefore examined IST use in eculizumab-treated patients with refractory gMG.

Methods: The REGAIN open-label extension (OLE) enrolled 117 adults with refractory anti-acetylcholine receptor antibody-positive gMG who had completed the 6-month, randomized, double-blind, placebo-controlled REGAIN study of eculizumab. Eligible patients had received ≥2 ISTs for ≥1 year or ≥1 IST with intravenous immunoglobulin or plasma exchange ≥4 times in 1 year, without symptom control. During REGAIN, changes in concomitant MG therapies were not permitted; during the OLE, they were permitted at the investigators' discretion. Participants received eculizumab 1,200 mg every 2 weeks for up to 4 years; concomitant prednisone and related corticosteroids (PRED), azathioprine (AZA), and mycophenolate mofetil (MMF) use was recorded. Changes in MG Activities of Daily Living and Quantitative MG total scores, MG exacerbations, and adverse events were also recorded.

Results: At last OLE assessment, 88.0% (103/117) of participants were using ≥1 IST vs. 98.3% (115/117) at OLE baseline. During the OLE, 76.9% (90/117) of patients experienced a total of 719 IST changes. Almost half of participants [48.7% (57/117)] stopped or decreased ≥1 IST owing to MG symptom improvement, representing 38.9% (280/719) of all changes. In patients who decreased and/or stopped ≥1 IST, mean daily doses of PRED, AZA, and MMF decreased between OLE baseline and last assessment by 60.8% [standard deviation (SD), 28.07; $P < 0.0001$], 89.1% (SD, 25.77; $P < 0.0001$), and 56.0% (SD, 32.99; $P < 0.0001$), respectively. Improved clinical outcomes were observed with eculizumab regardless of IST changes during the OLE, and eculizumab's safety profile was similar in patients who used PRED, AZA, and MMF.

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Conclusions: Use of ISTs by patients with previously refractory gMG decreased during eculizumab treatment in the REGAIN OLE. Clinical improvements with eculizumab were maintained by patients in all groups, including those who decreased and/or stopped concomitant ISTs.

Trial registration: www.clinicaltrials.gov: NCT01997229, NCT02301624.

Keywords: eculizumab, myasthenia gravis, immunosuppressive therapy, refractory, acetylcholine receptor

INTRODUCTION

Generalized myasthenia gravis (gMG) is an immune-mediated neuromuscular disorder characterized by fatigable muscle weakness. Most patients with MG (70–88%) have autoantibodies to the acetylcholine receptor (AChR) (1–6). These autoantibodies cause accelerated degradation of AChRs and activation of the complement cascade, resulting in structural damage to the neuromuscular junction (7–13) and thus impairing neuromuscular transmission and muscle strength (7–9, 14).

The current guidelines for the management of MG recommend that immunosuppressive therapy (IST), including prednisone and related corticosteroids (PRED), should be used in all patients with MG who have not met treatment goals after an adequate trial of pyridostigmine (15). Most patients with MG receive long-term IST (16), but use of these treatments is often associated with unwanted effects (especially in the case of prolonged use) and may adversely impact quality of life (17, 18). It is recommended that the IST dose be tapered once patients experience a stable response (15), an approach that is favored by both clinicians and patients (19). Approximately 10–15% of patients with MG have refractory disease on the basis that they do not respond adequately to ISTs, they require maintenance intravenous immunoglobulin or plasma exchange treatment, or they experience intolerable adverse events associated with ISTs (15, 20, 21).

REGAIN, a 6-month, phase 3, randomized, placebo-controlled study, and its open-label extension (OLE) demonstrated rapid and sustained efficacy and tolerability of the terminal complement inhibitor eculizumab in adults with refractory anti-AChR antibody-positive (AChR+) gMG (14, 22). During REGAIN, participants continued their previously established IST regimens with no changes permitted (14). However, adjustment of concomitant MG therapies, such as ISTs (including PRED), was permitted at the discretion of the investigator during the OLE (22).

This analysis examined changes in the use of ISTs, including PRED, azathioprine (AZA), and mycophenolate mofetil (MMF), in patients receiving eculizumab during the OLE of the REGAIN study. Clinical outcomes in subgroups of patients defined by changes in IST use were also examined.

Abbreviations: AChR, acetylcholine receptor; AChR+, anti-acetylcholine receptor antibody-positive; AZA, azathioprine; gMG, generalized myasthenia gravis; IST, immunosuppressive therapy; MG, myasthenia gravis; MG-ADL, MG Activities of Daily Living; MMF, mycophenolate mofetil; OLE, open-label extension; PRED, prednisone and related corticosteroids; QMG, quantitative MG; SD, standard deviation.

METHODS

Study Design and Participants

REGAIN was a 6-month (26-week), phase 3, randomized, placebo-controlled clinical trial that assessed the efficacy and tolerability of eculizumab in patients aged 18 years or older with refractory AChR+ gMG (www.clinicaltrials.gov: NCT01997229) (14). In addition to confirmed AChR+ gMG and an MG Activities of Daily Living (MG-ADL) total score $\geq$6, eligible patients had to have refractory disease: they must have received two or more ISTs for at least 1 year or one or more ISTs with intravenous immunoglobulin or plasma exchange treatment at least four times in 1 year, without symptom control. Full eligibility and exclusion criteria have been published previously (14). Within 2 weeks of completing REGAIN, participants could enroll in the OLE (www.clinicaltrials.gov: NCT02301624) to receive open-label eculizumab for up to a maximum of 4 years. All participants were required to have received *Neisseria meningitidis* vaccinations at least 2 weeks before the first dose of study drug (or prophylactic antibiotics until 2 weeks after vaccination) and to be revaccinated according to local guidelines. The first patient was enrolled in the REGAIN study on April 30, 2014, and the extension study was completed in January 2019 (22). Data reported here are from the final follow-up of all patients in the OLE.

All patients provided written, informed consent. Independent ethics committees or institutional review boards provided written approval for the study protocols and all amendments.

Treatment

Eculizumab and placebo administration during REGAIN and the OLE have been described previously (14, 22). During the OLE, participants received open-label eculizumab 1,200 mg every 2 weeks for up to 4 years after a 4-week blinded induction period.

During REGAIN, patients who had previously received ISTs were required to maintain their pre-study IST type, dose, and schedule (14). During the OLE, modifications to IST type, dose, and schedule were permitted at the investigators' discretion, although they were not required by the study protocol (22).

Concomitant ISTs included, but were not limited to, PRED (prednisone, prednisolone, methylprednisolone, methylprednisolone sodium succinate, and meprednisone), AZA, and MMF.

Assessments

Use and dosages of concomitant ISTs, including PRED, AZA, and MMF, were reported at all scheduled visits, and at unscheduled visits for MG crises/exacerbations, from OLE baseline (day 1) to last assessment (study discontinuation or end of study). PRED doses and dose changes were expressed as prednisone equivalents: doses of methylprednisolone sodium succinate, methylprednisolone, and meprednisone were converted to prednisone equivalents by multiplying them by 1.25. The numbers of change events during the OLE, as well as the nature of and reasons for these changes, were reported for PRED, AZA, and MMF. Due to the small numbers of participants using other ISTs during REGAIN and the OLE (cyclosporine, tacrolimus, methotrexate, and cyclophosphamide; $n \leq 17$ for each), change events for these ISTs during the OLE were not included in this analysis.

Changes in MG-ADL and Quantitative MG (QMG) mean total scores from eculizumab start (REGAIN baseline for eculizumab/eculizumab group and OLE baseline for placebo/eculizumab group) to last assessment were evaluated for all patients. The proportions of patients with exacerbations that did and did not meet the protocol definition [MG crisis, significant symptomatic worsening (an increase either by 2 points or to a score of 3 for any single MG-ADL item, excluding ocular items), or health in jeopardy without rescue therapy according to the treating physician] or who required rescue therapy were also recorded over this time frame.

Adverse events were recorded and coded by preferred term using the Medical Dictionary for Regulatory Activities Version 20.1.

Statistical Analysis

Analyses were largely based on descriptive data. Mean percentage changes in IST doses were analyzed using one-sample *t*-tests, and median percentage changes in IST doses were analyzed using the Wilcoxon signed-rank test.

RESULTS

Patient Disposition and Characteristics at Open-Label Extension Baseline

Nearly all (117/118) patients who completed REGAIN continued into the OLE (eculizumab/eculizumab, 56; placebo/eculizumab, 61) and were included in the efficacy and safety analyses (22). Of these, 87 patients completed the OLE (eculizumab/eculizumab, 43; placebo/eculizumab, 44), and 30 discontinued (eculizumab/eculizumab, 13; placebo/eculizumab, 17) owing to adverse events ($n = 7$), death ($n = 3$), patient withdrawal ($n = 13$), withdrawal by physician ($n = 6$), or "other" reason ($n = 1$) (23). The median duration of eculizumab treatment from OLE baseline to last assessment was 972.0 days (range, 1–1,372 days).

Patient demographics at OLE baseline have been reported previously (22). Baseline demographics were similar between groups of patients using PRED, AZA, or MMF at baseline, except that smaller proportions of Asian than white patients used AZA or MMF (**Table 1**). Also, patients receiving AZA at baseline showed a tendency to have previously used fewer ISTs than those receiving PRED or MMF, while prior plasma exchange was more common in those receiving MMF than in those receiving PRED or AZA (**Table 1**).

TABLE 1 | Demographic and disease characteristics by concomitant immunosuppressive therapy (IST) in patients using prednisone and related corticosteroids (PRED), azathioprine (AZA), or mycophenolate mofetil (MMF) at open-label extension (OLE) baseline.

Characteristic	PRED $n = 90$	AZA $n = 39$	MMF $n = 30$	All patients $N = 117$
Age[a], mean (SD), years	48.3 (16.52)	46.7 (16.87)	49.4 (17.52)	47.4 (16.70)
Sex, *n* (%)				
Male	34 (37.8)	14 (35.9)	9 (30.0)	38 (32.5)
Female	56 (62.2)	25 (64.1)	21 (70.0)	79 (67.5)
Race, *n* (%)				
Asian	18 (20.0)	2 (5.1)	1 (3.3)	19 (16.2)
Black/African American	0 (0.0)	0 (0.0)	1 (3.3)	2 (1.7)
White	67 (74.4)	34 (87.2)	26 (86.7)	88 (75.2)
Unknown	1 (1.1)	1 (2.6)	0 (0.0)	1 (0.9)
Multiple	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.9)
Other	4 (4.4)	2 (5.1)	2 (6.7)	6 (5.1)
Region, *n* (%)				
North America	31 (34.4)	16 (41.0)	11 (36.7)	43 (36.8)
South America	9 (10.0)	7 (17.9)	1 (3.3)	12 (10.3)
Europe	34 (37.8)	15 (38.5)	18 (60.0)	46 (39.3)
Asia Pacific	5 (5.6)	1 (2.6)	0 (0.0)	5 (4.3)
Japan	11 (12.2)	0 (0.0)	0 (0.0)	11 (9.4)
Duration of MG[b], mean (SD), years	9.87 (8.13)	9.67 (8.17)	10.31 (8.59)	10.21 (8.23)
MGFA classification by randomization stratification at screening, *n* (%)				
IIa or IIIa	47 (52.2)	21 (53.8)	18 (60.0)	58 (49.6)
IVa	3 (3.3)	3 (7.7)	1 (3.3)	6 (5.1)
IIb or IIIb	36 (40.0)	13 (33.3)	10 (33.3)	47 (40.2)
IVb	4 (4.4)	2 (5.1)	1 (3.3)	6 (5.1)
Prior IST use, *n* (%)				
2 ISTs only	42 (46.7)	32 (82.1)	9 (30.0)	52 (44.4)
3 ISTs only	27 (30.0)	5 (12.8)	14 (46.7)	39 (33.3)
≥4 ISTs	20 (22.2)	2 (5.1)	6 (20.0)	24 (20.5)
Prior IVIg use, *n* (%)	70 (77.8)	29 (74.4)	24 (80.0)	92 (78.6)
Prior plasma exchange use, *n* (%)	39 (43.3)	17 (43.6)	17 (56.7)	57 (48.7)

A total of 90 patients were using PRED at OLE baseline, 39 were using AZA, and 30 were using MMF. PRED, AZA, and MMF could be used as monotherapies, in combination with each other, or with other ISTs.

[a] *On day 1 of OLE.*

[b] *Time from MG diagnosis to first dose date in the OLE.*

IVIg, intravenous immunoglobulin; MG, myasthenia gravis; MGFA, Myasthenia Gravis Foundation of America; SD, standard deviation.

Changes in Immunosuppressive Therapy Use During the Open-Label Extension

At OLE baseline, 98.3% (115/117) of patients were using at least one IST. During the OLE, 99.1% (116/117) of patients used at least one IST at some point. At the last assessment, 88.0% (103/117) of patients were using at least one IST.

TABLE 2 | Changes in immunosuppressive therapy (IST) use during the open-label extension.

Type of/reason for change in IST use	IST change events, *n* (%)[a] *N* = 719	Patients, *n* (%)[b] *N* = 117
Change in IST use	719 (100.0)	90 (76.9)
Decrease in daily dose of 1 IST	386 (53.7)	74 (63.2)
Increase in daily dose of 1 IST	158 (22.0)	51 (43.6)
Stoppage of an existing IST	101 (14.0)	51 (43.6)
Start a new IST	69 (9.6)	37 (31.6)
Increase in daily dose of >1 IST	3 (0.4)	3 (2.6)
Decrease in daily dose of >1 IST	2 (0.3)	2 (1.7)
Stoppage or decrease in dose of ≥1 IST	489 (68.0)	84 (71.8)
MG symptoms improved	280 (38.9)	57 (48.7)
MG symptoms worsened	3 (0.4)	3 (2.6)
New indication other than MG for IST use	13 (1.8)	7 (6.0)
Side effects—intolerant to existing IST	49 (6.8)	18 (15.4)
Other[c]	144 (20.0)	47 (40.2)
Start or increase in dose of ≥1 IST[d]	230 (32.0)	71 (60.7)
MG symptoms worsened	111 (15.4)	44 (37.6)
New indication other than MG for IST use	19 (2.6)	12 (10.3)
Other[c]	98 (13.6)	43 (36.8)

[a] *Percentage of all changes in IST use.*
[b] *Any given patient may have experienced multiple changes under the same or different categories.*
[c] *"Other" category largely used to describe temporary dosing/treatment changes in response to conditions such as asthma/chronic obstructive pulmonary disease, conjunctivitis, and urinary tract infections, or to support surgery.*
[d] *Two reasons in this category were problematic (not applicable values).*
MG, myasthenia gravis.

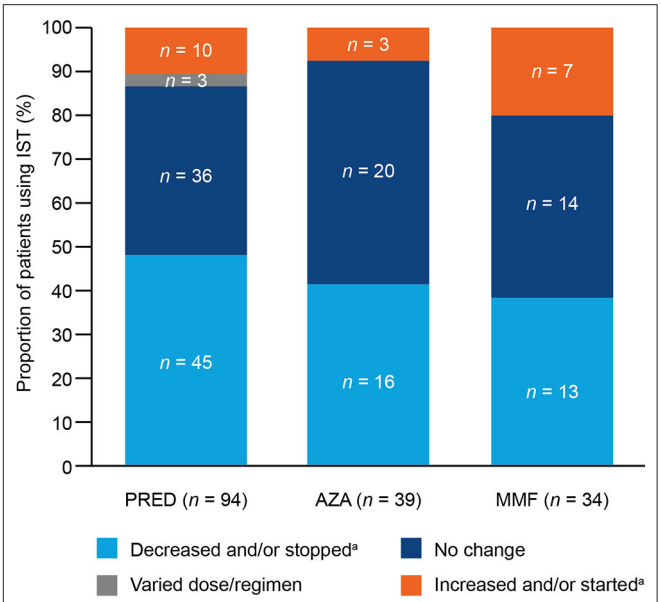

FIGURE 1 | Overall changes to immunosuppressive therapy (IST) at any time during the open-label extension (OLE) in patients using prednisone and related corticosteroids (PRED), azathioprine (AZA), or mycophenolate mofetil (MMF). During the OLE, 94 patients used PRED (90 patients at OLE baseline), 39 patients used AZA (39 patients at OLE baseline), and 34 patients used MMF (30 patients at OLE baseline). [a]Increases/decreases were calculated from the starting dose and the dose at the last assessment.

Over three quarters [76.9% (90/117)] of patients experienced a total of 719 change events in their IST regimens during the OLE. Stopping an IST or decreasing the dose of at least one IST [68.0% (489/719) of changes] was more common than starting an IST or increasing the dose of at least one IST [32.0% (230/719) of changes; **Table 2**]. In total, 71.8% (84/117) of participants stopped or decreased the daily dose of at least one IST at some point during the OLE. The most common reason for stopping or decreasing the dose was MG symptom improvement [48.7% (57/117) of participants on 280/489 occasions]. Conversely, among the 71/117 (60.7%) participants who started an IST or increased the daily dose of at least one IST at some point during the OLE, the most common reason for the change was MG symptom worsening [37.6% (44/117) of participants on 111/230 occasions; **Table 2**].

Changes in Prednisone and Related Corticosteroids, Azathioprine, and Mycophenolate Mofetil Use Between Open-Label Extension Baseline and Last Assessment

At OLE baseline, PRED were the most commonly used ISTs [being used by 90/117 (76.9%) patients], followed by AZA [39/117 (33.3%) patients], and then MMF [30/117 (25.6%) patients]. PRED and AZA were used in combination by 30/117 (25.6%) patients, and PRED and MMF were used in combination by 22/117 (18.8%) patients. Compared with those at OLE baseline, there were fewer patients at the last assessment using PRED [76/117 (65.0%)], AZA [26/117 (22.2%)], PRED and AZA combined [16/117 (13.7%)], and PRED and MMF combined [18/117 (15.4%)], with little change in the number using MMF [31/117 (26.5%)]. Most patients who used PRED, AZA, or MMF during the study had decreased and/or stopped or had no changes in their doses at the last assessment (**Figure 1**).

Of the patients who used PRED during the OLE, almost half [47.9% (45/94)] decreased and/or stopped their PRED dose, and 38.3% (36/94) had no change in dose (**Figure 1**). At the last assessment, 10 of the 90 (11.1%) patients who had been using PRED at baseline were no longer using PRED (**Figure 2A**). Of the patients using PRED at baseline, the proportion using more than 10 mg of PRED per day decreased from 58.9% (53/90) at baseline to 38.9% (35/90) at the last assessment. Significant reductions in mean daily PRED dose from OLE baseline to last assessment were observed among all patients [16.4% (SD, 72.12; $P = 0.0335$); 3.8 mg/day (SD, 10.89; $P = 0.0014$); **Figure 2A**] and among those who decreased and/or stopped PRED [60.8% (SD, 28.07; $P < 0.0001$); 10.5 mg/day (SD, 7.49; $P < 0.0001$)].

Of the patients who used AZA during the OLE, 41.0% (16/39) decreased and/or stopped their AZA dose, and 51.3% (20/39) had no change in dose (**Figure 1**). One third [13/39 (33.3%)] of patients who had been using AZA at baseline were no longer using AZA at the last assessment (**Figure 2B**). Of the patients using AZA at baseline, the proportion using more than 150 mg of AZA per day decreased from 46.2% (18/39) at baseline to

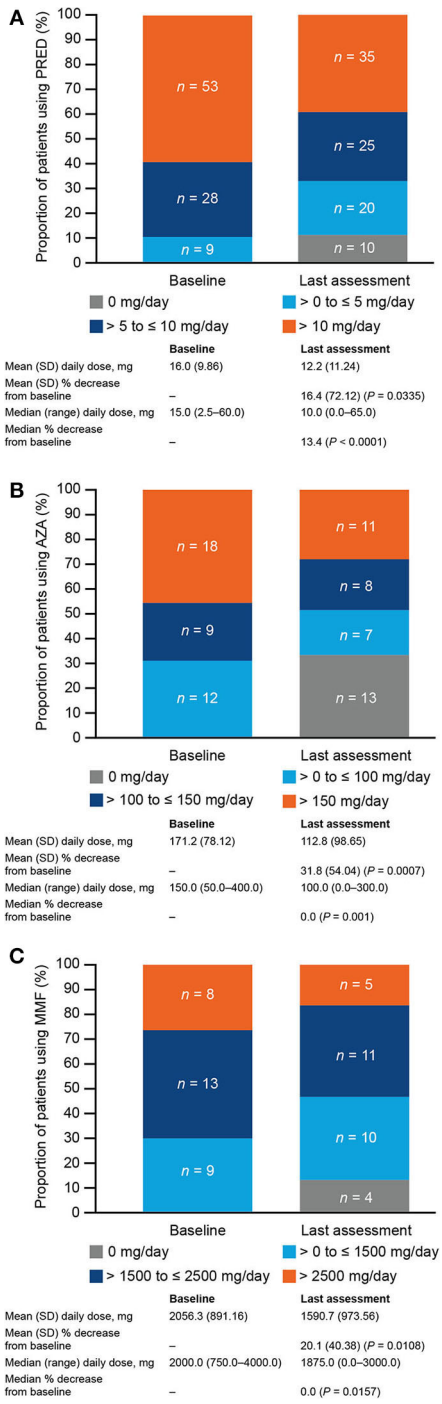

FIGURE 2 | Immunosuppressive therapy (IST) doses at open-label extension (OLE) baseline and last assessment in patients using **(A)** prednisone and related corticosteroids (PRED; $n = 90$), **(B)** azathioprine (AZA; $n = 39$), or **(C)** mycophenolate mofetil (MMF; $n = 30$) at OLE baseline. A total of 90 patients were using PRED at OLE baseline, 39 were using AZA, and 30 were using MMF. The distribution of IST doses at OLE baseline and last assessment, mean and median daily doses at OLE baseline and last assessment, and mean and median dose reductions from OLE baseline to last assessment are shown for these patients. *P*-values for mean percentage changes were calculated using the one-sample *t*-test; *P*-values for median percentage changes were calculated using the Wilcoxon signed-rank test. SD, standard deviation.

28.2% (11/39) at the last assessment. Significant reductions in mean daily AZA dose from OLE baseline to last assessment were observed among all patients [31.8% (SD, 54.04; $P = 0.0007$); 58.3 mg/day (SD, 96.88; $P = 0.0006$); **Figure 2B**] and among those who decreased and/or stopped AZA [89.1% (SD, 25.77; $P < 0.0001$); 151.6 mg/day (SD, 87.31; $P < 0.0001$)].

Of the patients who used MMF during the OLE, 38.2% (13/34) decreased and/or stopped their MMF dose, and 41.2% (14/34) had no change in dose (**Figure 1**). Four of the 30 (13.3%) patients who had been using MMF at OLE baseline were no longer using MMF at the last assessment (**Figure 2C**). Of the patients using MMF at baseline, the proportion using more than 2,500 mg of MMF per day decreased from 26.7% (8/30) at baseline to 16.7% (5/30) at the last assessment. Significant reductions in mean daily MMF dose from OLE baseline to last assessment were observed among all patients [20.1% (SD, 40.38; $P = 0.0108$); 465.7 mg/day (SD, 872.48; $P = 0.0067$); **Figure 2C**] and among those who decreased and/or stopped MMF [56.0% (SD, 32.99; $P < 0.0001$); 1,228.5 mg/day (SD, 788.58; $P < 0.0001$)].

Clinical Outcomes in Patients With Changes in Immunosuppressive Therapy Between Eculizumab Start and Open-Label Extension Last Assessment

Mean MG-ADL and QMG total scores decreased between the start of eculizumab therapy (REGAIN baseline for eculizumab/eculizumab group and OLE baseline for placebo/eculizumab group) and OLE last assessment across most groups of patients defined by change in IST use during the OLE (**Table 3**). The only exceptions to this were for changes in mean QMG total score in patients who increased and/or started PRED (0.2 increase; $n = 10$) and for changes in mean MG-ADL total score in those who increased and/or started AZA (0.3 increase; $n = 3$).

During the OLE, 27 patients experienced protocol-defined exacerbations, and five patients experienced exacerbations that did not meet the protocol definition. Of these patients, nine experienced protocol-defined exacerbations after decreasing and/or stopping PRED, AZA, or MMF; and two experienced exacerbations that did not meet the protocol definition following a decrease in PRED dose. Additionally, one patient experienced a protocol-defined exacerbation following a decrease in cyclosporine dose.

Safety

Eculizumab was well tolerated during both REGAIN and its OLE (14, 22). The most common adverse events that occurred in patients receiving eculizumab during these two studies were headache (44.4%) and nasopharyngitis (38.5%) (23). One meningococcal infection, which was resolved with antibiotic treatment, was reported during the OLE; three deaths occurred in patients with important comorbidities (22). The proportions of patients who experienced treatment-emergent adverse events were similar between groups of patients who used PRED, AZA, and MMF during the OLE (**Table 4**).

TABLE 3 | Mean changes in Myasthenia Gravis Activities of Daily Living (MG-ADL) and Quantitative MG (QMG) total scores from eculizumab start[a] to open-label extension (OLE) last assessment by immunosuppressive therapy (IST) and nature of dose change during the OLE.

IST	Nature of dose change during the OLE	MG-ADL total score		QMG total score	
		Eculizumab baseline[a], mean (SD)	Change to OLE last assessment, mean (SD)	Eculizumab baseline[a], mean (SD)	Change to OLE last assessment, mean (SD)
Total	All patients, $N = 117$	8.9 (3.60)	−3.6 (4.14)	15.9 (5.69)	−4.1 (5.81)
PRED[b]	Patients who decreased and/or stopped, $n = 45$	8.6 (3.57)	−4.7 (3.92)	16.0 (5.49)	−5.6 (5.15)
	Patients with no change, $n = 36$	8.9 (3.59)	−2.3 (4.11)	15.4 (5.49)	−1.5 (4.98)
	Patients who increased and/or started, $n = 10$	8.7 (3.16)	−0.7 (4.16)	14.9 (5.65)	0.2 (4.92)
AZA[b]	Patients who decreased and/or stopped, $n = 16$	7.6 (3.08)	−3.4 (4.00)	15.3 (4.61)	−3.8 (6.76)
	Patients with no change, $n = 20$	9.0 (4.15)	−4.7 (3.77)	16.0 (6.35)	−5.1 (5.26)
	Patients who increased and/or started, $n = 3$	7.7 (6.66)	0.3 (2.31)	13.3 (8.02)	−2.7 (4.93)
MMF[b]	Patients who decreased and/or stopped, $n = 13$	8.5 (2.57)	−5.1 (3.64)	14.1 (2.36)	−4.9 (3.52)
	Patients with no change, $n = 14$	9.0 (2.75)	−2.5 (3.37)	16.1 (4.92)	−1.6 (4.01)
	Patients who increased and/or started, $n = 7$	12.6 (2.23)	−5.3 (3.55)	20.0 (6.81)	−7.9 (5.24)

[a] *Eculizumab baseline is REGAIN baseline for the eculizumab/eculizumab group and OLE baseline for the placebo/eculizumab group.*
[b] *PRED, AZA, and MMF could be used as monotherapies, in combination with each other, or with other ISTs.*
AZA, azathioprine; MMF, mycophenolate mofetil; PRED, prednisone and related corticosteroids; SD, standard deviation.

TABLE 4 | Treatment-emergent adverse events by concomitant immunosuppressive therapy during the open-label extension (OLE) in patients using prednisone and related corticosteroids (PRED), azathioprine (AZA), or mycophenolate mofetil (MMF) at OLE baseline.

	PRED $n = 90$	AZA $n = 39$	MMF $n = 30$	All patients $N = 117$
Total patients with events, *n* (%)	87 (96.7)	38 (97.4)	29 (96.7)	114 (97.4)
Treatment-emergent adverse events occurring in >15% of all patients, *n* (%)				
Headache	34 (37.8)	17 (43.6)	9 (30.0)	47 (40.2)
Nasopharyngitis	34 (37.8)	8 (20.5)	9 (30.0)	42 (35.9)
Diarrhea	17 (18.9)	14 (35.9)	7 (23.3)	29 (24.8)
MG[a]	23 (25.6)	10 (25.6)	12 (40.0)	29 (24.8)
Upper respiratory tract infection	21 (23.3)	15 (38.5)	6 (20.0)	28 (23.9)
Arthralgia	18 (20.0)	10 (25.6)	5 (16.7)	23 (19.7)
Cough	13 (14.4)	8 (20.5)	5 (16.7)	22 (18.8)
Influenza	15 (16.7)	9 (23.1)	6 (20.0)	22 (18.8)
Nausea	16 (17.8)	9 (23.1)	6 (20.0)	22 (18.8)
Urinary tract infection	9 (10.0)	4 (10.3)	6 (20.0)	19 (16.2)
Pain in extremity	14 (15.6)	8 (20.5)	3 (10.0)	18 (15.4)

The number (%) of patients who experienced treatment-emergent adverse events is provided for each group. A total of 90 patients were using PRED at OLE baseline, 39 were using AZA, and 30 were using MMF.
[a] *Worsening (increased frequency and/or intensity) of a pre-existing condition, including MG, is considered to be an adverse event.*
MG, myasthenia gravis.

DISCUSSION

Immunosuppressive Therapy Use During the REGAIN Open-Label Extension

There is a burden associated with the use of ISTs in gMG (17, 18); it is therefore important to better understand what impact the addition of the complement inhibitor eculizumab may have on their use. In the REGAIN OLE, physician-directed changes in IST use were permitted. The present analysis demonstrates that concomitant IST use decreased during the OLE, over a median of 32 months. More patients stopped or decreased the dose of an IST than started or increased the dose of an IST; by the last assessment, over 10% of patients had stopped using concomitant ISTs. From baseline to the last assessment, the mean daily doses of PRED, AZA, and MMF were significantly reduced.

During eculizumab treatment in REGAIN and its OLE, improvements were observed in both patient-reported activities of daily living (MG-ADL total score) and physician-evaluated neurologic function related to MG (QMG total score) (14, 22). In this analysis, we sought to examine whether IST use impacts this response. We found that improvements were experienced regardless of the type of IST used or the nature of IST dosing change during the OLE. These improvements are notable considering that, at the start of REGAIN, all study participants had treatment-refractory gMG.

The long-term safety and tolerability of eculizumab have been reported from over 10 years of clinical use in atypical hemolytic

uremic syndrome and paroxysmal nocturnal hemoglobinuria (24–28). Safety data from the final analysis of the OLE were consistent with interim OLE safety data and the known safety profile of eculizumab in gMG (14, 22). Incidences of adverse events during the OLE were similar between patients who used PRED, AZA, and MMF.

Limitations of the Study

All adjustments of concomitant ISTs during the OLE were at the discretion of study investigators, with no protocol-specified procedures for IST tapering. IST changes during the OLE were therefore likely to have been heterogeneous, making it difficult to draw conclusions about whether clinical outcomes associated with them reflect patients' changing needs or the way in which the changes were implemented.

This analysis was largely based on descriptive data, and although the analysis of changes in IST use was pre-specified for the OLE, reasons for IST changes, clinical outcomes in patients with IST changes, and safety outcomes by IST type were analyzed *post-hoc*. The open-label design of the extension study is also a limitation of this analysis; however, selection or reporting biases are unlikely because over 90% of REGAIN participants continued into the OLE.

Future Directions

This study presents data on concomitant IST use with eculizumab in a strictly defined population of patients with refractory AChR+ gMG who were recruited for REGAIN. Data on the real-world use of eculizumab and concomitant ISTs in AChR+ gMG are limited (29); however, recruitment has recently been initiated for the Registry of Participants with Generalized Myasthenia Gravis Treated with C5 Inhibition Therapies (www.clinicaltrials.gov: NCT04202341). This registry will collect data for up to 5 years from ∼500 participants who are receiving, or who have received, Alexion C5 inhibition therapy, including details of concomitant IST use. These data will provide further information about IST use in patients with gMG treated with eculizumab and will extend the results of this study to reflect real-world clinical practice.

CONCLUSIONS

IST use by patients with previously refractory gMG decreased in the REGAIN OLE. Importantly, clinical improvements were experienced by patients across IST change categories, including those who decreased and/or stopped IST. These results suggest that individuals with gMG who are treated with eculizumab may be able to successfully reduce their IST use, which is likely to ease their treatment-related burden. Individualized tapering of ISTs, guided by best practice standards, should therefore be considered in patients who respond to eculizumab.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available. Alexion will consider requests for disclosure of clinical study participant-level data provided that participant privacy is assured through methods like data de-identification, pseudonymization, or anonymization (as required by applicable law), and if such disclosure was included in the relevant study informed consent form or similar documentation. Qualified academic investigators may request participant-level clinical data and supporting documents (statistical analysis plan and protocol) pertaining to Alexion-sponsored studies. Further details regarding data availability and instructions for requesting information are available in the Alexion Clinical Trials Disclosure and Transparency Policy at http://alexion.com/research-development. Requests to access the datasets should be directed to https://alexion.com/contact-alexion/medical-information.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by ethics committees (see **Appendix 2** in **Supplementary Material**). The patients/participants provided their written informed consent to participate in these studies.

AUTHOR CONTRIBUTIONS

RN, JH, MY, and FO'B contributed to the concept and design of the study. FO'B performed the statistical analyses. RN, SM, SB, FO'B, MY, and JH contributed to data acquisition, analysis or interpretation, drafting and critical revision, and final approval of the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2020.556104/full#supplementary-material

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REVIEW
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Myasthenia Gravis: Autoantibody Specificities and Their Role in MG Management

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Myasthenia gravis (MG) is the most common autoimmune disorder affecting the neuromuscular junction, characterized by skeletal muscle weakness and fatigability. It is caused by autoantibodies targeting proteins of the neuromuscular junction; ∼85% of MG patients have autoantibodies against the muscle acetylcholine receptor (AChR-MG), whereas about 5% of MG patients have autoantibodies against the muscle specific kinase (MuSK-MG). In the remaining about 10% of patients no autoantibodies can be found with the classical diagnostics for AChR and MuSK antibodies (seronegative MG, SN-MG). Since serological tests are relatively easy and non-invasive for disease diagnosis, the improvement of methods for the detection of known autoantibodies or the discovery of novel autoantibody specificities to diminish SN-MG and to facilitate differential diagnosis of similar diseases, is crucial. Radioimmunoprecipitation assays (RIPA) are the staple for MG antibody detection, but over the past years, using cell-based assays (CBAs) or improved highly sensitive RIPAs, it has been possible to detect autoantibodies in previously SN-MG patients. This led to the identification of more patients with antibodies to the classical antigens AChR and MuSK and to the third MG autoantigen, the low-density lipoprotein receptor-related protein 4 (LRP4), while antibodies against other extracellular or intracellular targets, such as agrin, $K_V1.4$ potassium channels, collagen Q, titin, the ryanodine receptor and cortactin have been found in some MG patients. Since the autoantigen targeted determines in part the clinical manifestations, prognosis and response to treatment, serological tests are not only indispensable for initial diagnosis, but also for monitoring treatment efficacy. Importantly, knowing the autoantibody profile of MG patients could allow for more efficient personalized therapeutic approaches. Significant progress has been made over the past years toward the development of antigen-specific therapies, targeting only the specific immune cells or autoantibodies involved in the autoimmune response. In this review, we will present the progress made toward the development of novel sensitive autoantibody detection assays, the identification of new MG autoantigens, and the implications for improved antigen-specific therapeutics. These advancements increase our understanding of MG pathology and improve patient quality of life by providing faster, more accurate diagnosis and better disease management.

Keywords: autoimmunity, myasthenia gravis, autoantibody, diagnosis, therapy, acetylcholine receptor, MuSK, LRP4

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INTRODUCTION

Myasthenia gravis (MG) is an antibody-mediated autoimmune disorder affecting skeletal muscles, characterized by fluctuating muscle weakness and abnormal fatigability. MG is caused by autoantibodies, which target proteins of the neuromuscular junction (NMJ), damaging the postsynaptic muscle membrane and impairing signal transmission from motor neurons to the muscle (1, 2).

The organization of the NMJ is crucial for effective signal transmission (3, 4). Acetylcholine receptors (AChRs) on the muscle cell membrane bind acetylcholine released from the axon terminals and open to allow inflow of ions, which leads to depolarization of the membrane. The AChRs are clustered at the NMJ resulting in a localized high density of receptors, which ensures the efficiency of signal transmission. Neural agrin, released from nerve terminals, binds to low-density lipoprotein receptor-related protein 4 (LRP4) on the muscle membrane, activating it to form complexes with muscle specific kinase (MuSK). This results in the phosphorylation and activation of MuSK, which in turn leads to rapsyn-mediated AChR clustering at the NMJ (**Figure 1**).

MG is heterogeneous in terms of symptom presentation, as well as pathophysiology, since different proteins of the NMJ can be targeted (6, 7). MG symptoms usually manifest initially at the ocular muscles and in ∼15% of patients they remain localized, commonly referred to as ocular MG (OMG). In the majority of patients, however, the symptoms progress within a couple of years to other skeletal muscles leading to generalized MG (GMG). In terms of age of onset the disease presents with two peaks of incidence: the first well below the age of 50, termed early-onset MG (EOMG), more commonly affecting women and the second above the age of 50 (late-onset MG, LOMG) more common among men.

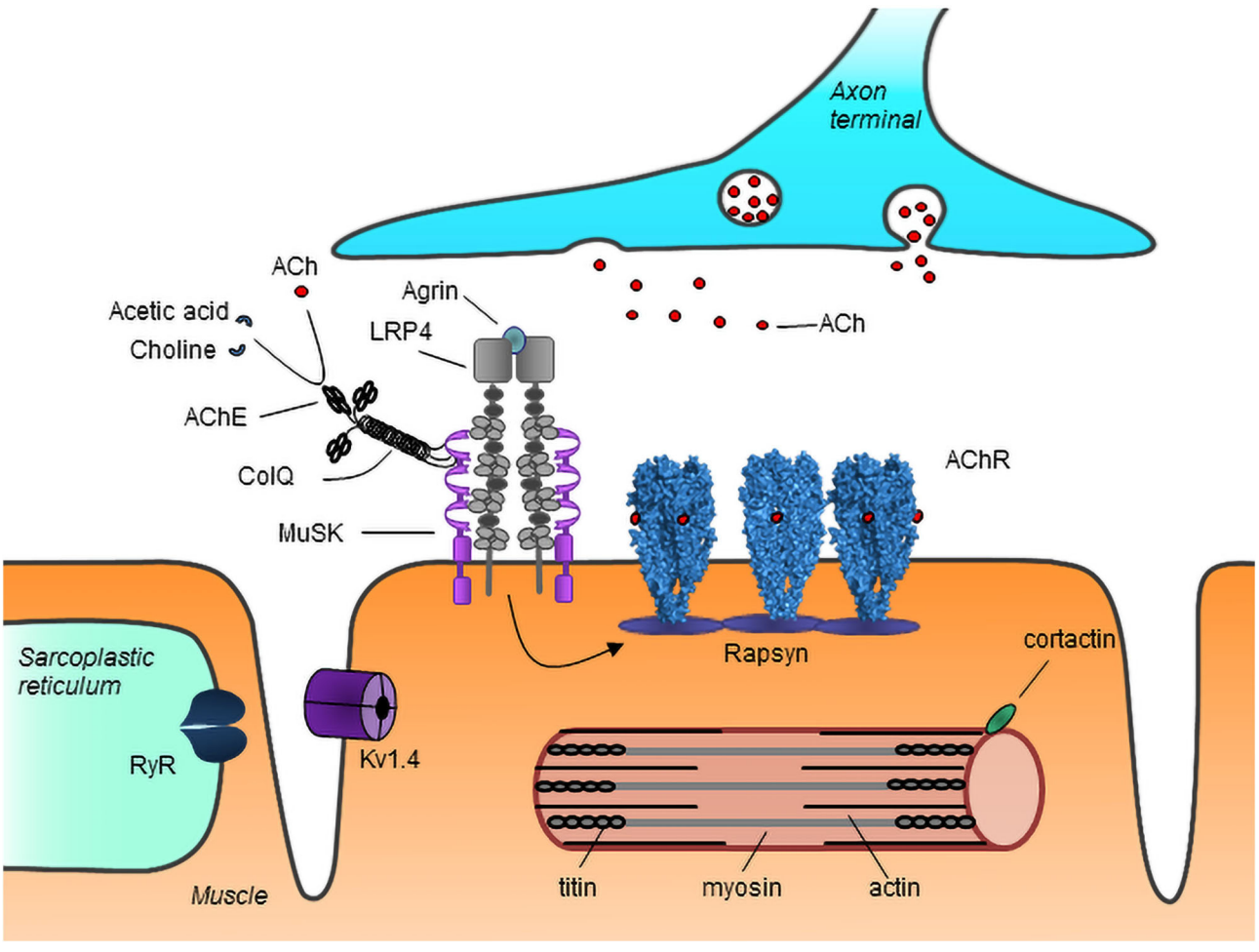

FIGURE 1 | Schematic representation of major neuromuscular junction and myotube proteins targeted by autoantibodies in MG. Neuron-released agrin activates LRP4 on the muscle membrane, initiating a pathway which via MuSK leads to rapsyn-dependent AChR clustering at the NMJ. Acetylcholine (ACh) released from the nerve terminal binds to AChRs causing their activation. ACh is broken down by AChE into choline and acetate, thus terminating its action. AChR, acetylcholine receptor; MuSK, muscle specific kinase; LRP4, low-density lipoprotein receptor-related protein 4; RyR, ryanodine receptor; ColQ, collagen Q; AChE, acetylcholinesterase; Kv1.4, voltage gated potassium channel 1.4. Image from Lazaridis and Tzartos (5).

Although MG is a relatively rare disease with a prevalence of 150–300 per million population and an incidence of ∼10 per million per year (8), it is considered a model antibody-mediated autoimmune disorder, due to the extensive characterization of the main autoantibodies and target antigens. In more detail, in most patients (∼85%) the autoantibodies target the muscle AChR. In ∼6% of patients the autoantibodies target MuSK, while autoantibodies targeting LRP4 are found in about 2% of MG patients. The pathogenicity of autoantibodies has been demonstrated by the improvement of patients' symptoms following plasmapheresis and by the onset of passive transfer experimental autoimmune MG (EAMG) when they are injected into experimental animals (9–13). Patients without detectable autoantibodies are referred to as seronegative (SNMG). Some MG patients have antibodies against a number of other extracellular or intracellular targets. Although the pathogenicity of these autoantibodies is often uncertain or unlikely, they can still be very valuable as disease biomarkers.

The detection of autoantibodies is crucial for MG diagnosis and for the differential diagnosis of many disorders with similar presentation. We will review the main autoantibodies found in MG, their implications for diagnosis and patient stratification, and recent advances for improved diagnostics based on more sensitive tests or the discovery of new target antigens. In addition, we will present an overview of recent efforts to develop targeted therapeutic methods aiming only at the antigen-specific components of the immune system, further highlighting the importance of autoantigen determination in MG diagnosis.

MG CLASSIFICATION BASED ON AUTOANTIBODY SPECIFICITY

Patients With AChR Antibodies

AChRs are located at the end plate of the muscle post-synaptic membrane, opposite the axon terminals. They are composed of five homologous subunits with a stoichiometry of $\alpha_2\beta\delta\varepsilon$ in adult and $\alpha_2\beta\gamma\delta$ in fetal or adult denervated muscles (14). The autoantibodies target the N-terminal extracellular domains (ECDs) of the AChR subunits. About half of the autoantibodies bind the AChR α subunit and studies in experimental rats have suggested that these are the most pathogenic (10). A region of the α subunit composed mainly by amino acids 67–76 with some contribution from other segments has been identified to be particularly targeted, commonly referred to as the main immunogenic region (MIR) (15–17). However, autoantibodies against all five subunits, including the γ subunit of the fetal AChR, can be found, even in the same patient (18–21).

AChR antibodies confer their pathogenicity by three mechanisms. Firstly, they can activate the complement cascade, since they belong mainly to the IgG1 and IgG3 subclasses, thus causing destruction of the post-synaptic membrane (22, 23). The loss of the typical local architecture results in a severely diminished efficiency of signal transduction between nerve and muscle. Secondly, they can cross-link AChRs causing their internalization and destruction by a process called antigenic modulation, thus leading to a reduction in the number of functional receptors in the post-synaptic membrane (24). Lastly, antibodies that bind close to the AChR ligand binding site are thought to directly block acetylcholine binding and receptor activation (25).

The detection of serum AChR antibodies has been an invaluable tool in the diagnosis of MG. Although the AChR antibody titer does not correlate with disease severity across different MG patients, the temporal variation of titers from individual patients appear to be associated not only with symptom severity but with response to treatment as well (26). Therefore, in addition to diagnosis, AChR antibody measurement can be useful for MG patient monitoring. Nonetheless, in very rare cases AChR antibodies can be found in non-MG patients with other autoimmune disorders or with thymoma (27).

Patients With MuSK Antibodies

MuSK is a key player involved in NMJ organization and maintenance. It is located on the muscle membrane where it interacts with LRP4 propagating the signal for AChR clustering, while it is involved in tethering acetylcholisteresase (AChE) via interactions with collagen Q (ColQ). MuSK is a transmembrane protein with an extracellular domain containing three immunoglobulin-like regions and a frizzled-like region, a transmembrane helix domain and a cytoplasmic domain with tyrosine kinase activity. Most of the MuSK antibodies are directed against the immunoglobulin-like regions of the extracellular domain (28, 29). This binding appears to block the interactions of MuSK with LRP4 or ColQ resulting in reduction of both agrin-dependent and agrin-independent AChR clustering (30–32). Antigenic modulation and complement activation are not thought to be significant in pathology, since MuSK antibodies are mostly of the IgG4 subclass, which does not activate complement and is functionally monovalent (33, 34). Nonetheless, since IgG1-−3 MuSK antibodies are also present in patients' sera, they could have pathogenic activity, although their relative contribution remains unclear.

MuSK antibodies are found in about 6% of MG patients, accounting for 40% of patients without AChR antibodies. However, their prevalence varies among countries possibly due to genetic and environmental factors, with northern European countries presenting lower rates than those in south Europe and the Mediterranean (29, 35–39), while in Japanese populations they are even less common with a prevalence of 2–3% (40). Similarly to AChR antibodies, their detection is crucial for MG diagnosis and monitoring. Interestingly, their titer has been shown to positively correlate with symptom severity not only in individual patients but in the population as well (41, 42).

Patients With LRP4 Antibodies

LRP4 is a transmembrane protein, containing several low-density lipoprotein domains, expressed in skeletal muscles and in motor neurons in the brain. In the muscle, it binds neural agrin released from the nerve terminals initiating the signal via MuSK for AChR clustering (43). LRP4 antibodies belong mostly to the IgG1 subclass and *in vitro* they have been shown to be capable of complement-mediated cell lysis (13, 44). However, the

contribution of complement activation in their pathogenicity is still unclear and the main *in vivo* mechanism at play is thought to be inhibition of interaction with MuSK, causing disruption of normal NMJ organization (13, 44–46).

The overall prevalence of LRP4 antibodies in MG patients appears to be around 2% [i.e., ∼19% of SNMG patients (47)], although there was considerable variation among initial studies with reported rates of 2–45%, possibly due to differences in the detection method used, the source of the antigen (animal or human) and the populations studied (44–46). A lower prevalence has been reported among Chinese MG patients accounting for 0.8–1.7% of total and 1–2.9% of SNMG patients (48, 49). Interestingly, LRP4 antibodies have also been reported in 10–23% of amyotrophic lateral sclerosis (ALS) patients (50, 51) and in 3.6% patients with other neurological diseases but not in healthy controls (47). Despite their frequent detection in ALS, their detection is a significant aid in MG diagnosis in parallel with the clinical presentation of the patients.

Patients With Other Antibody Specificities

In addition to the main MG antibody specificities discussed above, a number of other autoantibody targets, both extracellular and intracellular, have emerged in MG patients.

Extracellular Antigens

Activation of the LRP4/MuSK complex to drive AChR clustering is caused by neural agrin. Agrin antibodies have been detected in 2–15% of MG patients, though in most cases they were also positive for antibodies against AChR, MuSK, or LRP4 (52–55). Agrin antibodies have also been found in 14% of ALS patients (50). However, they have been shown to inhibit agrin-induced MuSK activation *in vitro*, and immunization with neural agrin caused MG symptoms in experimental animals, suggesting that these antibodies are involved in MG pathology (54, 56). Their detection can be valuable for disease management, as they have been shown to be associated with moderate to severe symptoms and moderate response to treatment (52).

In some MG patients antibodies against the voltage gated potassium channel α-subunit Kv1.4 have been found, which in addition to the central nervous system is expressed in skeletal and heart muscles. A prevalence of 11–18% among MG patients has been reported, although the associated symptom severity appears to depend on the population studied. In a Caucasian patient cohort Kv1.4 antibodies were associated with LOMG patients and mild disease, often remaining purely ocular (57), while in Japanese patients they correlated with increased disease severity, myasthenic crises and the presence of thymoma (58–60). Furthermore, since in the Japanese cohort myocarditis or abnormal ECG findings were present in as many as 27 and 60%, respectively of Kv1.4 antibody positive patients, they could be an important marker of myocarditis or cardiac dysfunction among Japanese MG patients.

The activity of acetylcholine on AChRs is controlled by the enzyme AChE, which breaks down acetylcholine to choline and acetate thus terminating its action. AChE is located close to the postsynaptic membrane, where it is anchored on MuSK via molecules of ColQ (61). Antibodies against both AChE and ColQ have been found in some MG patients. AChE antibodies have been reported in 5–50% of MG patients, but they are not specific for MG since they are also found in many patients with other autoimmune diseases, while no correlation has been identified with clinical characteristics or symptoms (62–64). ColQ antibodies have so far been detected in about 3% of MG patients, including among SNMG, although again they do not appear to be MG specific and no evidence of pathogenicity has been found yet (65). Finally, antibodies against collagen XIII, a transmembrane collagen, have been detected in the serum of about 7% of MG patients with AChR antibodies and 16% of SNMG, but their presence did not correlate with symptom severity (66). Furthermore, they too are not specific for MG, since they are also found in patients with Grave's ophthalmopathy (67). Overall, the lack of MG-specificity of AChE, ColQ, and collagen XIII antibodies as well as the lack of association with clinical characteristics, which might have attributed a prognostic value, make the usefulness of these antibodies in MG diagnosis uncertain and further investigation is required.

Intracellular Antigens

The first autoantibodies, after AChR antibodies, to be identified in MG were the striational antibodies, named after the characteristic staining patterns produced in sarcomere sections by patients' sera. The term in fact collectively refers to several antibodies directed against different muscle proteins including titin, the ryanodine receptor (RyR), actin, myosin, tropomyosin, filamin, and others (68–71). Although the pathogenicity of these antibodies is unlikely, due to the intracellular localization of their target antigens, the diagnostic and prognostic value for titin and RyR antibodies has long been established.

Titin is the largest protein known to date, a filamentous molecule with a molecular weight of up to 4,200 kDa (72). Interestingly, titin antibodies only bind to a 30 kDa domain, called MGT30, located near the A/I band junction (73). Until recently, titin antibodies were only found in MG patients with AChR antibodies, being detected in 20–40% of them. These antibodies show a strong correlation with disease age of onset, since they are present in about 6% of EOMG but 50–80% of non-thymomatous LOMG patients (74–78), but in 50–95% of EOMG with thymoma and only few non-thymoma patients, so their presence provides a strong indication for thymoma (69, 75, 78–82). Additionally, they appear to be prognostic of more severe disease in all age groups (78, 80, 81, 83). More recently, low-titer titin antibodies were detected in SNMG as well (84). These low titin antibody titers did not correlate with the presence of thymoma, in accordance with previous findings that thymoma is unlikely in MG patients without AChR antibodies (82).

RyR is a transmembrane protein forming a calcium channel in the sarcoplasmic reticulum, where it mediates Ca^{2+} release into the cytoplasm, facilitating muscle contraction in response to stimulation. Similarly to titin antibodies, RyR antibodies are found in few EOMG but in up to 40% of LOMG patients, while they are found in 75% of thymomatous MG patients and their presence is prognostic of more severe disease progression (81, 85–88).

Rapsyn is a scaffolding protein, which in the muscle plays a role in AChR clustering by linking the intracellular domains of the receptors (89). Antibodies against rapsyn have been found in about 15% of MG patients, including among SNMG (90). However, rapsyn antibodies have also been found in various other autoimmune disorders decreasing their value as MG specific diagnostic markers, while no correlation with disease severity has been identified (91).

Cortactin is a cytoplasmic protein also involved in AChR clustering downstream of MuSK. Cortactin antibodies have been detected in about 9.5% of AChR antibody positive MG patients and 24% of SNMG patients, while they seem to be associated with mild disease (92–94). Nonetheless, their importance for MG diagnosis is still unclear, since they are also found in ∼12.5% of patients with other autoimmune diseases and 5% of healthy controls (93), as well as up to 26% of patients with polymyositis, dermatomyositis, and immune-mediated necrotizing myopathy (95).

Relevance in MG Diagnosis

Although involvement in pathogenicity of most of the above antibodies against extracellular targets is often not clear yet, their detection can be valuable for MG diagnosis, especially in the case of otherwise seronegative patients. However, further validation or improvement of the detection assays is necessary, since in many cases they appear to lack good specificity for MG. The detection of antibodies against intracellular antigens, has proven invaluable as markers of disease severity, or identification of comorbidities, such as titin antibody detection for thymoma in EOMG. Furthermore, these antibodies, although unlikely to be pathogenic themselves, can play a significant role in diagnosis of SNMG patients, where the pathogenic autoantibodies may not be detectable by current assays, like anti-titin antibodies detected by RIPA in AChR-seronegative patients (84).

METHODS FOR SEROLOGICAL DIAGNOSIS OF MG

Serological tests for the detection of autoantibodies play a vital role in MG diagnosis. Being minimally invasive methods, they do not present a major barrier for testing and a single serum sample could potentially be tested by several assays if required, without the need for repeated hospital visits by patients. Although a final diagnosis may rely on additional tests, such as electrophysiological examination or assessment of response to AChE inhibitors, the high specificity of many MG antibody assays considerably facilitates diagnosis (**Table 1**).

Radioimmunoprecipitation assays (RIPA) are to this day the golden standard of serological MG tests, due to their high sensitivity and their ability to provide quantitative data allowing detailed patient monitoring. RIPAs are widely applied for the detection of AChR, MuSK, and, less frequently, other antigens. The AChR antibody assay is based on indirect labeling of solubilized AChR with ^{125}I-α-bungarotoxin, a highly specific AChR antagonist (102, 103). AChR can be obtained from human muscle from amputees or, currently more common, from AChR-expressing cell lines, such as CN21, which have been engineered to express both the fetal and adult types of the receptor, thus also detecting antibodies against the AChR γ subunit (104). The wide use of the AChR RIPA owes to the ∼99% specificity of the assay and its high sensitivity, which amounts to about 85% among GMG patients and 50% for OMG (105). In fact, many of the "seronegative" by AChR RIPA OMG patients have been found positive by other assays and/or for other antigens including: cell based assay (CBA) for AChR clusters [up to 50% (96)], for LRP4 [up to 27% (47)], for MuSK [16% (100)], or RIPA for titin antibodies [12% (84)] with some double positives; yet a few false-positives have been also referred by these assays. It is unknown whether the remaining "seronegative" OMG patients are true seronegative or have yet undetectable antibodies to known or yet unknown antigens. The fact that those OMG patients with AChR antibodies have generally low antibody titers may suggest that some of the remaining "seronegative" have yet undetectable AChR antibodies. Assays for the detection of blocking antibodies, i.e., antibodies that bind to the receptor binding site, which may not be detected by the conventional RIPA, have been developed and are also commercially available. The added value from the use of these assays is limited since most patients will have non-blocking antibodies as well, while ACh binding competition appears to be less important for pathogenesis compared to complement activation. MuSK antibodies are commonly detected using directly ^{125}I-labeled MuSK, with very high specificity for MG (106). The detection of AChR and MuSK antibodies in the same patient by RIPA is rare (107, 108). Recently, we developed a RIPA for the detection of titin antibodies with ^{125}I-labeled MGT30 and used it to test a large cohort of samples from European MG patients, including 372 SNMG, which do not usually have detectable titin antibodies by current methods. We found that 13.4% of SNMG patients had titin antibodies, as well as 14.6 and 16.4% of patients with MuSK and LRP4 antibodies, respectively (84). The RIPA-detected titin antibodies in SNMG were not predictive of more severe disease. Nonetheless, titin antibodies detected by RIPA are a valuable biomarker for the diagnosis of otherwise SNMG patients.

Efforts to improve the sensitivity of the classical RIPA have resulted in the development of modified assays, using much larger serum volumes in order to detect antibodies at lower titers. Different approaches have been explored in order to minimize non-specific binding, which would render the use of large serum volumes impossible. In the case of AChR antibodies, semi-purified anti-human IgG was used as secondary antibody, allowing an increase of serum volumes by 16-fold and consequently reducing the positivity titer cut-off value from 0.5 to 0.1 nM (109). The application of this method allowed the detection of AChR antibodies in 20 of 81 previously SNMG patients tested. For MuSK antibody detection a two-step approach has been proposed, initially semi-purifying the MuSK antibodies by affinity chromatography with sepharose-immobilized MuSK and then using the concentrated antibodies for standard RIPA (110). This modification allowed the use of up to 50 times larger serum volumes for the assay, which resulted in the

TABLE 1 | Autoantibody specificities in MG with clinical associations and common detection assays used.

	Target antigen	Detection assay*	Clinical presentation	References
Extracellular	AChR	RIPA: Good specificity (∼99%) and sensitivity (∼85% for GMG and ∼50% for OMG). Requirement for specialized equipment and use of radioactivity.	The major MG subgroup. Practically all MG symptoms may be present. The presence of AChR antibodies is very rare in other diseases. Thymic abnormalities (mostly thymic hyperplasia) are common, and thymoma in ∼10% of patients.	Several references, including (7, 96–99)
		ELISA: Various assays developed with reported specificities ranging between 96.1 and 99% and sensitivity for GMG 79.5–91.5%. Easier to adopt in non-specialized laboratories.		
		CBA (clustered AChR): Allows detection of antibodies bound only to high density AChRs, or those whose epitopes are altered during receptor solubilization. Detection of ∼20% of previously SNMG. Requirement for specialized equipment.		
	MuSK	RIPA: very good specificity. Detection of antibodies in ∼40% of AChR antibody negative patient	Usually manifested by bulbar symptoms. Moderate to severe symptoms. No thymic abnormalities.	(29, 37, 40, 100, 101)
		ELISA		
		CBA: Detection of 8–13% of patients negative for AChR and MuSK antibodies by RIPA. Can detect up to ∼99% of RIPA-positive samples and has ∼100% specificity when IgG Fc-specific 2nd antibodies are used.		
	LRP4	ELISA	Milder symptoms than AChR antibody positive MG. No thymoma.	(47, 50)
		CBA: Detection in ∼6–19% of SNMG patients, but also in 10–23% of ALS patients.		
	Agrin	ELISA or CBA: Detected in up to 15% of MG patients, mostly seropositive. They have also been found in 14% of ALS patients.	Associated with more severe symptoms and moderate response to treatment.	(50, 53)
	Kv1.4	Immunoprecipitation of ^{35}S-labeled cells extracts followed by SDS-PAGE.	In Japanese patients they are associated with more severe disease and myocarditis, while in Caucasian patients they are associated with LOMG and mild symptoms	(57, 59)
	AChE	ELISA: 5–50% of MG patients positive, but also several patients with other autoimmune diseases.	No association with thymic pathology and symptom severity.	(63, 64)
	ColQ	CBA: Found in ∼3% of MG patients, but lack specificity.	Not determined.	(65)
	Collagen XIII	ELISA: Found in ∼16% of SNMG. They are also associated with Grave's ophthalmopathy.	No association with disease severity apparent.	(66, 67)
Intracellular	Titin	ELISA: Detection of titin antibodies only in AChR Ab positive MG.	More common in LOMG, rare in non-thymomatous EOMG, but present in 50–95% of EOMG with thymoma. Their presence corelates with increased symptom severity	(69, 71, 78, 81, 84)
		RIPA: Detection of titin antibodies in all MG subgroups, including 13.4% of SNMG (low titers).	MG biomarker in "seronegative" MG. Low titer antibodies detected by RIPA αre not prognostic of more severe disease or thymoma.	
	RyR	Immunoblots or ELISA: Detection of RyR antibodies only in AChR Ab positive MG.	Present in 75% of thymomatous MG patients. Their presence corelates with increased symptom severity.	(85, 88)
	Rapsyn	Immunoblots: Detected in ∼17% of SNMG, but they were also detected in 10 and 78% of OND and SLE patients, respectively.	No association with disease severity apparent.	(90)
	Cortactin	ELISA or Western blot: Detected in up to 24% of SNMG, but not specific–also present in 12.5% of other autoimmune diseases and up to 26% of myositis patients.	They have been reported to be prognostic of mild disease.	(92, 93)

**Not all assay are available for routine diagnosis yet.*

detection of previously SNMG patients, without a compromise in specificity.

Enzyme-linked immunosorbent assays (ELISAs) have also been in use for the detection of AChR and MuSK antibodies, though less commonly than RIPA (97, 111). The ELISA has advantages as it does not involve the use of radioactivity and can be performed with standard equipment in most laboratories. For AChR antibodies, different ELISAs have been developed, either directly coating AChR onto ELISA plates followed by serum incubation, or by preincubation of AChR with serum in solution followed by measuring the inhibition of binding to a set of AChR monoclonal antibodies (immobilized and in solution). Although some studies have found the ELISA as specific and at least as sensitive as the RIPA, in others the ELISA presents with lower specificity and sensitivity, perhaps explaining its limited adoption (97, 98, 111). Assays aiming at the detection of modulating or blocking antibodies have also been developed, but they did not improve the sensitivity significantly compared to the standard RIPA (112, 113). On the other hand, ELISA with immobilized titin MGT30 domain is currently the most widely used method for the detection of titin antibodies. Other antibodies usually tested for by ELISA include cortactin and RyR antibodies using as antigen recombinant protein domains (114).

Several efforts have been made to produce other non-radioactive alternatives to RIPA with comparable sensitivity. A promising solution appears to be fluorescence immunoprecipitation assay (FIPA), which involves labeling of the target antigen with a fluorescent dye. In one approach for AChR labeling, the α, γ, and ε subunits were tagged with EGFP, before transfection together with the remaining subunits into HEK293 cells, while for MuSK the extracellular domain only was used labeled with maccherry and expressed in insect S2 cells (115). The overall sensitivity was shown to be very close to that of the RIPA for both AChR and MuSK antibodies. Furthermore, by labeling each antigen with a different fluorescent dye both AChR and MuSK antibodies could be detected simultaneously in the same assay, thus potentially reducing the cost and time for diagnosis. A similar method based on labeling recombinant fragments of the AChR α subunit with *Renilla luciferase* has been developed with good specificity (97%), but it was able to detect AChR antibodies only in 32% of MG patients, potentially due to the use of part of the α subunit rather than whole AChR (116). Further investigation with respect to the diagnostic value of assays employing AChR fragments is necessary.

The application of CBAs in MG diagnosis has been expanding over the last years. The method involves the transient or stable expression of the target antigen in a cell line, followed by incubation of the cells with test serum and the detection of autoantibody binding by fluorescence microscopy using labeled secondary or tertiary antibodies.

In the case of AChR antibody CBA, co-transfection of the cells with rapsyn, in addition to the AChR subunits, induced clustering of the receptors, thus permitting detection of antibodies that bind only to high density AChRs mimicking their clustering at the NMJ, or of antibodies whose epitopes are altered by the detergent solubilisation of membranes during the isolation of AChR antigen. Despite initial reports of high seropositivity found among SNMG with CBA for AChR antibodies (96, 99, 100, 109, 117, 118), routine diagnosis suggests that the overall frequency of antibodies against clustered AChRs in SNMG patients is around 20% or less (100, 119). Autoantibody titration can be achieved by using serial dilutions of sera, but based on our experience with both assays, it cannot reach the accuracy of the RIPA. Nonetheless, CBA has become invaluable for the diagnosis of SNMG patients, with several studies reporting detection of AChR antibodies that were undetected by other current diagnostics (115, 118, 120, 121). On the other hand, even for sera found positive for AChR antibodies by RIPA, a CBA test could be useful to confirm that the detected antibodies bind on the cell embedded AChR. The use of both fetal and adult forms of the AChR not only appears to increase the sensitivity of the assay, but also enables the discrimination among fetal or adult AChR directed antibodies (122). The latter is important for the diagnosis of transient neonatal MG not associated with maternal MG, a condition arising from the presence in the mother of antibodies against only the fetal AChR, which may not cause MG symptoms in the mother but can be detrimental for the new-born (20, 123, 124).

CBAs have also contributed significantly in the detection of MuSK and LRP4 antibodies in previously SNMG patients, including Asian populations where MuSK-MG is less common (99, 115, 120, 125). We have used CBAs for MuSK and LRP4 to test a cohort of sera from 13 European countries including over 630 samples from SNMG patients. We found that about 13% of SNMG samples were positive for MuSK antibodies, with a variation in the rates among countries, ranging from 5–22% (100). The MuSK CBA has allowed the detection of antibodies in SN-OMG patients as well, which is not common with RIPA (100, 115). Of note, most of the MuSK antibodies detected belonged to the IgM rather than IgG class. Using the LRP4 CBA, 19% of SNMG were found positive for LRP4 antibodies with an inter-country variability of 7–33% (47). The percentage of patients positive for more than one antibody specificities has increased by the use of CBAs. In more detail, 7.5% of AChR antibody positive and 15–20% of MuSK antibody positive sera have also been found positive for LRP4 antibodies, while 0.5–12.5% AChR antibody positive patients were reported positive for MuSK antibodies as well (47, 48, 52, 100).

Although the presence of antibodies only detectable by CBA is associated with milder disease and better response to treatment (118), these antibodies have also been shown to be pathogenic. Indeed, antibodies against clustered AChRs belong to the complement-activating subclasses and cause complement depositions on the cell surface (96). Furthermore, MuSK IgG antibodies, but not IgM, detected by CBA were shown to inhibit agrin-induced AChR clustering on the surface of C2C12 myotubes (101).

The specificity of the secondary antibodies and by extension the antibody classes detected by CBA appears to be important (119). For example, anti-human antibodies directed against the intact light and heavy IgG chains can also bind to IgM, and possibly other antibody classes as well. A study using such secondary antibodies for MuSK CBA resulted in a significant decrease in specificity (11 and 19% positives among the healthy

and disease controls, respectively), as well as in sensitivity (101). On the other hand, the use of a secondary antibody specific for the Fc part of the IgG heavy chain, which does not cross-react with other Ig classes, resulted in the detection of 99% of MuSK RIPA positive samples and 100% specificity, although this was accompanied by a decrease in the number of positives among SNMG (101). Since IgM may not be pathogenic, the importance of discrimination of the antibody classes for diagnosis remains to be fully assessed.

Recently, a modified CBA approach was developed based on the generation of stably-transfected HEK293 cell with the target antigen and, following incubation with the test sera, autoantibodies were measured by FACS analysis, providing more quantitative results. The assay has been used for the detection of antibodies against various antigens such as Kv1.4 and even the intracellular titin (126). In fact, the cytometric CBA showed improved sensitivity compared to the ELISA for titin. Furthermore, it could facilitate the diagnosis of Kv1.4 antibodies despite the somewhat lower sensitivity compared to the currently used method, which is relatively complicated and laborious, involving the immunoprecipitation of ^{35}S-labeled cell extracts from rabdomyosarcoma and leukemic cells followed by electrophoresis analysis. The presence of a 70 kDa Kv1.4 protein band in the former but not the latter extracts is considered a positive finding (59).

A significant disadvantage of most of the aforementioned methods is the requirement of specialized equipment and expertise. Efforts are made for the development of fast, easy to perform and instrument-free assays. The use of such assays in decentralized small clinics and doctors' offices could reduce the time to diagnosis significantly, improving disease management. To this end, we have developed an assay based on the immobilization of antigen on a stick-type solid surface (immunostick) at high density. The immunostick can be immersed in succession into the undiluted test serum, secondary antibodies and substrate solution, similar to standard ELISA, but with much reduced incubation times, allowing completion in less than an hour. Furthermore, immobilization of various antigens in different zones of the immunostick could allow the simultaneous detection of more than one MG autoantibodies. Evaluation of this method for the detection of AChR antibodies, showed that it had very good specificity and sensitivity (99 and 91%, respectively) (127). A similar approach based on a modified dot-blot method, using AChR preparations immobilized onto nitrocellulose membrane, achieved the same sensitivity as the ELISA (128).

DEVELOPMENT OF THERAPIES BASED ON AUTOANTIGEN SPECIFICITY

In addition to its value for MG diagnosis, the determination of autoantibody specificities is important for efficient management of the disease. For example, the differentiation among AChR and MuSK antibody positive patients has important implications for therapy, since the latter can present with adverse effects when treated with AChE inhibitors, a common first line AChR-MG treatment, thymectomy, or the use of complement inhibitors does not appear to be beneficial to them (129). On the contrary, MuSK antibody positive patients usually respond very well to rituximab or therapeutic plasma exchange (130–132). Of note, the detection of any autoantibody specificity could provide an indication for the use of neonatal Fc receptor (FcRn) inhibitors, which work by blocking IgG recycling via the FcRn, thus reducing IgG half-life and which show potential for MG treatment in recent clinical trials (133).

Current common treatments for MG include AChE inhibitors, immunosuppressive drugs, thymectomy, intravenous immunoglobulin (IVIG) and plasmapheresis (1, 134). However, these approaches are to a large extent not specific and can thus be accompanied by various side effects. The problem is augmented given the long-term immunosuppression that may be required, increasing the risk of infections or neoplasia. Furthermore, a number of patients may remain unresponsive to current treatments (132). The development of antigen-specific therapies targeting only the pathogenic components of the immune system would, therefore, greatly benefit MG patients. Knowledge of the autoantibody repertoire of each patient is vital for such approaches to be implemented, further underlining the role of serological diagnostics.

One approach would be antigen-specific immunoadsorption, which is based on the selective removal of the autoantibodies from the patient's circulation. The procedure is a modification of plasmapheresis, whereby the isolated plasma, instead of being discarded, is passed through a matrix allowing the removal of the autoantibodies, before being returned to the patient (135) (**Figure 2**). Since no replacement fluids would be needed as in plasmapheresis, an additional advantage of the approach will be the reduction in risk of infection or allergic reactions. Efforts to develop such a matrix have been made by immobilization of recombinant extracellular domains of AChR or MuSK onto sepharose. Expression of the recombinant proteins has been optimized to achieve sufficient production yield and purity together with maximum antibody binding (110, 136). A number of *in vitro* experiments have established the efficiency, speed and specificity of AChR or MuSK autoantibody binding from sera of immunized experimental animals or MG patients (28, 137). Especially in the case of MuSK antibodies, immunoadsorption resulted in almost complete removal of the autoantibodies from all the patient sera tested. Furthermore, *ex vivo* immunoadsorption has been performed in rats with experimental autoimmune MG (EAMG), induced by immunization with human AChR or MuSK ECDs. The procedures resulted in quick and significant reduction of symptom severity, without the emergence of any adverse effects (138, 139). Although such an approach would not be a permanent cure as the autoantibodies would inevitably re-emerge, it would be greatly beneficial as a treatment option, providing immediate relief from symptoms when required, such as during myasthenic crises or pre-operatively.

Another approach for antigen-specific therapy would be to induce immunosuppression or immune tolerance in a targeted manner. In this case treatment would not have an immediate impact, but it would aim at a long-lasting or permanent

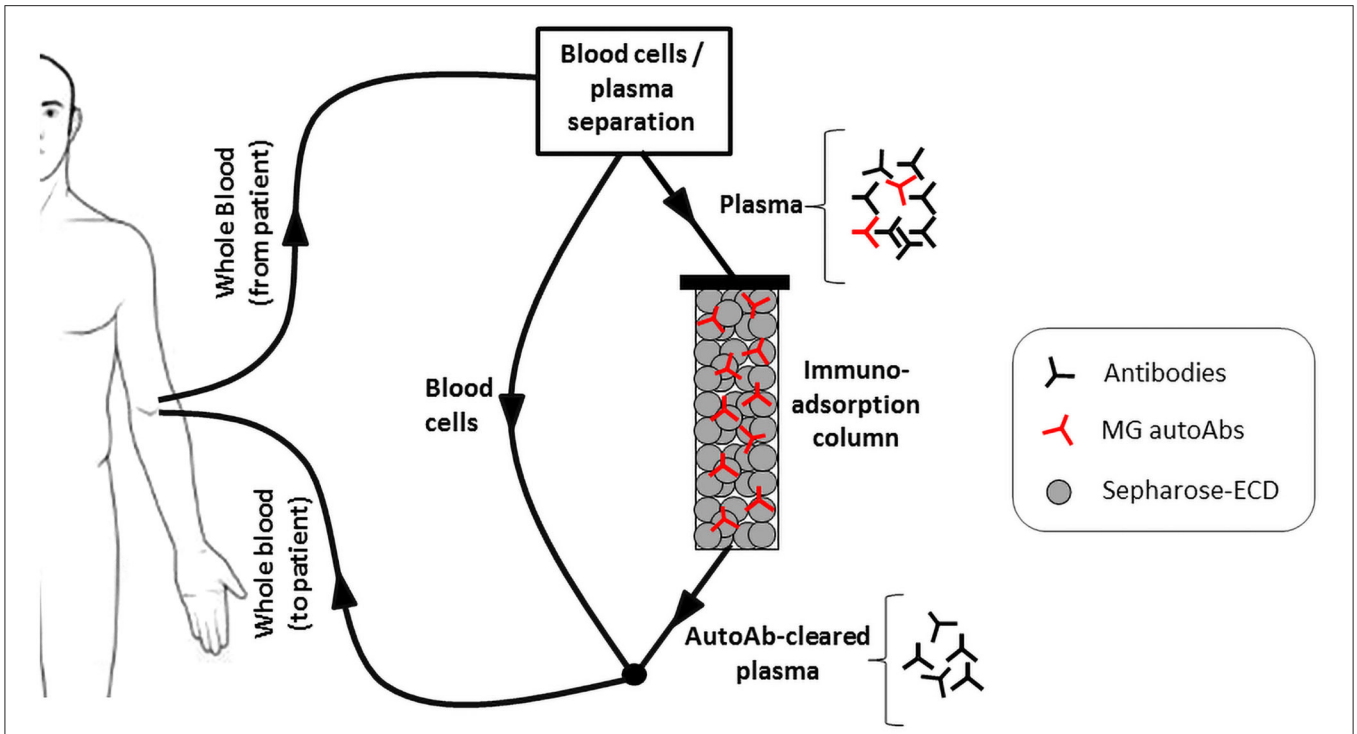

FIGURE 2 | The antigen specific immunoadsorption therapy approach. Recombinant extracellular domains (ECDs) of the AChR or MuSK are immobilized onto sepharose and packed into a column. During treatment, the patient's plasma is passed through it, allowing the selective binding and removal of only the MG autoantibodies. The autoantibody-depleted plasma is then returned to the patient.

effect. Indeed, studies have shown that EAMG symptoms can be prevented or ameliorated by oral or nasal administration of AChR or MuSK domains (140–144). In most studies the extracellular domain of the AChR α subunit has been used, while the response was not affected by the use of syngeneic (rat) or xenogeneic (human) AChR sequences (145). The therapeutic efficacy appeared to depend on the conformation of the administered antigens, with denatured proteins having a more pronounced effect (146). In fact an α subunit domain lacking some of the B cell epitopes has been found more efficient for treatment (147), suggesting that destruction of conformation-dependent B cell epitopes was responsible for the increased efficacy of denatured antigens. Furthermore, the use of AChR peptides corresponding to dominant T cell epitopes orally has been shown to ameliorate disease symptoms (148). Interestingly, a beneficial effect was also observed when dominant T cell epitopes were administered in the form of subcutaneous immunization in the presence of adjuvant (149). Conjugation of antigen derived peptides to immunomodulating protein domains as a means of targeting has also been explored to improve treatment potency with promising results (150). In most cases of tolerance induction, a shift in the T cell responses from Th1 to Th2 and/or Th3 was involved in mediating the therapeutic effect, evidenced by changes in the respective cytokine levels, mostly reduction in IFN-g, IL-2 and IL-12 and increase in IL-10 and TGF-β expression, accompanied by changes in the AChR IgG subclass distribution (144, 151–153).

The identification of peptides derived from the human AChR α subunit as T cell dominant epitopes, lead to the construction of altered peptides with single amino acid substitutions (termed altered peptide ligands, APL), some of which were found to inhibit T cell proliferative responses *in vitro* (154). Furthermore, oral administration of a dual APL (two APL peptides in tandem) in mice with EAMG, resulted in improvement of clinical manifestations and reduction of autoantibody titers (155). The therapeutic effect was marked by downregulation of the IFN-g and IL-2, upregulation of IL-10 and TGF-β, and induction of immunoregulatory CD4+CD25+ T cells (156, 157).

A different strategy relied on the administration of peptides incorporating only the intracellular domains of the AChR subunits, which have been shown to be incapable of disease induction (158). Although oral or nasal administration of the intracellular polypeptides was able to prevent and, in some cases, treat ongoing EAMG, the effect was greater when treatment was given as subcutaneous vaccination (142, 159). The mechanism of action appears to involve diverting the immunological response away from the production of ECD-targeting pathogenic antibodies, toward epitopes of the intracellular domains, and possibly causing apoptosis of AChR-specific plasma cells (160).

CONCLUSION

The clinical presentation of MG, its underlying pathophysiology and the response to treatment vary depending on the targeted

autoantigens. Assays for the detection of MG autoantibodies are central in diagnosis, and they often serve as early diagnostics in cases of clinically suspected MG. Furthermore, since serological tests can identify the autoantibody specificities in MG patients, their role extends beyond disease diagnosis as invaluable tools for MG management. Patients with suspected MG but initially negative for autoantibodies, should be retested since usually the antibody titers increase and there is epitope spreading with disease progression. Nevertheless, some MG patients remain seronegative, making the discovery of novel antigenic targets or the development of more sensitive assays against known antigens invaluable. To this end, several new antigens recognized by autoantibodies in MG patients' sera have been identified over the last years, but the diagnostic relevance for most of them remains to be fully established.

RIPAs for AChR and MuSK antibodies have been the most widely used assays, owing to their very high sensitivity and specificity. The use of CBAs in routine diagnosis, mostly for clustered AChRs, MuSK and LRP4, is being slowly introduced during the recent years, contributing in reducing the number of SNMG patients. However, a significant disadvantage of CBAs currently is their limited capability of providing accurate titer information, which in addition to the lack of commercial kits, has resulted in their use mostly for patients negative by the standard RIPAs. Cytometric CBAs providing more quantitative results have already been proposed as a useful alternative, but their value for routine diagnosis remains to be assessed. Furthermore, simpler assays designed for quick instrument-free sample analysis are being developed, which should decrease the time to diagnosis and contribute to the improvement of patients' care when they become commercially available. Finally, not surprisingly due to the nature of serological tests, irrespective of sensitivity, there is currently no single assay detecting all seropositive patients. Therefore, the potential need to ultimately use different assays for the diagnosis of these few patients must not be overlooked by the clinicians.

The identification of the antigen targeted in individual MG patients, presents the unique opportunity to develop personalized antigen-specific therapies that would selectively target the autoimmune components of the immune system. Among the approaches studied are specific removal of the autoantibodies, induction of tolerance and diversion of the immune response from the targeted autoantigen. Several studies have shown their therapeutic potential, but further pre-clinical trials are required before they can progress to clinical application. The development of such personalized approaches would increase the treatment efficacy and reduce side effects, thus significantly improving the patients' quality of life, and should be the focus of further efforts.

AUTHOR CONTRIBUTIONS

KL and ST researched the bibliography for the review, made substantial contributions to the content, and reviewed and edited the manuscript. KL wrote the first draft. All authors contributed to the article and approved the submitted version.

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Controversies in Ocular Myasthenia Gravis

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Myasthenia gravis (MG) with symptoms limited to eye muscles [ocular MG (OMG)] is a rare disease. OMG incidence varies according to ethnicity and age of onset. In recent years, both an increase in incidence rate, particularly in the elderly, and a lower risk for secondary generalization may have contributed to the growing disease prevalence in Western countries. OMG should be considered in patients with painless ptosis and extrinsic ophthalmoparesis. Though asymmetric muscle involvement and symptom fluctuations are typical, in some cases, OMG can mimic isolated cranial nerve paresis, internuclear ophthalmoplegia, and conjugate gaze palsy. Diagnostic confirmation can be challenging in patients negative for anti-acetylcholine receptor and anti-muscle-specific tyrosine kinase antibodies on standard radioimmunoassay. Early treatment is aimed at relieving symptoms and at preventing disease progression to generalized MG. Despite the absence of high-level evidence, there is general agreement on the efficacy of steroids at low to moderate dosage; immunosuppressants are considered when steroid high maintenance doses are required. The role of thymectomy in non-thymoma patients is controversial. Prolonged exposure to immunosuppressive therapy has a negative impact on the health-related quality of life in a proportion of these patients. OMG is currently excluded from most of the treatments recently developed in generalized MG.

Keywords: neuromuscular junction, acetylcholine receptor antibodies, muscle-specific kinase antibodies, autoimmune disease, ophthalmoparesis

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INTRODUCTION

The impairment of neuromuscular transmission (NMT) in myasthenia gravis (MG) is due to loss of acetylcholine receptors (AChRs) and end-plate alterations caused by autoantibodies (Abs). The AChR, the muscle-specific tyrosine kinase (MuSK), and low-density lipoprotein receptor-related protein 4 (LRP4) are the main Ab targets. Extracellular proteins, like neuronal Agrin and collagen Q (ColQ), have recently been recognized as additional antigens (1, 2). In clinical practice, patient subgrouping based on disease-specific Abs is a prerequisite for personalized management (3).

Anti-AChR Abs induce MG through complement activation, AChR cross-linking, and internalization, and, to a lesser extent, by interfering with ACh binding (1). MG with AChR Abs affects around 85% of patients. It has a bimodal incidence pattern with a peak in young women and a larger peak in elderly men and is associated with thymus hyperplasia and thymoma, both playing a role in autoimmunization against AChR (3). On clinical grounds, AChR-MG shows broad variability in weakness severity and extension.

Anti-MuSK Abs are found in 30–40% of AChR-negative patients, with high prevalence in women.

Clinical phenotype is dominated by cranial and bulbar weakness (4). MuSK Abs are mostly IgG4 that interfere with the protein function inhibiting MuSK activation and leading to reduced AChR clustering (1). Anti-LRP4 Abs are detected in a proportion of AChR and MuSK-negative [double seronegative (dSN)] patients, generally in association with mild disease (5, 6), and can be also found in some AChR and in some MuSK-positive cases (5). Anti-LRP4 Abs are IgG1/2 with a potential to activate complement (7). Abs to Agrin (6, 8) and to ColQ (9) have been found so far in few AChR/MuSK/LRP4-negative MG patients, and the associated clinical aspects are not defined. Lastly, some patients, often with juvenile onset and limited disease, do not have detectable serum Abs.

Dysfunction of ocular motility is common in MG and very few patients fail to experience ptosis or diplopia at some point of their disease. The term "ocular MG" (OMG) refers to the disease clinically confined to extrinsic ocular muscles (EOMs). Hereinafter, we review OMG pathophysiology and clinical aspects and discuss issues that are still controversial in its management.

PATHOPHYSIOLOGICAL AND CLINICAL ASPECTS

Gaze control requires the precise and sustained activity of the oculomotor system and, in normal individuals, EOM contraction is stable under high rate motoneuron firing (10). EOMs have a unique biological organization with different compartments and six distinct fiber types according to innervation (singly and multiply innervated), metabolic arrangement, and protein expression pattern (10, 11). EOM increased susceptibility to MG can be related to structural and molecular properties different from those in other striated muscles. In EOMs, neuromuscular junctions (NMJs) often show underdeveloped postsynaptic folding (12), with a decreased content of AChRs (13). In addition, low expression of complement regulators, as the decay-accelerating factor (DAF) (14), can increase NMJ vulnerability to the effect of complement-activating AChR Abs and, possibly, LRP4 Abs. Lastly, it is well-known that EOMs express both the adult ($\alpha 2\beta\varepsilon\delta$) and the embryonic ($\alpha 2\beta\gamma\delta$) isoforms of the AChR (11). While the relevance of the fetal AChR as Ab target is unclear, its functional characteristics may foster susceptibility to MG. Fetal AChR has a longer open time and higher affinity for agonists (15) and, in a recent study, was found to recover more slowly from desensitization than the adult isoform (16). Such characteristics may reduce EOM adaptability to high-rate innervation and lead to impairment of NMT (16).

OMG should be suspected in patients with painless ophthalmoparesis and intact pupillary reflexes. Symptom fatigability, fluctuations in severity, and a remitting–relapsing course increase the likelihood of OMG diagnosis. Initial manifestations may consist of unilateral ptosis or diplopia due to weakness of a single EOM (17–19). Nonetheless, at the first examination, most patients have ptosis and diplopia with multiple muscle pareses (17–20). Weakness of the orbicularis oculi (which is a facial muscle), although uncommon at presentation (20), is frequent in the later course of the disease (18). AChR Ab-positive and dSN patients share a similar clinical pattern (19). Ptosis is usually asymmetrical, with rapid fluctuations and shifting from one eye to the other. EOMs can be involved in different combinations with a broad variability of unconjugated pareses. Complete external ophthalmoplegia occurs rarely and mostly in chronic disease (18). In patients with MuSK, Abs ocular symptoms tend to be less evident, often consisting in symmetrical gaze limitations with transitory diplopia and bilateral, largely symmetrical, ptosis (21–23). The pattern of ocular dysfunctions associated with anti-LRP4 other Abs has not been described.

INCIDENCE AND PREVALENCE

MG epidemiology has changed over the last decades with a steady increase in incidence (24–26) and prevalence rates (27, 28), particularly among elderly males. From recent data, it seems that these changes also include OMG.

It is generally accepted that, among adult Caucasians, more than 50% of MG patients present with ocular symptoms. The majority of these cases eventually develop generalized MG (GMG), most often within 2 years from onset, and up to 20% remain affected by OMG (29, 30). In a recent population-based survey, the annual incidence of OMG was 1.13/100,000 (31) at twice the rate previously reported (32), and contemporary studies have consistently shown an increased proportion of males with late-onset disease among incident cases (19, 31, 33). OMG prevalence depends on the generalization rate, which is related to several factors, such as disease duration, treatment, and, above all, ethnicity and age at onset. In Asian countries, particularly in China, a high proportion of patients present in childhood and remain affected with OMG (34, 35), and, irrespective of ethnicity, progression to GMG is more rare in children with prepubertal onset than in adults (35–37). Presence of thymoma (38), signs of NMT failure in limb muscles on electrophysiological testing (39, 40), detection of AChR (31, 41) and MuSK Abs (42), and increased serum levels of microRNA miR-30e-5p (43) were found to be associated with increased risk of secondary generalization. A protective role of immunosuppression was found in some studies (41, 44, 45), but was not confirmed by others (46).

Overall, generalization rate appears to be lower in current studies than in earlier reports based on immunosuppression-naïve patients. Moreover, in subjects treated early with steroids, disease progression may be delayed and become evident after treatment tapering or withdrawal. Recent data support this possibility showing that conversion time can be considerably longer than previously reported (47).

DIAGNOSIS

OMG is easily suspected in patients with fluctuating asymmetric ptosis and diplopia caused by involvement of multiple EOMs, as very few conditions can mimic such a pattern. On the other hand, the diagnosis can be tricky when ocular symptoms can be due to single nerve paresis or fatigability is not obvious.

OMG confirmation relies on serological, electrophysiological, and bed-side tests.

Serological Testing

AChR and MuSK Ab detection by radioimmunoassay (RIA) is highly specific, as AChR Abs are rarely found in subjects with other diseases or thymoma without MG (1, 48), and MuSK Abs have never been reported in non-MG patients. When MG is clinically suspected, AChR Abs are tested first, and MuSK Abs are assayed in AChR-negative cases. The detection of either Ab confirms the diagnosis, with no actual necessity for electrophysiological or clinical tests.

The sensitivity of AChR Abs in OMG is generally thought to be around 50% (19, 30). However, recent studies reported positivity rates higher than 70%, particularly in male patients (31, 49). These results strengthen the value of AChR Ab standard assay and warrant confirmation. Conversely, there have been very few reports of MuSK-positive OMG (22, 23), although MuSK Ab prevalence in this population has not been systematically investigated.

More recently, the development of sensitive cell-based assays (CBAs), in which Abs bind to antigens concentrated on cell membranes, has expanded the serological diagnosis of MG. Disease-specific "clustered AChR" Abs were reported in 16–45.8% of dSN patients (50–52) and, in a study (52), were strongly associated with prepubertal onset and OMG. In addition, MuSK Abs were detected by an IgG-specific CBA in 8% of dSN sera and, interestingly, 38% of these patients had OMG (53). Although these results are encouraging, it must be considered that CBAs require specific skills and facilities and are not largely available.

With different assays, LRP4 Abs were detected at variable rates (from 18.7 to 2.9%) in dSN patients and, in these studies, OMG frequency ranged 22–53% (5, 54). LRP4 Ab testing has the same limitations and, apparently, lower specificity than AChR and MuSK detection by CBA (55).

Methodological standardization and studies involving large cohorts are needed to establish the diagnostic yield of new Abs (56). At present, these assays should be reserved for dSN patients with positive results on electrophysiological testing or responsive to cholinesterase inhibitors (ChE-Is).

Abs to cortactin have been described in 9/38 dSN-MG patients with mild generalized or purely ocular disease (57). Cortactin, an intracellular muscle protein expressed at the NMJ, contributes to the stabilizations of AChR clusters (58). Abs to cortactin are likely not pathogenic and are not diagnostic of MG. Their possible role as marker of OMG (57) needs confirmation.

Electrophysiological Studies

In MG, low-rate repetitive nerve stimulation (RNS) typically elicits a >10% decrement of the compound muscle action potential between the first and the fourth or fifth stimuli, and single fiber-EMG (SF-EMG) shows an abnormally increased jitter and, when NMT impairment is severe, intermittent blocking of the second potential. Electrophysiology diagnostic yield depends on testing weak muscles, although SF-EMG can detect an increased jitter in subclinical MG (59).

RNS has limited sensitivity in patients with OMG. In different studies, positivity rates ranged from 16.7 to 44% (19, 60–62), consistently associated with high specificity (63). SF-EMG, when performed in the orbicularis oculi muscle, was found to be 79–100% sensitive for the detection of OMG (19, 63–66), but it is time consuming and not largely available. An altered SF-EMG is commonly found in other disorders of NMT like Lambert-Eaton myasthenic syndrome (LEMS) and botulism, and both low-rate RNS and SF-EMG are frequently positive in congenital myasthenic syndromes (CMS). However, these diseases rarely manifest with purely ocular symptoms. On the other hand, finding an increased jitter in conditions that can closely mimic OMG, as chronic progressive ophthalmoplegia, incomplete Miller-Fisher syndrome (MFS), and ptosis following botulinum toxin injection, may complicate the diagnosis (67).

Repetitive ocular vestibular evoked myogenic potentials (RoVEMPs) can detect muscle fatigability through direct recording from EOMs. RoVEMPs, at stimulation rates of 20–30 Hz and recording from inferior oblique muscles, effectively distinguished MG patients from healthy controls (sensitivity 71–89% and specificity 64–86%) (68–70) and from patients with other neuromuscular diseases (sensitivity 67% and specificity 82%) (70). This non-invasive technique is a promising diagnostic tool and warrants confirmation in further studies.

Response to Cholinesterase-Inhibitors

In MG patients, ChE-Is improve NMT by prolonging ACh half-life at the motor endplate. A positive response as unequivocal improvement strongly supports the diagnosis.

The infusion of the short-acting agent edrophonium chloride (max dosage 10 mg) has been used for several decades as confirmatory test. In OMG patients, clinical improvement can be readily quantified when obvious ptosis and/or severe restriction of ocular motility are present. In different studies, response rates ranged between 88 and 95% (19, 63, 71). Edrophonium injection often elicits lacrimation, sweating and fasciculations; as more serious adverse effects (AEs) as bronchoconstriction and severe bradycardia can occur, atropine should always be kept at reach. Responsiveness to ChE-Is can also be tested with neostigmine (1–2 mg, i.m.) or pyridostigmine (60 mg, orally), with clinical evaluation after 15–30 min and 45–60 min, respectively. These slow-acting ChE-Is may have a lower diagnostic sensitivity than edrophonium given the more gradual clinical effect. A positive reaction to ChE-Is is observed in most CMS and to a lesser extent in LEMS and botulism. False responses have been described in amyotrophic lateral sclerosis, peripheral neuropathy, and, rarely, in patients with mitochondrial myopathy (72) and intracranial tumors (73).

Other Bedside Tests

The ice pack, rest, and sleep tests are particularly helpful in patients with ptosis. Clinical evaluation of ptosis and ocular motility are performed before and immediately after an ice pack has been placed over the patient's closed eyelids for 2–5 min (ice pack test), the patient has kept his/her eyelids closed for 5 min (rest test), or the patient has slept or rested for 30 min (sleep test). A positive response consists in clear-cut

TABLE 1 | Sensitivity and specificity of diagnostic tests in ocular myasthenia gravis.

	Sensitivity (% positivity)	Specificity
Anti-acetylcholine receptor antibodies by radioimmunoassay	38[19]-48[62]-70.9[49]-73[31]	98[63]-100[19]
Repetitive nerve stimulation	16.7[62]-24[19]-33[61]-44[60]	83[62]-84[19]-94[63]
Single-fiber electromyography	79[64]-90[19]-94[66]-100[62]	80[64]-92[63]-100[19]
Repetitive ocular vestibular evoked potentials	80[70]-89[68]	64[68]-82[70]
Response to cholinesterase inhibitors	88[19]-92[63]	50[19]-97[63]
Ice-pack test	80[19]-86[66]-92[76]-96[75]	25[19]-79[66]-79[76]-88[75]

Numbers superscripted in square brackets are the related references.

symptom relief. The ice pack test sensitivity was 76.9% in patients with diplopia (74) and 92–96% in those with ptosis with specificity ranging 79–98% (66, 74–76). When the effects of the ice pack test and the rest test were compared in patients with ptosis, the former produced a stronger response (77). These assessments can be safely performed in patients with contraindications to edrophonium. In a recent study, the combination of positive results of the ice pack test and SF-EMF was associated with higher specificity (66). **Table 1** summarizes the sensitivity and specificity of the main diagnostic tests in OMG. Variability among studies may reflect differences in the study population.

OMG may mimic intracranial lesions, ocular neuropathy, migraine, internuclear ophthalmoplegia, MFS without obvious ataxia and pupillary abnormalities, progressive external ophthalmoplegia, levator aponeurosis, and orbital inflammatory disease. Thyroid disease is frequently associated with OMG (78). Magnetic resonance imaging of the brain and orbits is indicated when OMG is uncertain and even in patients with established diagnosis with atypical symptoms (79).

In patients diagnosed with OMG, particularly those with AChR Abs, a chest computed tomography is mandatory to rule out a thymoma.

THERAPEUTIC OPTIONS

Ptosis and, even more, diplopia interfere with daily activities and impact on health-related quality of life (QoL) (80). In addition, patients are concerned about the possibility of symptom generalization and frequently ask whether this may be prevented. Clinical management is complicated by lack of Class I evidence (81).

ChE-Is are first-line treatment in nearly all OMG patients. Oral pyridostigmine (90–300 mg/day) may sufficiently relieve mild to moderate ptosis but is less effective in resolving diplopia (82, 83). ChE-Is are generally well-tolerated and AEs, mostly consisting of diarrhea, hyperhidrosis, and muscle cramps, can usually be controlled by dose adjusting. These agents do not reduce the risk for secondary generalization. In addition, patients with chronic symptomatic OMG can develop permanent ophthalmoparesis and muscle atrophy, with reduced chance of recovery (30).

Patients with unsatisfactory response to ChE-Is are candidate for immunosuppressive treatment. In retrospective studies, prednisone and prednisolone were effective in relieving symptoms, with response rates ranging 66–86% (19, 84–86) and the rate of disease progression was much lower in patients under steroids than in those receiving pyridostigmine only (41, 44, 45, 85, 86). The EPITOME trial investigated the safety and efficacy of prednisone in OMG patients with unsatisfactory response to pyridostigmine (87). The study was closed early because of slow enrollment (11 patients were randomized of the 88 planned). Although severely underpowered, this trial showed a clear superiority of prednisone over placebo, as 83% of patients receiving prednisone (and no patient on placebo) achieved the status of minimal manifestations (MM) (88). No patient progressed to GMG (87).

Oral treatment with prednisone or prednisolone is first-choice immunosuppression in patients with disabling OMG. Treatment can be started at full dosage or with an escalating regimen, but maximum doses (25–50 mg/day) are generally lower than in GMG (83, 89, 90). Once symptom control has been achieved, prednisone is slowly tapered to the lowest effective dose or withdrawal. Maintenance doses $\leq$5 mg/day are well-tolerated with a favorable impact on QoL (91). Prednisone is largely available and has a rapid effect, and, in OMG, the risk of "early deterioration" is not a concern. In a recent study comparing OMG response to two steroid regimes, i.e., high-dose intravenous methyl prednisolone (IVMP) and low-dose oral prednisone, IVMP was associated with faster improvement (92). This finding deserves confirmation in further studies. Steroid-sparing agents are frequently used in long-term therapy, with the same criteria and treatment regimens as in GMG (89, 90). In uncontrolled studies, azathioprine (85), mycophenolate mofetil (19, 93), and tacrolimus (94) were beneficial both in relieving symptoms and in preventing disease progression.

OMG is deemed refractory when patients do not respond to immunosuppression or require high-dose regimens with intolerable AEs (95). In these cases, treatment options are far more limited than in GMG. Plasma exchange was tried in very few patients and resulted in no benefit (19, 96). In a RCT, no response to intravenous immunoglobulin was detected in a small OMG population (97). B cell depletion with rituximab, a chimeric anti-CD20 monoclonal Ab, has gained increasing popularity in the treatment of GMG (4, 90, 98). Rituximab was very effective in the few OMG subjects treated so far (99, 100) and, although evidence is scarce, may be considered in refractory disease. Lastly, OMG patients were not included in RCTs investigating novel therapeutic options based on complement inhibition or competition with IgG for the Fc neonatal receptor (101).

Thymectomy, when feasible, is obviously indicated in thymoma patients. Conversely, the role of therapeutic thymectomy (i.e., in non-thymoma patients for the treatment

of MG) has been the object of a long-standing debate. In the past decades, when the recommended surgical technique was extended trans-sternal thymectomy, it was considered a too aggressive option for a limited disease. Currently, minimally invasive surgery can make thymectomy more acceptable in this population. As available evidence comes from retrospective heterogeneous studies, no firm conclusions can be drawn on the efficacy of thymectomy in OMG. Some investigations reported no clear benefit (102–104), while others found early thymectomy to be associated with an increased probability of remission and improvement (105–108). In a metanalysis, the pooled remission rate after surgery was 50% with a better outcome in pediatric patients (108). In current practice, thymectomy is considered on an individual basis (109), as initial treatment in early-onset AChR-positive OMG (110) or for patients with unsatisfactory response to immunosuppression (83).

Supportive measures as crutch glasses for severe ptosis and prisms in patients with diplopia are helpful in treatment-resistant OMG. Ptosis surgical correction is effective and well-tolerated when exposure keratitis is avoided (90, 111); the possibility of diplopia worsening should be discussed beforehand. Topical naphazoline, a mainly α2-agonist, was found to be effective in relieving mild to moderate ptosis (112). Strabismus surgery can be considered in patients with stable ocular misalignment (113), but recurrences can complicate the long-term course.

CONCLUSIONS

There are still considerable challenges in the diagnosis and treatment of OMG. In dSN patients, borderline results on SF-EMG and ambiguous response to ChE-Is may be misleading and the diagnostic utility of new Ab assays is not yet established. Early steroid treatment is often required to improve symptoms and may reduce disease progression. OMG prognosis is generally good in patients who can achieve and maintain symptom control with low-dose treatment, but it is much less favorable in those with relapsing disease requiring prolonged immunosuppressive therapy at high-dose regimens. At present, there are few treatment options for refractory OMG. Clinical management would greatly benefit from RCTs and prospective studies in large cohorts.

AUTHOR CONTRIBUTIONS

AE drafted the manuscript. RI revised the manuscript for intellectual content. Both authors contributed to the article and approved the submitted version.

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REVIEW
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Immunopathology of Autoimmune Myasthenia Gravis: Implications for Improved Testing Algorithms and Treatment Strategies

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Myasthenia gravis (MG) is a heterogeneous condition, characterized by autoantibodies (Abs) that target functionally important structures within neuromuscular junctions (NMJ), thus affecting nerve-to-muscle transmission. MG patients are more often now subgrouped based on the profile of serum autoantibodies, which segregate with clinical presentation, immunopathology, and their response to therapies. The serological testing plays an essential role in confirming MG diagnosis and guiding disease management, although a small percentage of MG patients remain negative for antibodies. With the advancements in new highly effective pathophysiologically-specific immunotherapeutic options, it has become increasingly important to identify the specific Abs responsible for the pathogenicity in individual MG patients. There are several new assays and protocols being developed for the improved detection of Abs in MG patients. This review focuses on the divergent immunopathological mechanisms in MG, and discusses their relevance to improved diagnostic and treatment. We propose a comprehensive "reflex testing," algorithm for the presence of MG autoantibodies, and foresee that in the near future, the convenience and specificity of novel assays will permit the clinicians to consider them into routine systematic testing, thus stimulating laboratories to make these tests available. Moreover, adopting treatment driven testing algorithms will be crucial to identify subgroups of patients potentially benefiting from novel immunotherapies for MG.

Keywords: CBAs 2, LRP4, Musk, AChR, myasthenia gravis (MG), autoantibodies (Abs), RIPA

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INTRODUCTION

Myasthenia gravis (MG) is an autoimmune disorder, caused by autoantibodies (Abs) that target functionally important components at the neuromuscular junction (NMJ) in the postsynaptic muscle membrane (1, 2). MG is a heterogeneous condition with remarkably distinct immunopathology, autoimmune profile, and the multifaceted immune response (2–4). MG patients are subgrouped based on the presence of Abs as well as their clinical phenotypes, thymus pathology, and age at onset (4–7). Antibody testing has a crucial role for clinical diagnosis confirmation and treatment. Majority of MG patients (around 80–85%) develop Abs against the acetylcholine receptors (AChR; AChR MG), whereas muscle-specific kinase Abs (MuSK; MuSK MG) are detected in 1–10% patients, depending on detection techniques used and the differences

between the source population (5, 8, 9). Interestingly, Abs are not detected in around 1–15% of MG patients [that is, negative for AChR, and MuSK Abs with current gold standard methods; seronegative MG (SNMG)] (4, 5). It is reasonable to believe that SNMG patients probably have a low affinity/low titer Abs against known antigens that are below the detection levels of currently available gold standard tests. It is also speculated that the target antigens in the NMJ are not yet fully discovered (10). Consequently, considerable efforts have been made to develop improved Abs detection methods as well as finding novel target antigens at the NMJ. In recent years, new Abs have been discovered in some of the MG patients targeted against lipoprotein-receptor-related protein 4 (LRP4), agrin, acetylcholinesterase (AChE)/collagen Q (ColQ), anti-striational muscle [that is, Kv1.4 potassium channel, titin, and ryanodine receptors (RyR)] and cortactin antigens at NMJ (11, 12). Unfortunately, as most of these Abs co-exists with anti-AChRAbs (AChRAbs) and/or MuSK Abs, it is difficult to generate strong scientific evidence to prove their direct contribution to MG pathogenicity. The anti-LRP4, anti-striational, and anti-cortactin Abs are of particular interest as they are associated with distinct clinical pathology in MG patients, although future research is needed to define the potential for these antibodies in the clinic (10, 11).

With the development of novel therapeutic regimens customized for different MG subgroups, it is particularly important to identify the specific Abs with more sensitive diagnostics methods. One of the major progresses in the field has been the development of novel live cell-based assays (CBAs) for the detection of Abs in SNMG patients (13). The improved specificity and sensitivity that CBAs offer has significantly changed the MG diagnostics algorithms (5, 10). The CBAs are now increasingly used in comprehensive testing for the detection of clustered AChR, MuSK, and LRP4 Abs in MG patients (4, 14). The CBAs can also generate quantifiable and highly accurate results when the target antigen-Abs interactions are measured using flow cytometry (10, 15–17).

The distinct immunopathology of MG is strongly associated with heterogeneity that is observed among different subgroups of MG patients. The typical clinical feature of MG is muscle weakness that fluctuates and worsens with active muscle use, and improves with rest. Initial weakness often starts with extraocular muscles [Ocular MG (OMG)], with a classic presentation of intermittent drooping of the upper eyelid (ptosis) and rapidly progressive double vision (diplopia) (18–20). In ∼15% of patients, the symptoms remain ocular, however, for the majority of patients (85%) symptoms progress to limb and bulbar muscles, resulting in generalized MG (GMG), usually within the first 2 years (4, 5). Respiratory muscles can also be affected (4, 8). It is interesting to note that weakness in myasthenia can be alleviated by applying cold on the weak muscle thus blocking the effect of acetylcholine esterase and improving strength. This is the basis of the ocular ice-test. The OMG without anti-AChRAbs generally is a harbinger of a milder disease, if it does not become generalized in the first 2 years. Thymic abnormalities (thymoma-associated MG; TAMG) are common in GMG patients with almost 50% having thymic hyperplasia, and 10–15% having a thymic tumor (5). The thymus can show follicular hypertrophy and secretes AChRAbs (21), particularly in younger patients with AChR MG (21, 22).

The TAMG is more often seen with anti-striational muscle antibodies, (mainly titin or to RyR) almost always in the context of AChRAbs positivity (23–26). Gender and age at onset also play a critical role in AChR MG pathogenicity. The disease has two typical peaks of onset; early-onset MG (EOMG, <50 years), with a predominance of females and late-onset MG (LOMG, >50 years), that have a larger proportion of males (18, 24). In contrast, neonatal and juvenile MG is relatively uncommon and symptoms are usually less severe and limited to OMG form (18, 27–30). Genetic studies have revealed strong relationship between human leukocyte antigen, HLA-DQA1, DQB1 with thymoma, while HLA-DQB1and DRB1alleles were associated with EOMG, LOMG and OMG (31, 32). Modern epidemiological studies show that the incidence of Myasthenia is increasing in the aged (33).

On the opposite of the scale, MuSK MG has a more dangerous prognosis with prominent bulbar, neck and respiratory muscle involvement and frequent respiratory crisis (8). Characteristic of the clinical picture is the midline tongue atrophy, even-though patients may present with other classic MG symptoms including GMG and OMG. The thymus does not appear to be involved (no LFH, no thymoma) in patients with MuSK MG (8). Interestingly, MuSK MG has a marked female dominance with a female to male ratio of 9:1 (4, 7). Strong association with HLA-DRB1, DQB1, DQ5, and DR14 has been reported in patients positive with MuSK Abs (31, 34). Fortunately, IgG4 subtypes predominate in MuSK MG and responds well to B-cell depletion therapy with Rituximab (8). In contrast, LRP4 associated MG has a female to male ratio of only 2.5:1 (35). The disease is generally associated with late-onset age, and a milder phenotype with variable thymus pathology (35, 36). However, a recent multicentric study demonstrated that LRP4 patients have a more severe presentation than quadruple seronegative MG (negative for AChR, MuSK, LRP4, and agrin) patients (37). The combination of antibodies to agrin and LRP4 produces more severe symptoms than LRP4 alone (37).

In this review, we focus on immunopathological mechanisms of the most common muscle Abs that have been associated with MG, and their relevance for developing improved testing algorithms and therapies. Major clinical MG subtypes, common detection methods, and treatment of choices are summarized in **Table 1**.

NEUROMUSCULAR JUNCTION AND IMMUNOPATHOLOGICAL MECHANISMS

The NMJ is a synaptic connection between the presynaptic motor nerve terminal and postsynaptic skeletal muscle membrane. NMJ is responsible for transmitting action potential from nerve-to-muscle cells. The antigens which are targeted by Abs in MG are located throughout the post-junctional region and can be classified under two main groups: transmembrane or extracellular antigens and cytoplasmic or intracellular antigens (51). A deeper understanding of the mechanisms of

TABLE 1 | Summary of the major clinical MG subtypes, common detection methods, and treatment of choices.

MG subtypes	Clinical phenotypes/IgG subclass	Detection methods	Treatments	References
AChR MG	Thymoma associated MG, OMG, GMG, early onset MG, late onset MG, refractory GMG, /IgG1, IgG3	RIPA, ELISA, FIPA, dot-blots	TAMG-Thymectomy OMG, GMG-pyridostigmine, prednisone, IVIG, and PLEX Refractory GMG-eculizumab	(4, 34–36, 38)
Clustered AChR MG	Milder symptoms than AChR MG/IgG1, IgG3	Live CBAs	Treatments similar to AChR MG	(5, 12–14)
MuSK MG	Bulbar symptoms, refractory GMG /IgG4	RIPA, Live CBAs, ELISA, FIPA	PLEX and prednisone Refractory GMG-rituximab	(1, 5, 39–42)
LRP4 MG	Mild to severe symptoms, Variable thymoma/IgG1, IgG2	Live CBAs, ELISA	Treatments similar to AChR MG	(6, 43–45)
Striational muscle MG	Titin and RyR Abs in Thymoma/N/A	Immunofluorescence, RIPA, ELISA	N/A	(4, 46–48)
Cortactin MG	OMG, mild GMG/N/A	ELISA, western blots	N/A	(4, 49, 50)

immunopathology is critically important to develop improved diagnostics and customized treatment plans to their respective MG subgroups.

AUTOANTIBODIES TARGETING TRANSMEMBRANE OR EXTRACELLULAR ANTIGENS

AChR Antibodies (AChRAbs)

The muscle AChR of the NMJ is the most common targets for Abs attack in MG patients. The muscle AChR is a transmembrane pentameric structure that exists in two developmentally regulated subtypes: fetal/embryonic and adult AChR. The fetal or embryonic AChR glycoprotein is made up of 2α: β: ɤ: δ subunits, whereas in the adult AChR, the expression of the ϵ-subunit is replaced by the ɤ subunit within the AChR pentameric structure (52, 53). Each of these subunits is composed of an extracellular domain, four transmembrane domains, and an intracellular domain (4). The AChRAbs can target extracellular domains of all five subunits, including ɤ- subunit of the fetal AChR although Abs targeting α-subunit are the main immunogenic region (MIR) and more pathogenic (53, 54). AChRAbs primarily belong to IgG1 and IgG3 subclasses (that can activate complement cascade) and can be detected in around 80–85% of GMG patients and 50–75% of patients with OMG (22, 55). Interestingly, nearly 100% of patients with TAMG have detectable serum AChRAbs (22). The presence of AChRAbs is specific for MG diagnosis as false-positives are uncommon in healthy individuals as well as with other neuroimmunological conditions. The immunopathologic mechanisms by which these Abs can affect the signal transmission are: cross-linking of AChR leading to increased endocytosis; activation of complement cascade causing AChR loss and destruction of the postjunctional membrane; and also by directly blocking the acetylcholine binding to AChR site (**Figure 1A**) (56, 57).

For the past several years, the radioimmunoprecipitation assay (RIPA) method has been the gold standard test for the detection of AChRAbs, with nearly 100% test specificity. That is, if the patient with muscle weakness tested positive for AChRAbs by RIPA, clinical diagnosis of MG can be confirmed (58). Human AChR used in RIPA is usually obtained from human muscles or AChR-expressing cell lines, such as TE671 cell line (that expresses fetal AChR), or CN21 cell line, (that expresses both fetal and adult AChR) (59). The RIPA is based on the labeling of human AChR antigens with 125I-α-bungarotoxin and then precipitating the complex of labeled AChR-with patients AChR binding Abs using a secondary antibody "in solution." The precipitate is counted and compared with healthy control serum. If the test result is positive then, blocking with cold α-BT (unlabeled) is performed to verify binding results (51, 58). It is important to point out that although blocking with cold α-BT blocks the major AChRAbs target (the AChR α-subunit), false-negative blocking results are possible if other subunits of AChR pentamer are being targeted by AChRAbs. The overall sensitivity of the RIPA assay is reasonably good (80% in GMG and 50% in OMG) (51), however, it can be further improved if a mixture of both the adult and fetal forms of the receptors are used. Additionally, if exceptionally high radiation values (CPM; count per minute) are reported with positive results, repeat testing is recommended to avoid any human/technical errors. On the other hand, confounded test results, for example, false negatives are possible, if patients have received treatments including intravenous immunoglobulin (IVIG) or plasma exchange (PLEX) within 6 weeks of their antibody test or monoclonal antibodies (mAbs; Rituximab, Eculizumab) within 24 weeks of their test (personnel experience) (60). Therefore, any unexpected RIPA findings should always be confirmed with independent confirmatory methods, for example with highly specific CBAs, to provide a definitive diagnosis.

Although RIPA is the most commonly used detection method for the presence of AChRAbs, not all clinically relevant antibodies bind well to 125I-α-bungarotoxin labeled AChR antigens "in solution." In contrast, the AChRAbs that have low affinity for the soluble antigens, used in standard RIPA binds better to clustered AChR in its native form in the live CBAs (12–14). Typically, the HEK293 cells are transfected with fetal or adult AChR subunits (at a density similar to that at the NMJ), and rapsyn (to promote AChR clustering on the cell surface, clustered AChR) (61). The

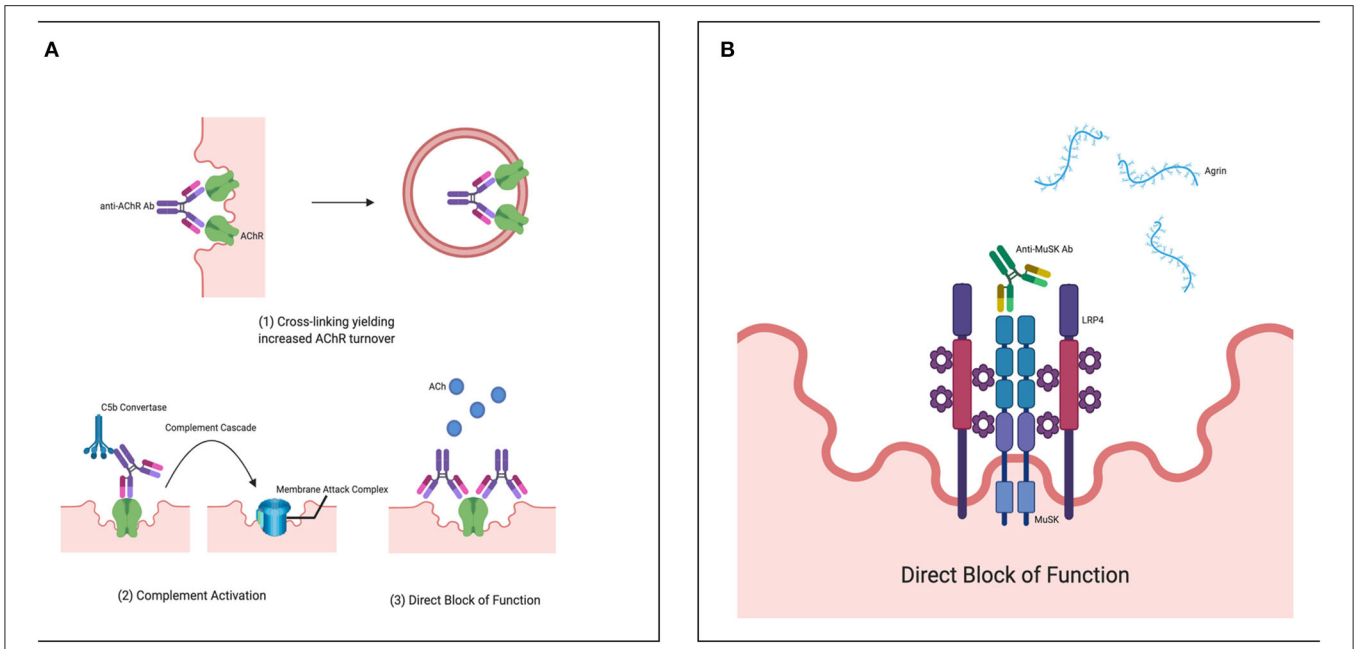

FIGURE 1 | (A) Immunopathologic mechanisms by which pathogenic AChR autoantibodies affect signal transmission. (1) By cross-linking of AChR leading to increased endocytosis (2) By activating complement cascade causing AChR loss and destruction (3) and also by directly blocking the acetylcholine binding to AChR site. Figures created with BioRender.com. **(B)** Immunopathologic mechanisms by which MuSK binding autoantibodies affect signal transmission: By binding directly to the extracellular domain of MuSK protein and block the MuSK protein interaction with LRP4-agrin complex that is required for AChR clustering at NMJ. Figure created with BioRender.com.

binding of the patient's serum is detected with a fluorescently labeled secondary antibody on a fluorescent microscope. Several studies have confirmed the CBAs ability for improved detection of AChRAbs that are usually not detectable by RIPA (5, 59, 61). In routine diagnostic settings, clustered AChRAbs are detected in around 20% of SNMG patients (5). Sensitivity of the live CBAs is here also further improved when both the adult and fetal forms of the AChR are used. The clustered AChRAb test is recommended as a reflex test in adult patients that tested negative for AChRAb by RIPA and have a clinical suspicion of MG. Moreover, the clustered AChR Abs positive patients are usually younger with higher OMG prevalence, and better treatment response (61). This is particularly useful in children, as they tend to have OMG or milder GMG disease (61). Additionally, for the pediatric population, the importance of distinguishing between acquired and congenital MG makes this high sensitivity clustered CBAs test a first-line option. In a recent study conducted at our laboratory: 7 out of 44 SNMG children (16%) tested positive for clustered AChRAb CBAs. All these 7 children with positive results have been clinically confirmed as having acquired MG.

Although most MG patients develop Abs against AChR antigens, the titer of AChRAb generally does not correlate well with clinical severity (62, 63). It is important to highlight that the poor correlation with disease severity is due to the fact that both assays (RIPA and CBAs) that are currently being used to detect pathogenic AChRAbs in MG patients only measure the circulating antibodies that bind. However, given the heterogeneity of MG patients, it will be important to measure a combination of antibodies that bind complement or modulate the receptors, in order to provide quantitative titers that would correlate better with disease severity.

Unfortunately, commercial CBA test kits are not yet available and the assay is highly complex, making it relatively difficult to incorporate the test for routine clinical diagnosis. The other limitation of the CBAs is that it is a semiquantitative method and cannot provide antibody titer information that might be used for individual patient management (5, 13, 14). The detection of AChRAb by quantitative flow cytometry offers a viable alternative to current CBAs and is being further evaluated for clinical application (16, 17, 64). Anti-AChR is also detected by enzyme-linked immunosorbent assay (ELISA), fluorescence immunoprecipitation assay (FIPA) and dot-blot methods, however, overall sensitivity and specificity are considerably lower than the RIPA assay or the live CBAs, making it difficult to rely on for clinical diagnosis (10, 65).

MuSK Antibodies (MuSKAbs)

The muscle MuSK of the NMJ is the second most common target for Abs attack in MG patients. MuSK is an anchoring protein, that has an extracellular domain, a transmembrane domain, and an intracellular domain with tyrosine kinase activity (39–41). The extracellular domain has three immunoglobulin-like regions (Ig1, Ig2, and Ig3) and a frizzled domain. MuSK protein is necessary for the maintenance of the NMJ structure and plays a crucial role in the process of AChR clustering (1, 41). Agrin released from the postsynaptic region binds to LRP4 protein (that is, LRP4-agrin complex), which in turn binds at Ig domains of the extracellular domain and activates MuSK (42). Activated MuSK

drives the clustering of AChR with the help of rapsyn protein that bridges the AChR at NMJ. MuSK Abs primarily belong to IgG4 subclass (that is, unable to activate complement cascade and not binding to FcReceptor thus unable to activate the feed-back loop controlling IgG synthesis). It can be detected in 1–10% of all MG patients and 10–40% among AChRAbs negative MG (66). MuSK pathogenic Abs bind directly to extracellular Ig domains and block the MuSK protein interaction with the LRP4-agrin complex that is required for AChR clustering at NMJ (**Figure 1B**) (8).

The MuSK antibodies can be detected by RIPA (as a reflex test in patients that are seronegative for AChRAbs and have a clinical suspicion of MG), which is a highly specific assay. The diagnosis of MuSKAbs in patient serum confirms the clinical diagnosis of MG, as false-positives results are uncommon among healthy individuals. However, some of the conformation-dependent MuSKAbs fail to bind to 125I-α-bungarotoxin labeled MuSK antigen in solution. In contrast, MuSK cell-based assay (MuSK-CBAs; HEK293 cells transfected with MuSK recombinant antigen) has been reported to have increased sensitivity (6–10%) due to additional detection of conformation-dependent MuSK Abs (5, 8). The titer of MuSK Abs correlates well with clinical improvements, thus laboratory testing of serial samples is recommended to monitor the clinical progress as well as after the therapy of the individual patients (67). Unfortunately, commercial test kits are not yet available for MuSK CBAs, limiting its use in routine clinical practices. The detection of MuSKAbs by quantitative flow cytometry is also being further evaluated for clinical application (68, 69). MuSKAbs could also be detected by ELISA, and FIPA methods, however, rigorous evaluations are required before their use in routine clinical practice (10, 63).

Lipoprotein-Receptor-Related Protein 4 (LRP4) Antibodies (LRP4Abs)

LRP4 has been recognized as a third autoimmune target in MG patients. On NMJ, LRP4 is a single transmembrane protein with one large extracellular domain (70). LRP4 acts as a muscle receptor for agrin and forms LRP4-agrin complex which in turn binds and activates MuSK kinase and promotes AChR clustering at NMJ (71, 72). The LRP4 pathogenic Abs are of IgG1/IgG2 subclass (and thus can activate the complement cascade and negative signal on IgG synthesis) that blocks the LRP4-agrin signaling, inactivate MuSK and inhibit AChR clustering at NMJ (43).

The LRP4Abs reflex testing is recommended in SNMG patient sera by CBAs (HEK293 cells transfected with LRP4 recombinant protein) or ELISA, although in CBAs the expression of LRP4 transmembrane protein has been difficult (10). The transport of LRP4 to the cell surface improves when the chaperon Mesdc2 is co-expressed, however, the effects is not profound (35, 44). Alternatively, transfected cells can be fixed and permeabilized, however, the accuracy of the permeabilized assay needs to be first optimized (35). Quantitative LRP4 assay has also been optimized using a flow cytofluorimetric detection system. LRP4Abs are reported with a wide variation range (2–45%) depending on the detection methods used and the differences between geographical locations (5, 45, 72). However, LRP4Abs are also present in around 8% of AChRAbs positive patients, 15–20% of MuSK positive patients, and 3.6% of patients with other neurological conditions (11, 45, 73). Furthermore, prevalence of LRP4Abs is also reported among population of amyotrophic lateral sclerosis (ALS) patients (10–23%); thus, more research is required to establish its specificity and clinical utility for MG diagnosis (60, 74). As such the detection of LRP4Abs in patient blood alone may not establish MG diagnosis and any positive laboratory results should always be analyzed with the clinical correlation of the patient's symptoms.

Agrin Antibodies (AgrinAbs)

Agrin is a proteoglycan released from the motor nerve that binds to LRP4 and forms LRP4-agrin complex that is critical for MuSK activation and AChR clustering at NMJ (4). The agrinAbs are tested in patient sera by CBAs (HEK293 cells transfected with recombinant agrin proteins) or ELISA method (11). AgrinAbs were detected in ∼50% of known triple seronegative MG patients (that is, AChR, MuSK or LRP4 antibodies negative) (45, 72). However, agrinAbs are also detected in MG patients (2–15%) with or without AChRAbs and MuSK antibodies (5, 14). Moreover, high levels of agrinAbs are found among ALS patients (60), suggesting that the detection of agrinAbs are not specific from a diagnostics standpoint. Furthermore, in a recent study, although most agrin positive patients were presented with severe form of disease, they responded well to standard MG therapy (37).Thus the clinical utility of routine agrinAbs testing is currently not evident.

Acetylcholinesterase (AChE)/Collagen Q (ColQ) Antibodies (ColQAbs)

ColQ proteins expressed in the extracellular matrix at NMJ are crucial for anchoring and concentrating AChE (i.e., AChE/ColQ complex) (10, 14). At synaptic basal lamina the interaction with MuSK protein anchors this complex. ColQ Abs possibly disrupt the AChE/ColQ complexes, thus reducing the amounts of AChE on the cell surfaces (75). In addition, the MuSK Abs can block ColQ-MuSK interactions that subsequently may reduce AChR clustering. Anti ColQ fused with the transmembrane domain of contactin-associated protein-like 2 (CASPR2) in CBAs have detected ColQAbs in 3% of MG patients, although similar frequencies are reported in the controls (76, 77). Currently, ColQAbs has no role in clinical testing.

Striational Antibodies (Kv1.4 Antibodies) (Kv1.4Abs)

Voltage-gated potassium channel Kv1.4 are membrane proteins present in skeletal and heart muscles (78, 79). Kv1.4 Abs against the α-subunit of Kv1.4 are detected in 10–20% of MG patients. The Kv1.4Abs can be tested in patient sera by CBAs (HEK293 cells transfected with recombinant Kv1.4 proteins) or SDS-PAGE method (80). In Japanese patients the presence of Kv1.4Abs has been associated with mild to severe disease, myasthenia crisis, and thymic abnormalities (81, 82). In a recent study, a flow cytometric CBAs has detected Kv1.4 Abs with increased sensitivity from MG patients with myositis and/or myocarditis as well as late onset MG and thymoma associated MG (15). Although Kv1.4 positive tests can predict thymoma-associated

MG and disease severity, they currently have a limited clinical role in and CAT scanning is the test of choice (15).

AUTOANTIBODIES TARGETING INTRACELLULAR PROTEINS

Although intracellular localization of these antigens makes them unlikely to play a direct role in MG pathogenicity, however, they could be useful biomarkers for clinical characteristics, and/or thymus pathology in MG patients (4, 10, 83).

Striational Antibodies [TitinAbs and Ryanodine Receptor (RyRAbs)]

Titin is the largest known intracellular protein in striated muscle cells. The titin Abs are usually tested in patient sera by commercial immunofluorescence, ELISA, and RIPA tests (4, 84). TitinAbs are detected in around 20–40% AChRAb positive MG patients, with associated symptoms of late onset MG and thymoma-associated MG, therefore the presence of titin Abs in early onset MG patients could be a biomarker for thymoma (24–26, 81, 85, 86). Titin Abs are also detected in approximately 13% of known triple seronegative MG patients (that is, AChR, MuSK, or LRP4 Abs negative) (46–48). Similar to titin, RyRAbs are also associated with late onset MG and thymoma (15, 87). The RyRAbs are detected by ELISA or western blot methods. However, recently, flow cytometric CBAs have been used for the quantification of these antibodies with higher sensitivity than ELISA (14, 15). In addition, the MG patients with myositis as well as late onset MG and thymic abnormalities associated MG tested positive for the presence of anti-titin, and RyRAbs (26, 88–90). Additional research is required to define full potential for these antibodies in the clinic.

Cortactin Antibodies (CortactinAbs)

Cortactin is an intracellular protein that promotes actin assembly and MuSK mediated AChR clustering at NMJ. The cortactinAbs can be detected by ELISA or western blots. CortactinAbs are detected in 20% of SNMG, however, they are also detected in 10% of AChR MG patients and 5% of healthy controls (49, 50). Interestingly, most of the patients with cortactin Abs are associated with ocular or mild GMG (10, 50, 91). The role of cortactinAbs in the clinical meaning is still to be clarified and probably should be performed only in research settings.

IMPLICATIONS FOR THERAPIES

Accurate antibodies detection is crucial for diagnosis and prognosis, together with other factors, such as thymus histology, age and clinical features. For instance, AChR antibody-positive patients tend to have follicular hyperplasia of the thymus and practically all cases of thymoma are AChRAbs positive, thus thymectomy (surgical removal of thymus) is a first-line treatment choice in AChR MG, excluding patients with only OMG (29, 92–94). In addition, refractory AChR-MG is usually present in patients with thymoma. Thymectomy is a preferred option in AChR Abs positive patients that are also positive for anti-striational Abs, as TAMG is more often seen in presence of anti-striational muscle antibodies (23–25, 62, 95). In contrast, benefits of removal of thymus are uncertain in MuSK MG, LRP4 MG, and Agrin MG patients as thymic abnormalities are very rare in these patients (4, 63, 94, 96, 97).

Standard treatment choices for MG includes AChE inhibitors (pyridostigmine), corticosteroid (prednisone), IVIG and PLEX, although the distinct MG subgrouping has a strong influence in order to adopt the best conventional therapeutic options (6, 63, 92). For example, MuSK Abs positive patients tend to have more severe symptoms and are less responsive to pyridostigmine and IVIG treatments. However, they do well with PLEX, prednisone and rituximab (RTX) treatments (98). In contrast, LRP-4 Abs positive patients generally have milder phenotype and they respond well to pyridostigmine, prednisone as well as IVIG treatments similar to AChR Abs positive patients (6, 45). Unfortunately, there are no clear guidelines yet for the management of SNMG patients (6).

Patients with refractory MG (lack of response with standard therapies) more frequently have AChR GMG (with or without thymoma) or MuSK MG (1, 4, 18, 99). Several groups have investigated the efficacies of antigen-specific novel immunotherapeutic options such as B cell targeting therapies for the treatment of refractory MG patients. In particular, the anti-CD20 mAbs rituximab has been a preferred second-line treatment choice in MuSK MG patients with a large ptoportion of complete stable remissions observed; still some patients do not respond (99–102). Furthermore, monitoring MuSK Abs titers could be useful to establish overall disease severity and/or clinical improvements after RTX therapy (103). On the other hand, improvement is less apparent for AChR MG patients with high relapse rates after RTX treatment (103–105). Moreover, as discussed above, the binding titers of AChR Abs do not correlate well with clinical severity in MG patients after RTX treatment (63, 106). In patients with refractory AChR positive GMG, a complement inhibitor humanized mAb, eculizumab has demonstrated significant improvements, although some patients do not respond (38). The eculizumab has been approved by the USFDA, Health Canada, and the European Medicines Agency for the treatment of refractory generalized, AChR Abs positive MG (38, 107–110). Its cost however is close to prohibitive (CAD 500,000/year). Refractory MG can also be managed by periodic IVIG infusions or PLEX or subcutaneous IG (SCIG) treatments (1, 4, 111). In addition, several early-stage novel immunotherapeutic trials including, the new generation of complement inhibitors, neonatal Fc receptor (FcRn) inhibitors, and proteasome inhibitors are currently underway, although the results are not yet fully available (4, 83, 112, 113). Unfortunately, such clinical experiences are currently lacking for SNMG or LRP4 MG patients (92, 112).

CONCLUSIONS

A deeper understanding of the different autoimmune mechanisms in MG disease is important in order to design

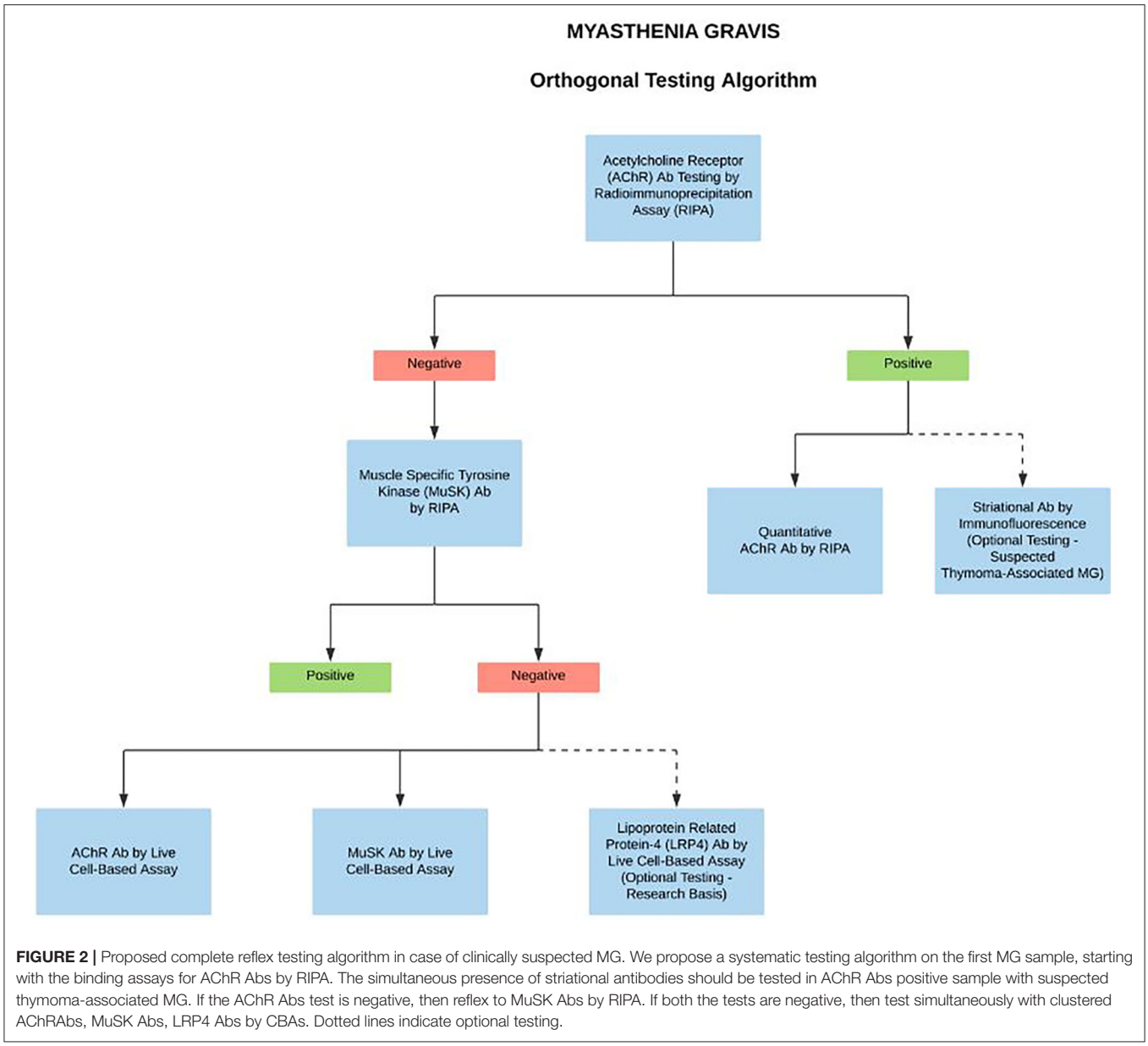

FIGURE 2 | Proposed complete reflex testing algorithm in case of clinically suspected MG. We propose a systematic testing algorithm on the first MG sample, starting with the binding assays for AChR Abs by RIPA. The simultaneous presence of striational antibodies should be tested in AChR Abs positive sample with suspected thymoma-associated MG. If the AChR Abs test is negative, then reflex to MuSK Abs by RIPA. If both the tests are negative, then test simultaneously with clustered AChRAbs, MuSK Abs, LRP4 Abs by CBAs. Dotted lines indicate optional testing.

better diagnostics and to personalize treatment options. There are several new assays currently under development for the detection of Abs in MG patients; however, perhaps one of the most significant developments in the overall MG field has been the recent launch of CBAs (4, 5, 14). The CBAs are highly specific and should be the method of choice for the systematic testing in case of clinical suspicion for clustered AChR MG, MuSK MG, and LRP4 MG (1, 4, 10). However, due to the unavailability of commercial CBA kits, they are currently used as a reflex test in highly specialized laboratories for patients that are seronegative by standard RIPA. Nevertheless, with the development of improved serological methods, and more importantly early and novel therapies targeting immune mechanisms specific to MG subtypes, there has been a recent proposed change to MG testing algorithms. Since many treatments influence the laboratory assay performance, if the patient is under the care of a neurologist or ophthalmologist, we propose a full reflex testing algorithm on the first pre-treatment sample in case of clinically suspected MG. Starting with the binding/blocking assays for AChR Abs by RIPA. The simultaneous presence of striational antibodies should be tested in AChR Abs positive sample with suspected thymoma-associated MG (optional test). If AChR Abs tested negative, then reflex to MuSK Abs by RIPA. If MuSK tests found negative, then concurrent testing with high sensitivity clustered AChRAbs, MuSK Abs, and LRP4 Abs by CBAs (optional, on research basis) (**Figure 2**). Importantly, the algorithm-based approach does not affect the test turnaround time and the delivery of care as the CBAs are performed and reported simultaneously. We anticipate that the sensitive and accurate detection algorithms

will be crucial for considering novel treatments for MG disease subtypes.

AUTHOR CONTRIBUTIONS

HF and PK performed the literature search, wrote and edited the manuscript. JO performed the literature search, reviewed and edited the manuscript. All authors contributed to the article and approved the submitted version.

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Immunoregulatory Cells in Myasthenia Gravis

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Myasthenia gravis (MG) is a T cell-dependent, B-cell mediated autoimmune disease caused by antibodies against the nicotinic acetylcholine receptor or other components of the post-synaptic muscle endplate at the neuromuscular junction. These specific antibodies serve as excellent biomarkers for diagnosis, but do not adequately substitute for clinical evaluations to predict disease severity or treatment response. Several immunoregulatory cell populations are implicated in the pathogenesis of MG. The immunophenotype of these populations has been well-characterized in human peripheral blood. $CD4^+FoxP3^+$ regulatory T cells (Tregs) are functionally defective in MG, but there is a lack of consensus on whether they show numerical perturbations. Myeloid-derived suppressor cells (MDSCs) have also been explored in the context of MG. Adoptive transfer of $CD4^+FoxP3^+$ Tregs or MDSCs suppresses ongoing experimental autoimmune MG (EAMG), a rodent model of MG, suggesting a protective role of both populations in this disease. An imbalance between follicular Tregs and follicular T helper cells is found in untreated MG patients, correlating with disease manifestations. There is an inverse correlation between the frequency of circulating IL-10–producing B cells and clinical status in MG patients. Taken together, both functional and numerical defects in various populations of immunoregulatory cells in EAMG and human MG have been demonstrated, but how they relate to pathogenesis and whether these cells can serve as biomarkers of disease activity in humans deserve further exploration.

Keywords: myasthenia gravis, regulatory T cells (Treg), follicular, circulating, regulatory B cells (Breg)

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INTRODUCTION

Myasthenia gravis (MG) is a chronic autoimmune disease characterized by muscle weakness and fatigue (1, 2). Pathogenic autoantibodies in MG target components of the post-synaptic muscle endplate located at the neuromuscular junction, impairing neuromuscular transmission (3). A vast majority of patients have antibodies against muscle nicotinic acetylcholine receptors (AChRs); a minority have antibodies against muscle-specific tyrosine kinase (MuSK) or low-density lipoprotein receptor-related protein 4 (LRP4) (2, 4). MG patients without detectable autoantibodies are referred to as having seronegative MG. Apart from autoantibody specificity, MG can be subclassified based on age of onset, clinical presentation, and thymic histopathology (3, 5). Heterogeneity of the disease makes predicting prognosis challenging (1, 6). Conventional treatment options, including symptomatic treatments and general immunosuppression, can help many but not all patients (5). Durable remission remains improbable, and chronic treatment with high doses of non-specific immunosuppressive drugs is usually necessary to maintain disease remission. Current therapeutic approaches lack specificity and are associated with a number of side effects

(1, 5). Identifying new biomarkers that can predict disease progression and treatment response and can be practically applied in clinical studies is highly desirable for the development of more specific and better tolerated treatments for MG patients.

The primary outcome measure of choice in MG trials is so far focused on the effect of clinical signs and symptoms (7). Single fiber electromyography represents the most robust biomarker of neuromuscular transmission, but is limited by factors related to accuracy, reproducibility, and availability of technical expertise (8). Antibody titers to AChR or MuSK have been used as a marker of the therapeutic response, but the correlation of this measure with disease severity has not been confirmed (9–12). Attempts to identify new biomarkers face challenges. Serum metabolomic profiling distinguishes patients with anti-AChR antibody-seropositive (AChR+) MG from those without (13), but whether metabolic analysis can predict therapeutic outcome remains to be explored.

Immunoregulatory cells operate in the periphery to modulate immune responses, especially those of autoreactive T and B cells that have escaped central tolerance (14–17). They are implicated in the pathogenesis of a variety of autoimmune diseases, including MG (18–21). Regulatory cells can be readily phenotyped and isolated on the basis of surface antigens and have been reported in a number of studies of MG (22–36). This review summarizes current knowledge of regulatory cells in MG, including their potential implication in pathogenesis.

IMMUNOREGULATORY CELLS

Immunoregulatory cell populations are diverse in their lineage and phenotype. Regulatory cells in the lymphoid lineage are represented by regulatory T (Tregs) (37–39), regulatory B (Bregs) (20, 40), and regulatory natural killer cells (41–43), while those in the myeloid lineage comprise myeloid-derived suppressor cells (MDSCs) (44–47), regulatory dendritic cells (DCs) (48–50), regulatory macrophages (51–53), regulatory neutrophils (54–57), and regulatory eosinophils (58, 59).

Regulatory T Cells

$CD4^+FoxP3^+$ Regulatory T Cells

As a principal player in peripheral tolerance, Tregs are among the most widely studied of the regulatory cells (38, 39). In humans and mice, Tregs are characterized as suppressive T cells, predominantly $CD4^+$, that constitutively express CD25 and the transcription factor forkhead box P3 (FoxP3) (37, 60). Human $CD4^+CD25^+FOXP3^+$ T cells are heterogeneous and have been labeled by additional surface antigens such as CD127, CD45RA/RO, and sialyl lewis x (CD15s) to further delineate naïve Tregs as $CD25^+CD127^{low}CD45RA^+FoxP3^{low}$, activated Tregs as $CD25^{high}CD127^{low}CD45RA^-FoxP3^{high}$, highly suppressive Tregs as $CD25^+CD127^{low}CD45RA^-CD15s^+FoxP3^+$, and non-suppressive T cells (also known as "non-Tregs") as $CD25^+CD127^{low}CD45RA^-FoxP3^{low}$ (61–66). Tregs have also been identified in domestic animal species, including dogs and cats (67–72), which are gaining traction as spontaneous models for many human diseases (73–79). Our previous work has revealed a conserved transcriptomic signature of Tregs among humans, mice, and dogs, vindicating the view that these cells are phenotypically and functionally related between these mammalian taxa. Thirty-one consensus transcripts were highly expressed in Tregs of all three species in comparison with their conventional T cell counterparts. Of the 31 consensus transcripts, six encode the Treg signature molecules CD25, FoxP3, IL-10, Helios, Galectin 3, and T-cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domains (TIGIT) (68). Many other T cell subsets possess regulatory function, including $CD8^+$ T cells (80–82), type 1 regulatory T (Tr1) cells (83, 84), γδ T cells (85, 86), and invariant natural killer T (iNKT) cells (87, 88). However, $CD4^+FoxP3^+$ Tregs (**Figure 1A** and **Table 1**) dominate research in this field (17, 21, 89).

A number of studies have characterized Tregs in human AChR+ MG patients based on CD25 and/or FoxP3 expression (22, 23, 25–32, 35). A majority of reports found no alteration in the frequency or absolute number of Tregs isolated from either peripheral blood or thymus of MG patients in comparison with those from healthy subjects (22, 23, 25, 26, 28, 29, 31, 32, 35). However, some studies made disparate observations. In the study of Fattorossi et al. (30), the number of circulating Tregs in untreated MG patients was lower than those in healthy subjects and MG patients treated with prednisone and azathioprine, which suggested that the clinical benefit of immunosuppressive therapy may in part be attributable to increasing Treg numbers. These authors also found that although thymectomy transiently inhibited the increase in frequency of circulating Tregs following immunotherapy, circulating Tregs in these patients eventually returned to a level similar to those of patients treated with immunotherapy without thymectomy. These data suggest that circulating Treg recovery during immunotherapy might be independent of the thymus. Li et al. (27) found lower frequency of circulating $CD4^+$ Tregs, but unaltered frequency of $CD8^+$ Tregs, in MG patients than in healthy controls. However, further studies with subgroup analysis is needed to discern the difference between the subtypes of MG and the effect of medications. In contrast to the lack of consensus on numerical perturbations of Tregs in MG, impaired function of Tregs has been consistently demonstrated by *in vitro* functional analysis (22, 23, 26, 28, 29, 32, 35). The dysfunction has been associated with attenuated FoxP3 expression, given the pivotal role of FoxP3 in Treg development and function (90–92). One study suggested a link between decreased FoxP3 expression and lowered phosphorylation of signal transducer and activator of transcription-5 (STAT5) (35). Furthermore, Luther et al. (26) reported that Tregs from prednisolone-treated MG patients had enhanced suppressive function *in vitro* compared to those from untreated patients, suggesting that prednisolone might augment Treg function. This result accords with the findings of Fattorossi et al. (30), which also showed augmentation of Treg numbers during immunosuppressive medication. Together, these data indicate a potential role of immunosuppressive therapy in restoring Treg number and function. However, both studies only compared treated and untreated patients at a single time point. A longitudinal study is needed to address this hypothesis. In

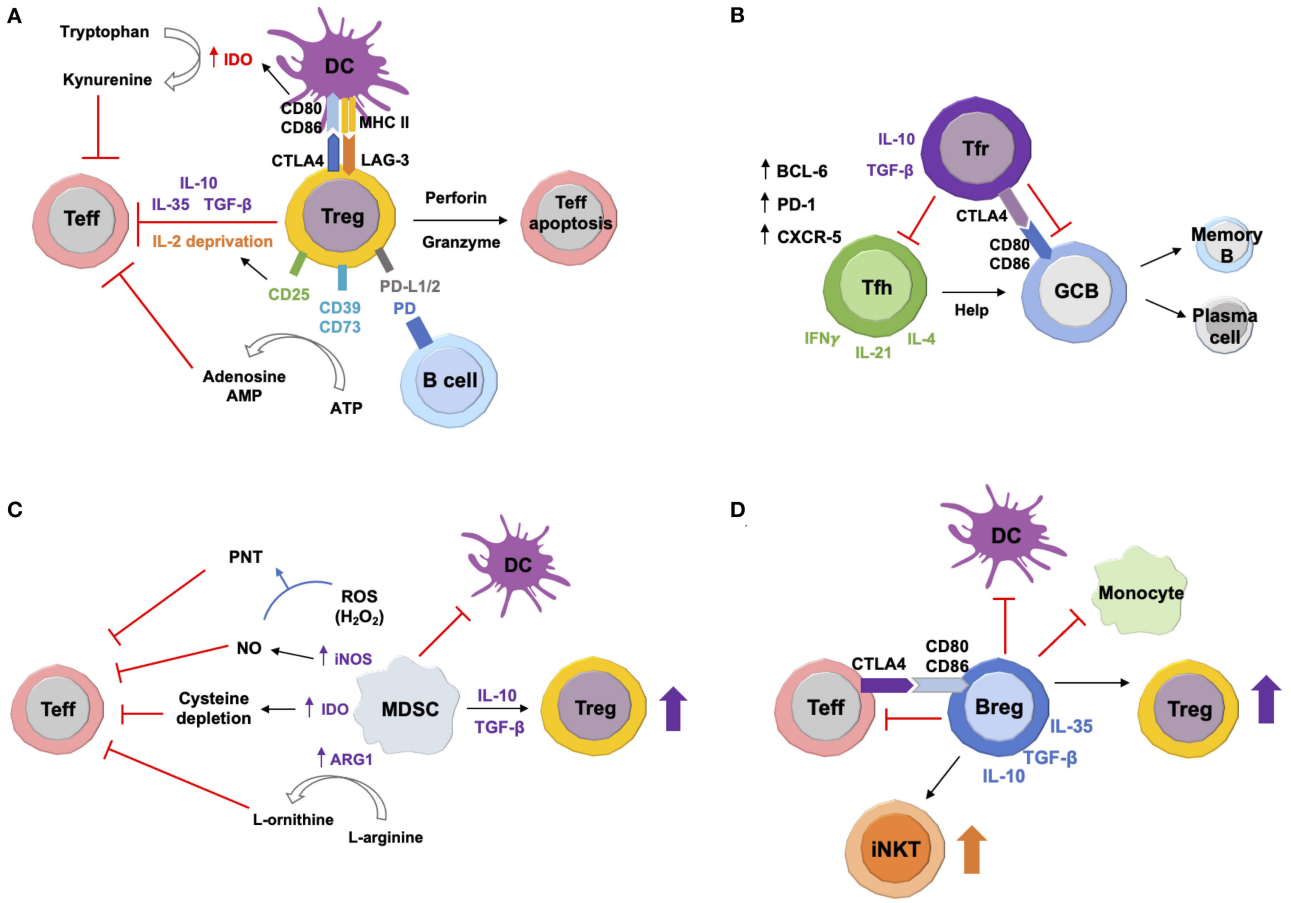

FIGURE 1 | Suppressive Mechanisms of Immunoregulatory Cells. **(A)** Tregs secrete anti-inflammatory cytokines IL-10, transforming growth factor beta (TGF-β), and IL-35, thus inhibiting target effector T (Teff) cells. Production of perforin and granzyme causes Teff cell apoptosis. Constitutively high expression of CD25 depletes IL-2, which attenuates Teff cell proliferation. Interaction with dendritic cells (DCs) *via* cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and lymphocyte-activation gene 3 (LAG-3) downregulates CD80/CD86 expression, which induces upregulation of indoleamine 2,3-dioxygenase (IDO). This enzyme expressed by DCs converts tryptophan to kynurenine, leading to Teff cell exhaustion. Surface expression of CD39 and CD73 converts extracellular adenosine triphosphate (ATP) to immunosuppressive adenosine and adenosine monophosphate (AMP). Tregs can also suppress autoreactive B cells *via* programmed death (PD) ligands 1 and 2 (PD-L1/2). **(B)** In germinal centers (GCs), both follicular helper T (Tfh) and follicular regulatory T (Treg) cells express transcription factor B cell lymphoma 6 (BCL6), surface marker PD-1, and C-X-C motif chemokine receptor 5 (CXCR5). Tfh cells produce IL-4, IL-21, and interferon gamma (IFNγ). They provide help signals to GC B cells and promote their differentiation into antibody-secreting plasma cells and memory B cells. Tfr cells regulate GC responses by inhibiting both Tfh and B cells *via* anti-inflammatory IL-10 and TGF-β. Tfr cells can also directly suppress GC B cells *via* CTLA-4. **(C)** Myeloid-derived suppressor cells (MDSCs) produce high levels of inducible nitric oxide synthase (iNOS), arginase-1 (ARG1), and reactive oxygen species (ROS). iNOS generates nitric oxide (NO), which reacts with ROS to produce peroxynitrite (PNT). ARG1 converts L-arginine to L-ornithine. IDO expressed by MDSCs sequesters cysteine. All of these can inhibit Teff cells. MDSCs also induce Treg expansion *via* IL-10 and TGF-β. In addition, MDSCs suppress maturation, migration, and antigen presentation of DCs. **(D)** Regulatory B cells (Bregs) inhibit activation and differentiation of pro-inflammatory target cells, including Teff cells, DCs and monocytes *via* secretion of IL-10, IL-35, and TGF-β. Bregs can also directly suppress Teff cells *via* CTLA-4 and CD80/CD86 interaction. On the other hand, Bregs induce expansion and differentiation of Tregs and invariant natural killer T (iNKT) cells. (Suppressive mechanisms in this figure refer to general contexts, including homeostasis and all inflammatory conditions.)

addition, stability of Treg function is likely to be influenced by the inflammatory environment in MG. For instance, the inflammatory cytokine tumor necrosis factor alpha (TNF-α) negatively modulates human $CD4^+CD25^{high}$ Treg function (93). A more recent study showed that loss of FoxP3 expression by human Tregs mediated by TNF-α depends on the FoxP3 complex component Deleted in Breast Cancer 1 (DBC1) (94).

Studies on experimental autoimmune MG (EAMG) in rodents have provided additional insight into the role of Tregs. Aricha et al. (34) showed that myasthenic rats had a lower frequency of circulating $CD4^+CD25^{high}FoxP3^+$ T cells than healthy controls, while Nessi et al. (24) found no difference in frequency of $CD4^+CD25^{+/high}$ T cells in either spleens or lymph nodes between rats with EAMG and healthy controls. Both groups also investigated the therapeutic effect of passive transfer of Tregs. Aricha et al. (33, 34) reported that adoptive transfer of *in vitro*-induced polyclonal Tregs from either healthy or EAMG donors suppressed ongoing EAMG. Nessi et al. (24) found that $CD4^+CD25^+$ T cells isolated from naïve rats prevented the induction of EAMG, but did not suppress established disease.

This observation might reflect insufficient numbers of activated Tregs among administered $CD4^+CD25^+$ T cells.

Only a limited number of studies have investigated T cell populations in peripheral blood of human patients with MuSK+ MG. Yi et al. (95) found that $CD4^+$ T cells exhibit enhanced inflammatory Th1 and Th17 responses in MuSK+ MG, although no difference was found in either frequencies or CD39 expression of $FoxP3^+$ Tregs between MuSK+ MG and healthy controls, suggesting that increased pro-inflammatory T cell responses were not attributed to numerical or functional defects of Tregs. The same group (96) also reported that tacrolimus, an immunosuppressant for AChR+ MG, inhibited Th1 and Th17 responses, and reduced Treg frequencies of *in vitro* cultured peripheral blood mononuclear cells (PBMCs) from MuSK+ MG patients. Reuveni et al. (97) reported that a mouse model of MuSK+ EAMG had decreased Treg frequencies and FoxP3 expression, the latter of which was restored by oral administration of recombinant MuSK protein.

In summary, AChR+ MG is associated with functional defects of Tregs. Adoptive transfer of Tregs derived from either healthy rats or myasthenic rats can attenuate EAMG. In contrast, the pathogenic role of Tregs remains unclear in MuSK+ MG.

Follicular Regulatory T Cells

Follicular regulatory T (Tfr) cells (**Figure 1B** and **Table 1**) have emerged as a unique subset of $CD4^+$ Tregs that negatively regulate the proliferation of follicular helper T (Tfh) and B cells in germinal centers (GCs) (98, 99). Both Tfr and Tfh cells express common GC-associated antigens, including transcription factor B cell lymphoma 6 (BCL6), chemokine receptor CXCR5, programmed death-1 (PD-1), and inducible T-cell co-stimulator (ICOS) (100–103). However, unlike Tfh cells, Tfr cells concurrently express Treg-characteristic markers such as CD25, FoxP3, glucocorticoid-induced tumor necrosis factor receptor (GITR), and cytotoxic T-lymphocyte antigen 4 (CTLA-4) (100, 101). Tfr and Tfh cells regulate humoral immunity in opposite directions (104). Imbalance between these two populations dysregulates production of autoantibodies, promoting pathogenic autoimmunity (105, 106). Tfr and Tfh cells primarily reside in GCs (98). However, some studies have identified counterpart $CD4^+$ T cell subsets in peripheral blood, facilitating investigation of their pathogenic potential in the context of autoimmunity, including MG (107, 108).

The frequency of a population of $CD4^+CXCR5^+$ T cells was higher in the peripheral blood of untreated MG patients than in that of healthy controls (109). The cell frequency was positively correlated with disease severity. Thymectomy followed by glucocorticoid therapy reduced $CD4^+CXCR5^+$ T cell frequency in these myasthenic patients to the control level (109). In a similar observation, an increased frequency of circulating Tfh cells, defined as $CD4^+CXCR5^+PD\text{-}1^{high}/ICOS^{high}$ cells, was demonstrated in MG patients in comparison to healthy subjects (110). The study also showed a positive correlation between circulating Tfh cell frequency and serum anti-AChR antibody titer in these patients (110). In line with these clinical studies, EAMG mice also have a higher frequency of splenic $CD4^+CXCR5^+PD\text{-}1^+$ Tfh cells than controls, and their Tfh cell frequency is positively correlated with the concentration of anti-AChR antibodies in serum (111). All these findings collectively suggest that the frequency of circulating Tfh cells reflects disease activity in AChR+ MG. However, a shortcoming in these studies is the lack of distinction of Tfr and Tfh cells amongst circulating follicular T cells.

Three recent studies showed that AChR+ MG patients had a lower frequency of circulating Tfr cells, but a higher frequency of circulating Tfh cells than healthy controls, suggesting a link between the imbalance of the Tfr/Tfh ratio and disease manifestations (107, 112, 113). The Tfr/Tfh ratio showed an inverse correlation with AChR+ MG severity, and the imbalance was restored by steroid and cyclophosphamide therapy (107). Taken together, the ratio between circulating Tfr and Tfh cells is likely to predict the development of AChR+ MG. Similarly, a higher Tfh/Tfr ratio was found in MuSK+ MG patients, accompanying increased frequencies of Th17-producing Tfh cells and higher Tfh-promoted IgG synthesis (114). The pathological roles of Tfr and Tfh populations in MG need to be further investigated in animal models.

Other Regulatory Cell Populations

In contrast to Tregs, limited information is available on other regulatory populations in MG. To date, only MDSCs and Bregs have been examined in MG.

Myeloid-Derived Suppressor Cells

MDSCs (**Figure 1C** and **Table 1**) are a heterogeneous population of immature myeloid cells that accumulate in cancers and other diseases involving chronic inflammation (45). These cells suppress T cell responses and contribute to tumor progression and metastasis, emerging as a promising therapeutic target in cancer (46). MDSCs comprise two major subsets, polymorphonuclear (PMN)- and monocytic (M)-MDSCs (47). They are distinguished from conventional neutrophils or monocytes by surface antigens and density (44, 47). In humans, PMN-MDSCs are identified as $CD11b^+CD14^-CD15^+CD33^+$ or $CD11b^+CD14^-CD66b^+CD33^+$ hypodense myeloid cells, while M-MDSCs are identified as $CD11b^+CD14^+CD15^-CD33^+HLA\text{-}DR^{-/low}$ hypodense myeloid cells; both populations are found in the PBMC fraction after density gradient separation (44). The murine counterparts of PMN- and M-MDSCs are $CD11b^+Ly6G^+Ly6C^{low}$ and $CD11b^+Ly6G^-Ly6C^{high}$ cells, respectively (44). Our previous work has identified functional equivalents of these subsets in dogs based on the expression of CADO48A and CD14 (115). The role of MDSCs has been investigated in a variety of autoimmune diseases (116–122). The ability of *in vitro* generated MDSCs to suppress EAMG has been investigated in mice (123). Adoptive transfer of MDSCs improved muscle weakness, reducing both serum anti-AChR IgG levels and complement deposition at the endplates in EAMG mice. Splenocytes from MDSC-treated mice had a lower production of IFN-γ and IL-17 *in vitro*, demonstrating reduced Th1 and Th17 responses. MDSCs also directly inhibited pre-activated B cells both *in vitro* and *in vivo*. These results suggest that MDSCs suppress ongoing EAMG by inhibiting both autoreactive T and B cells (123).

TABLE 1 | Summary of Immunoregulatory Cells in AChR+ MG.

Cell types	Markers*	Cytokines*	Target cells*	Association with pathogenesis in MG	References
$CD4^+FoxP3^+$ Treg	$CD25^+$ or $CD25^{high}$, FoxP3, CTLA-4 (CD152), GITR, LAG-3, Neuropilin-1, $CD127^{-/low}$, Sialyl Lewis x (CD15s)	IL-10, TGF-β, IL-35	Teff cells, APCs, B cells	- Functional defect is associated with reduced FoxP3 expression and MG pathogenesis; - Decreased FoxP3 expression correlates with attenuated STAT5 signaling; - Numerical correlation remains controversial; - Adoptive transfer treats EAMG	(22–35, 37, 61, 64, 130)
Tfh	$CD4^+CXCR5^+PD\text{-}1^+/ICOS^+$	IL-21, IL-4, IL-17, IFNγ	GC B cells	- Cell frequency positively correlates with disease severity; - Tfr/Tfh ratio inversely correlates with disease severity	(102–113)
Tfr	$CD4^+CXCR5^+FoxP3^+$	IL-10, TGF-β	Tfh cells; GC B cells	- Cell frequency inversely correlates with disease severity; - Tfr/Tfh ratio inversely correlates with disease severity	(98–101, 107, 112, 113, 131)
PMN-MDSC	$CD11b^+CD14^-CD15^+CD33^+$ or $CD11b^+CD14^-CD66^+CD33^+$ (human); $CD11b^+Ly6G^+Ly6C^{low}$ (mouse); $CD11b^+CD14^-CADO48^+$ (dog)	IL-10, TGF-β	Teff cells; DCs; macrophages	Adoptive transfer of MDSC treats EAMG in mice	(44–47, 115, 123)
M-MDSC	$CD11b^+CD14^+CD15^-CD33^+HLA\text{-}DR^{-/low}$ (human); $CD11b^+Ly6G^-Ly6C^{high}$ (mouse); $CD11b^+CD14^+CADO48^-$ (dog)	IL-10, TGF-β	Teff cells; DCs; macrophages	Adoptive transfer of MDSC treats EAMG in mice	(44–47, 115, 123)
Breg	CD19, CD38, CD1d, CD24, CD27	IL-10, TGF-β	Teff cells; DCs; monocytes; iNKTs	Cell frequency and function inversely correlate with disease severity	(20, 36, 40, 124, 125)

**Markers, cytokines, and target cells refer to general contexts, including homeostasis and all inflammatory conditions.*
FoxP3, forkhead box P3; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; GITR, glucocorticoid-induced tumor necrosis factor receptor; LAG-3, lymphocyte-activation gene 3; TGF-β, transforming growth factor beta; Teff, effector T cells; APCs, antigen-presenting cells; STAT5, signal transducer and activator of transcription 5; CXCR5, C-X-C motif chemokine receptor 5; PD-1, programmed death 1; ICOS, inducible T cell co-stimulator; IFNγ, interferon gamma; GC, germinal center; DCs, dendritic cells; iNKTs, invariant natural killer T cells.

Regulatory B Cells

Bregs (**Figure 1D** and **Table 1**) have been identified in humans and mice as a heterogeneous population of immunosuppressive B cells that inhibit pro-inflammatory responses predominantly by means of IL-10 synthesis (40, 124). However, intracellular staining for IL-10 precludes functional studies of Bregs, prompting Breg isolation using surface markers such as CD19, CD38, CD24, CD1d, and CD27 (20, 40). Breg frequency and function are negatively correlated with disease activity of several autoimmune disorders, such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and multiple sclerosis (MS) (20). Two studies have shown reduced frequency and function of circulating Bregs in untreated AChR+ MG patients compared with healthy controls (36, 125). The proportion of circulating Bregs can be restored by thymectomy, but not by steroid therapy (125). A subset of Bregs, namely IL-10–producing B (B10) cells (126), repopulated at a faster rate in the patients with a favorable response to rituximab than in those with a poor response (36). In addition, Guptill et al. (127) also reported a reduction of B10 frequencies in MuSK+ MG patients compared to healthy controls. These results together suggest an immunopathogenic role of diminished Bregs in both AChR+ and MuSK+ MG. Adoptive transfer of Bregs has not yet been reported in MG. However, Bregs transferred into mice with experimental autoimmune encephalomyelitis induced $FoxP3^+$ Tregs and Tr1 cells, and correlated with disease remission (128). This observation suggests that Bregs might hold promise as an adoptive cellular therapy for MG.

DISCUSSION

Current data suggest that immunoregulatory cells may play significant roles in the pathogenesis of MG. In AChR+ MG patients, these populations show either functional defects ($CD4^+FoxP3^+$ Tregs) or numerical deficiency (Tfr), or both

(Bregs). They can be readily isolated from patients' peripheral blood and characterized by flow cytometry. Performing functional assays in the current routine clinical setting can be challenging, while numerical analysis of circulating Tfr, Tfh, or Breg cells shows promising utility in clinical practice. However, several drawbacks need to be addressed before these assays may be translated for clinical use.

First, current studies have extensively examined AChR+ MG cases, leaving a scarcity of knowledge for the less common, but equally debilitating, MuSK+, LRP4+, and seronegative phenotypes of MG — although the nature of a small subpopulation of a rare disease makes such studies challenging. Second, the current studies have treated all AChR+ MG patients as a homogeneous group, calling into question whether these assays can further differentiate subsets of MG patient groups, including classification based on clinical presentation, age of onset, gender, and thymic histopathology. Third, the low frequencies of circulating Tfr and Breg cells are a significant obstacle in accurate quantification of these populations. An alternative is to analyze the characteristic gene expression of these populations by qRT-PCR assay. Furthermore, antigen-specific regulatory cells may closely correlate with disease severity in MG, assessed using MHC-peptide tetramers or fluorescently-labeled antigens (129).

In conclusion, numerical measures of circulating Tfr, Tfh and B10 cells appear to correlate with disease activity of AChR+ MG; however, none of these populations shows sufficient sensitivity or specificity to serve as a biomarker for the disease. Mechanistic insight into the roles of immunoregulatory cells in the pathogenesis of MG will enable the development of more targeted therapies for this debilitating autoimmune disease in the future.

AUTHOR CONTRIBUTIONS

YW conceptualized and drafted the manuscript. OG and JL critically reviewed the manuscript. All authors contributed to the article and approved the final version.

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Outcome Measures in Clinical Trials of Patients With Myasthenia Gravis

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Myasthenia gravis (MG) is a heterogeneous disorder whose clinical presentation ranges from mild ocular deficits to severe widespread weakness. This variance poses a challenge when quantifying clinical deficits. Deficits and symptoms are quantified using standardized clinical scales and questionnaires which are often used as outcome measures. The past decades have seen the development of several validated outcome measures in MG, which are used in clinical trials to obtain regulatory approval. In recent years, emphasis has moved from objective assessments to patient-reported outcomes. Despite a growing body of literature on the validity of the MG-specific outcome measures, several unresolved factors remain. As several novel therapeutics are currently in clinical development, knowledge about capabilities and limitations of outcome measures is needed. In the present paper, we describe the most widely used clinical classifications and scales in MG. We highlight the choice of outcome measures in published and ongoing trials, and we denote whether trial efficacy was reached on these outcomes. We discuss advantages and limitations of the individual scales, and discuss some of the unresolved factors relating to outcome assessments in MG.

Keywords: myasthenia gravis, classification, clinical trials, review, outcome measure, rating scale

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INTRODUCTION

Myasthenia gravis (MG) is an autoimmune neuromuscular disease characterized by fatigable muscle weakness due to autoantibodies targeting components of the neuromuscular junction (1). Symptoms and deficits involve ocular, bulbar, respiratory and proximal limb muscles, and they fluctuate in a diurnal and day-to-day pattern. This fluctuating nature of symptoms challenges assessments of disease severity. Deficits and symptoms are measured using validated clinical scales. The past decades have seen the development of several clinical scales reflecting objective, patient-reported and composite measures of disease severity. These validated outcome measures are frequently employed as primary and secondary efficacy parameters in randomized controlled trials (RCT). Several RCTs of currently used immunosuppressants have produced ambiguous results concerning their efficacy in MG. This lack of efficacy may be due to trial-related factors, including sample size issues (e.g., low recruitment), design (e.g., length and inclusion criteria) and insufficiently sensitive outcome measures (e.g., floor and ceiling effects) (2). Accordingly, the current use of these treatments is based on expert consensus and convincing efficacy in daily clinical use. Treatment of MG has recently entered a new era with the development of monoclonal antibodies targeting

specific pathophysiologic culprits. As RCTs of these therapeutics may lead to regulatory approval of new treatments, knowledge of the capabilities and limitations of the clinical scales is imperative in understanding the efficacy of current and future treatments in MG.

CLINICAL CLASSIFICATION

MG is a heterogeneous disease with several possible classifications according to disease and patient-related factors (1). Type of autoantibody enables classification accordingly, which may directly affect treatment choice. Age at onset enables classification into early-onset and late-onset disease; the former having a female predominance and a higher frequency of thymic hyperplasia. Symptom distribution may be used to classify MG into ocular and generalized MG; and MG may be classified by presence or absence of thymoma.

Although subpopulations of MG are distinguishable, patients are often classified according to the severity of deficits using the Myasthenia Gravis Foundation of America (MGFA) Classification. In 2000, the MGFA Classification was defined as an iteration of previously used classifications (3–6). Patients are classified according to level of overall severity, spanning ocular-only (I), mild (II), moderate (III), severe (IV) and intubation (V), with additional subclassification related to axial/extremity (a) or bulbar (b) predominance. The MGFA Classification is not a recommended outcome measure owing to its poor correlation with summated rating scales (7, 8) and high dependence on physician interpretation. The MGFA Classification is a system broadly characterizing patients according to severity of disease and prognosis.

OUTCOME MEASURES

In the 1930s, the use of ephedrine (9), acetylcholine esterase inhibitors (10), pituitary extract (11) and thymectomy (12) enabled non-quantifiable individual-level descriptions of treatment-related improvements in MG. A rating scale specific to MG was not introduced until the 1980s, and the subsequent decades saw the development of several MG-specific clinical scales (**Table 1**). Several publications review the various measures in detail (13, 25). Currently, the QMG, the MGC, the MG-ADL, and the QOL15(r) are the most widely used scales in clinical trials. Recently, the MGII was developed and has several potential advantages, however this scale has not been used in any clinical trials yet. Accordingly, the QMG, the MGC, the MG-ADL, and the QOL15(r) will be described below. The MGII will be discussed in context of advantages and limitations of these scales.

The Quantitative Myasthenia Gravis (QMG) scale was introduced in 1998, serving as an objective measure of disease severity (16). The QMG encompassed eight items in the first version (26). It was later expanded to include 13 items (15). In a subsequent revision, the patient-reported items were replaced by physician examinations resulting in its current version (16). The QMG assesses muscle strength and fatigability using objective measures of double vision, ptosis, facial muscles, dysphagia, dysarthria, proximal limb, hand muscles, neck muscles and respiratory function. These assessments are somewhat time consuming and require equipment. Accordingly, in daily clinical practice use of the QMG is challenging. Each item is given a score of 0–3, resulting in an unweighted total score of 0–39. A higher score corresponds to more severe disease. Based on data from the cyclosporine trials (15, 16, 27), a 3-point change is considered clinically meaningful, with a modification in milder cases where a 2-point change is considered sufficient (13). Reliability is high and interobserver variability is low (16, 28, 29).

TABLE 1 | MG-specific outcome measures.

Name	Year	Type
Myasthenia muscle scale (13)	1983	Objective
MG score (14)	1987	Objective
Basta neurologic institute rating scale (5)	1988	Composite
Quantitative myasthenia gravis (15)	1998	Objective
MG activity of daily living (16)	1999	Patient-reported
MG questionnaire (17)	2002	Patient-reported
MG manual muscle test (6)	2003	Objective
Ocular-bulbar-facial-respiratory scale (18)	2006	Objective
MG composite (19)	2008	Composite
MG quality of life (20)	2008	Patient-reported
MG quality of Life 15 items (revised) (21, 22)	2008 (2016)	Patient-reported
MG disability scale (23)	2014	Patient-reported
MG impairment index (24)	2016	Composite

The MG Activity of Daily Living (MG-ADL) scale is a patient-reported outcome developed in 1999 (17) as a quickly administered set of questions examining frequency and severity of key MG symptoms. The MG-ADL was constructed as an expanded version of the patient-reported sub-items from another scale (15). Using a recall period of a few weeks, eight questions assess ocular function, speech, chewing, swallowing, respiratory function, and strength of proximal upper and lower extremities. Each item is scored from 0 to 3, which results in an unweighted total score of 0–24 points. A higher score indicates more severe symptoms. Based on a longitudinal study on the MG-ADL, the QOL15 and the physician impression of change (30), a 2-point change is considered clinically meaningful. Reliability is high (30).

The MG Composite (MGC) scale was developed in 2008 (20). It was constructed using the top performing items of the QMG, the MG-ADL and the Manual Muscle Test during a trial of mycophenolate. Six physician-assessed examinations evaluate ocular, neck and proximal limb muscles. Furthermore, four patient-reported items assess speech, chewing, swallowing and respiratory function. All patient-reported items are from the MG-ADL. A group of MG experts decided on item-score weighting based on symptom severity. Total score spans from 0 to 50; a higher score indicating more severe disease. A 3-point change is considered clinically meaningful based on physician's

impression of change (31). The MGC has been reported to have a high reliability (31).

The MG Quality of Life 15-items (QOL15) was developed in 2008 as a patient-reported outcome (22). It was based on a large 60-item MG questionnaire (21). The current 15 questions were based on feedback from patients and on responsiveness of the individual items during a trial of mycophenolate. Using a recall period of a few weeks [originally 4 weeks (22)]), these 15 questions assess ocular symptoms, swallowing, speech, proximal limb function, mobility, personal grooming, work, social life, activities, fluctuations and psychological items. Scoring is qualitative. Each question is scored from 0 to 4, resulting in a total score in the range of 0–60; a higher score indicates poorer quality of life. The QOL15 score was slightly revised to its present version during subsequent international validation (23). The QOL15r retains the original 15 questions using a slight re-phrasing of some items and reducing the item score to a range of 0–2. Reliability is high (23, 32). The responsiveness has not been studied or published. The questionnaire has been validated in various languages and cultures.

OUTCOME MEASURES IN PUBLISHED AND ONGOING MG TRIALS

Choice of primary and secondary endpoint(s) vary among the published and ongoing RCTs. In **Table 2**, trials with more than 30 participants are summarized, and their results are denoted according to the prespecified analysis.

The prespecified endpoints have not been reached in several trials (**Table 2**). This may be due to a lack of efficacy; however, lack of efficacy may also result from sample-size issues, trial design and choice of statistical analysis.

Prior to 2017, the primary endpoint was mainly objective assessments (15, 27, 33, 35–37, 39, 42), antibody titers (15, 27) and the steroid-sparing effect (27, 34, 38, 40–42). The REGAIN trial evaluating eculizumab (43) was published in 2017 and was the first trial to introduce the MG-ADL as a primary endpoint. Currently, most ongoing phase 3 trials rely on the MG-ADL as a primary endpoint (**Table 2**). A trial of rituximab applies a composite measure of QMG and steroid-sparing effect, and a trial of oral Salbutamol is using the QOL15 as primary endpoint. Recently, the QMG is mostly used as a secondary endpoint in phase 3 trials (**Table 2**) and as a primary endpoint in pilot studies and phase 2 trials, including trials of mycophenolate (2003) (47), terbutaline (2009) (48), eculizumab (2013) (49), belimumab (2018) (50), rozanolixizumab (2019) (46), iscalimab (2019), and zilucoplan (2020) (51).

ADVANTAGES, LIMITATIONS AND UNRESOLVED FACTORS

In recent years, the regulatory authorities have emphasized the use of patient-reported outcomes as primary efficacy parameter in clinical trials. Accordingly, several ongoing trials in MG use patient-reported outcomes as primary endpoint (**Table 2**). Symptoms fluctuate in MG; hence, objective assessments may not necessarily reflect patients' experienced symptom burden. Consequently, patient-reported outcomes are preferred as primary outcomes in MG trials.

Few patient-reported scales have been developed in MG (**Table 1**). The MG-ADL is validated, it has been tested in several trials, it is quick and easy to administer, and it assesses disease severity using questions specifically addressing MG symptoms. However, several symptoms of MG are not assessed, and the negative consequences of treatment (e.g., side-effects) are not addressed. Despite improvements in symptoms during treatment, the overall quality of life may be more severely affected due to, e.g., intolerable side-effects. Therefore, health-related quality of life measures may be considered more relevant outcome parameters. Using the QOL15 score introduces new challenges as factors unrelated to MG symptoms may affect quality-of-life scores (22, 52–55). Hence, relying on the QOL15 as primary endpoint may result in inadequate power to detect improvements in core MG-related symptoms. This may, in turn, result in issues relating to adequate trial recruitment. Improvements in the QOL15(r) score should therefore be considered as supplementary information when using the MG-ADL as primary endpoint. The use of a single patient-reported question assessing perceived degree of normal (Single Simple Question, SSQ) (56) has shown a high degree of correlation with the QOL15 and other MG measures, however this has not been tested prospectively. The Myasthenia Gravis Impairment Index (MGII) (57) is a newly developed composite outcome measure consisting of patient-reported items and physical examinations. The patient-reported subitems have excellent reliability as a stand-alone scale (57), however responsiveness and clinical meaningful change has only been published on the composite measure (58).

Some MG symptoms are poorly reflected by the MG-ADL. Neck weakness is not addressed although it is a debilitating symptom in some patients. Assessment of limb muscle fatigability is restricted to few shoulder and hip activities, although fatigability is one of the most relevant symptoms in patients with MG (59) potentially affecting several ADL functions. The QMG scale specifically addresses both complaints. The QMG is a well-established test providing evidence of responsiveness during various treatments; however, the QMG may be more sensitive to changes in ocular, limb and axial muscles than to changes in bulbar and respiratory functions (60). Thus, the QMG provides valuable objective information complementing the patient-reported outcomes, however objective assessments of respiratory and bulbar functions are still lacking.

MG symptoms contribute differently to the degree of clinical disability. Obviously, respiratory failure is more medically severe than persistent ocular symptoms. Hence, weighted scores as used in the MG-Composite may capture more clinically relevant information concerning disease severity. Thus, the MG-Composite may serve as an alternative to linear disease measures, complementing both the patient-reported outcomes and the QMG.

Degree of clinical disability is heterogeneous; hence, clinical scores should cover the entire spectrum ranging from mild to

TABLE 2 | RCTs in MG with ≥30 participants and available results.

Year	Trial	Primary Endpoint	Secondary Endpoint
1993	Cyclosporine (26)	MG Score* Steroid-sparring Antibody-titer*	Treatment failures
1997	IVIG vs. plasma exchange (32)	MMS	Antibody titer Time-to-effect
1998	Azathioprine (add-on to prednisone) (33)	Steroid-sparing* Treatment failure* Duration of remission*	Muscle strength (handheld dynamometry, walking time, swallowing time, forced vital capacity, subjective scoring)
2005	IVIG 2 vs. 1 g/kg for exacerbation (34)	MMS	Time-to-treatment response Forced vital capacity Antibody titer Intubation or nasogastric tube
2007	IVIG (35)	QMG*	SF-EMG RNS Post-intervention status*
2008	Mycophenolate (add-on to prednisone) (36)	QMG	MMT MG-ADL Forced vital capacity* SF-36 Treatment failure Global assessment of response Antibody type
2008	Mycophenolate (37)	Treatment response (post-intervention status steroid sparing effect, pyridostigmine dose)	Steroid sparing Pyridostigmine dose QMG SF-36 MG-ADL Global Assessment of Severity Antibody titer
2011	IVIG vs. plasma exchange (38)	QMG	SFEMG Post-intervention status Antibody titer
2011	Tacrolimus as steroid-sparing agent (39)	Steroid-sparing	QMG MG-ADL
2016	Methotrexate as steroid-sparing agent (40)	Steroid-sparing	QMG MG-ADL MMT QOL15 MGC*
2016	Thymectomy in non-thymomatous MG (41)	QMG* Steroid-sparing*	Treatment-associated symptoms* SF-36 MG-ADL* Post-intervention status* Use of immunosuppressants*
2017	Eculizumab in refractory MG (Phase 3) (42)	MG-ADL	QMG* MGC QOL15*
2017	Tacrolimus (43)	QMG	MGFA Classification MG-ADL MMT Steroid sparring
2018	Rituximab[1] NCT02110706	Steroid-sparing effect	MGC QMG
2019	Efgartigimod (Phase 2) (44)	Safety	MG-ADL* QMG* MGC QOL15r*
2019	Rozanolixizumab (Phase 2) (45)	QMG	MGC MG-ADL*
2019	Iscalimab (Phase 2)[1] NCT02565576	QMG	MGC MG-ADL QOL15
2020	Zilucoplan (Phase 2) (46)	QMG*	MG-ADL* MGC* QOL15r
2020	IVIG (Phase 2)[1] NCT02473965	Steroid-sparing effect	
Ongoing	Salbutamol (Phase 2/3)[1] NCT03914638	QOL15	MG-ADL QMG MGC NeuroQOL
Ongoing	Ravulizumab (Phase 3)[1] NCT03920293	MG-ADL	QMG QOL15r
Ongoing	Efgartigimod (Phase 3)[1] NCT03669588	MG-ADL	QMG
Ongoing	Rozanolixizumab (Phase 3)[1] NCT03971422	MG-ADL	MGC QMG MG Symptoms PRO
Ongoing	Zilucoplan (Phase 3)[1] NCT04115293	MG-ADL	QMG
Ongoing	Rituximab[1] NCT02950155	QMG & steroid-sparring effect	QMG MG-ADL MG-QoL

*Ongoing phase 3 RCTs in MG[1]. Statistical significance according to prespecified analysis is denoted by *.*

[1]Accessed 17th of August 2020 on ClinicalTrials.gov.

MMS, Myasthenia muscle scale; QMG, Quantitative Myasthenia Gravis; MMT, Myasthenia Gravis Manual Muscle Test; MG-ADL, Myasthenia Gravis Activities of Daily Living; QOL15, Myasthenia Gravis Quality of Life 15-items; SF-EMG, Single-fiber electromyography; RNS, Repetitive Nerve Stimulation; MGFA, Myasthenia Gravis Foundation of America.

severely affected cases. However, there is considerable floor-effect in the MG-ADL (61) limiting its use in milder cases. The MGII shows less floor-effect than both the MG-ADL and the MGC (57), and it was recently shown to provide clinically relevant supplementary information to the MG-ADL (61). Interestingly, the MGII correlates only moderately with the QMG and the QOL15 during follow-up (58). Until now, the MGII has not been used as an outcome measure in trials, but it has the potential as an attractive alternative to other secondary outcomes. Due to the emphasis by regulatory authorities on patient-reported outcomes, the MGII is currently best suited as a secondary endpoint. MGII may enable superior assessment of

efficacy covering a larger spectrum of disease severity if used as a primary endpoint, however this remains to be studied in RCTs. Further, the responsiveness and clinical meaningful change of the MGII patient-reported items as a stand-alone scale is unsettled.

Response to treatment is variable, and the overall treatment-effect consists of patients with both minor and larger improvements. Accordingly, the point-change required for a clinically meaningful improvement has been established on the MG-ADL, the QMG, and the MGC. This enables responder-analysis and assessments of clinical meaningful effects while negating minor placebo-effects and natural fluctuations. The pooled QMG response of several RCTs (62) detected significant effects over placebo on both continuous and categorical analysis. The MGFA Post Intervention System (MGFA-PIS) apply this required point-change on the MGC (recommended) or the QMG in order to address whether patients improve or deteriorate (2). Only few studies have applied the MGFA-PIS as an outcome measure (**Table 2**), however assessments or minimal manifestation and clinical remission are also included in the MGFA-PIS. Recently, to obtain patient acceptable symptom states (Patient Acceptable Symptoms Score, PASS) the cut-off values required on several clinical scales (the QMG, the MGC, the MG-ADL, the QOL15 and the MGII) were analyzed (63). It is currently unsettled whether dichotomized assessments of minimal manifestation or PASS is feasible in clinical trials.

No prospective study has analyzed the relations between the four most frequently used scales (the QMG, the MGC, the MG-ADL and the QOL15). Correlations between some of the scales have previously been published (30–32, 57, 58, 64), and the relations between objective (QMG and MGC) and patient-reported measures (MG-ADL and QOL15) seem attenuated during treatment and follow-up. One study (58) has applied the QMG, the MGC, the MG-ADL and the QOL15 to the same population; however, between-scale correlations were not published. It is unknown whether improvements on objective scores are accompanied by equal improvements on patient-reported outcomes (e.g., MG-ADL and QOL15/QOL15r).

There is a lack of information concerning how outcome measures are affected by basic patient characteristics and how the scales perform in various subpopulations. Such information is crucial in design of clinical studies, and it is critical when determining relevant change in burden of symptoms and deficits during routine care. Sex differences characterize early and late-onset subpopulations of MG; hence, females often have longer disease duration than males. Further, studies report sex differences in rates of refractory MG (65–67). Most recent and ongoing trials focus on severe or refractory patients; hence, trial populations may consist mainly of females, and participants may have long-standing disease. It is unsettled whether sex and disease duration affect potential for improvement on current outcome measures, and it is unknown whether current outcome measures are equally applicable in the various MG subpopulations.

When applying the current outcome measures, a major challenge is inability to capture all clinically relevant factors in MG. Fatigue is a relevant feature of MG in addition to muscular fatigability (68). Being a subjective feeling of exhaustion, fatigue is preferably quantified using patient-reported outcomes. Several generic fatigue scales have been used in MG, including the Neuro-QOL Fatigue Scale (68) and the Chalder Fatigue Scale (53). Only the REGAIN trial included fatigue as a secondary outcome (69). Although the QOL15 was not designed to specifically incorporate fatigue, a high degree of correlation has been established between fatigue and QOL15 (53, 69). This suggests some responsiveness to improvements in fatigue in addition to MG specific symptoms. Further, the patient-reported subitems of the MGII incorporate fatigue (57). Change-correlations between the MGII and the Neuro-QOL Fatigue Scale are moderate and equally directed (58). Whether fatigue scores complement improvement captured by the QOL15 or MGII scores remains to be studied.

Use of treatment as well as presence and severity of side effects are not systematically assessed in any of the outcome measures despite their clinical relevance. Steroids are frequently used during MG exacerbations and as effective bridging therapies when tapering immunosuppressive agents. Some patients require chronic steroid therapy due to inadequate symptomatic control. Several trials have used the steroid-sparing effect as an outcome measure (**Table 2**). Due to the side-effect profile of chronic steroid exposure, a reduced steroid dose is equated to improvement on MG scales. Reduction in other therapies (e.g., pyridostigmine or immunosuppressive agents) or a change in therapy (e.g., intravenous to subcutaneous immunoglobulin) may result in better quality of life despite stability in MG symptoms; however, this is only indirectly assessed by sub-items of the QOL15(r) and not addressed by any of the symptom-orientated scales. Risk of side effects may result in significant psychological stress, especially when considering cancer risk in young patients requiring long-term treatment or potentially teratogenic effects in fertile woman. Since MG is a chronic disease usually requiring treatment for decades, treatment satisfaction may be considered as important as symptomatic control. Treatment satisfaction is not systematically assessed using any of the current outcome measures.

In coming years, the use of tele-medicine will likely increase, especially due to the current global pandemic when monitoring immunocompromised patients. Further, virtual care may increase patient willingness to participate in RCTs owing to fewer physical attendances. Accordingly, validated measures assessing MG functioning through virtual care are needed. It is unsettled how the current MG scales function in a virtual setting. Some objective assessments are feasible, especially of ocular and bulbar involvement, however pure patient-reported measures will likely result in the most robust assessments. This area currently merits further research.

Patient-reported outcomes are often used as primary endpoints in establishing efficacy of novel treatments. Several of the recent trials focus on medically severe and refractory patients. However, a large proportion of patients are mild to moderately affected. New therapeutic options are warranted

addressing unmet medical needs in this large group of patients. None of the current patient-reported outcomes enables detection of improvement on the entire severity continuum. In addition, no single patient-reported scale captures both the quantitative and qualitative aspect of improvement in MG symptoms during treatment.

CONCLUSIONS

Several MG-specific outcome measures have been developed, reflecting objective disease burden, patient-reported symptom severity and health-related quality of life. Each scale has distinct advantages relating to MG assessments and complements information obtained from other outcome measures. Detailed assessments of treatment efficacy should currently incorporate patient-reported assessments (e.g., MG-ADL), quality-of-life measurements [e.g., QOL15(r)], objective assessments (e.g., QMG) and composite measures (e.g., MGC or MGII). Fatigue measures (e.g., NeuroQOL) may provide additional and relevant information. However, several clinically relevant issues are not addressed by any of the current scales, and the relation of several basic patient characteristics to current outcome measures remain unsettled. This restricts thorough assessment of treatment efficacy and may limit conclusions concerning validity across subpopulations in MG.

AUTHOR CONTRIBUTIONS

JT wrote and revised the manuscript. HA co-authored and revised the manuscript for intellectual content. Both authors contributed to the article and approved the submitted version.

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Vitamin D Receptor Polymorphism and Myasthenia Gravis in Chinese Han Population

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Myasthenia gravis (MG) is an autoimmune disease in which antibodies bind to acetylcholine receptors (AChR) or other functional molecules in the postsynaptic membrane at the neuromuscular junction. Vitamin D (VD) has a number of pluripotent effects, which include immune-regulation and bone metabolism. The immunomodulatory actions of 1,25(OH)2D3 are mediated by its binding to a vitamin D receptor (VDR). In the study, we undertook a case-control study to explore the association between VDR gene polymorphism and the susceptibility and severity of MG patients. Four hundred and eighty MG patients and 487 healthy controls were included and gene polymorphisms of VDR were determined with improved multiplex ligation detection reaction technique and SNPscan^{TM} technique. MG patients were classified into subgroups by essential clinical features and by a comprehensive classification. The frequencies of alleles and genotypes were compared between the MG group and the control group, between each MG subgroup and the control group, and between each pair of MG subgroups. There were no significant differences in frequencies of alleles and genotypes between MG patients and healthy controls, between MG subgroups and healthy controls, or between each pair of MG subgroups in the analysis of subgroups classified by essential clinical features (onset age, gender, thymoma, AChRAb positivity, onset involvement) and the maximal severity (modified Oosterhuis score). In the analysis of subgroups with a comprehensive classification, the frequencies of alleles and genotypes in rs731236 showed significant differences between adult non-thymoma AChRAb negative MG subgroup and the control group, as well as the adult non-thymoma AChRAb positive MG group. In the Chinese Han population, rs731236 was found to be possibly associated with adult non-thymoma AChRAb negative MG patients, although this needs further confirmation.

Keywords: myasthenia gravis, vitamin D receptor, polymorphism, susceptibility, severity

INTRODUCTION

Myasthenia gravis (MG) is an autoimmune disease in which antibodies bind to acetylcholine receptors (AChR) or to other functional molecules, such as muscle-specific kinase (MuSK) and lipoprotein-receptor-related protein 4 (LRP4), in the postsynaptic membrane at the neuromuscular junction (NMJ) (1). Various immune cells involving innate and adaptive immunity participate in the pathogenesis of MG, including dendritic cells and B and T lymphocytes (2). Immune-modulating molecules, such as cytokines, are major mediators. An aberrant regulation of the immune system is presumed to be involved in the susceptibility and the severity of MG. Immune response is also modulated by other molecules, such as vitamin D (3).

Vitamin D (VD) has a number of pluripotent effects, which include immune-regulation and bone metabolism. 1,25(OH)2D3 promotes the differentiation of monocytes and inhibits the maturation of dendritic cells (4). 1,25(OH)2D3 inhibits the proliferation and differentiation of T helper 1 cells and modulates cytokine production by reducing the expression of pro-inflammatory interleukin-2 and interferon-γ, stimulates T helper 2 cells with upregulation of the production of anti-inflammatory cytokines, suppresses the development of Th17 cells, inhibits the production of interleukin-17, and induces proliferation of regulatory T cells (5, 6). Moreover, 1,25(OH)2D3 inhibits proliferation and differentiation of plasma cells (6).

The immunomodulatory actions of 1,25(OH)2D3 are mediated by its binding to VDR. The Vitamin D receptor (VDR) is a transcription factor belonging to the glucocorticoid nuclear receptor family, which binds to 1α,25-dihydroxyvitamin D3 [1,25(OH)2D3] (7). VDRs are expressed in most immune cells, including monocytes/macrophages, dendritic cells, B lymphocytes, and T lymphocytes (3). The formation of the VD-VDR complex results in gene expression at the transcriptional level (3). The VDR gene is located in 12q14, which includes six untranslated exons (exon1a-1f) and eight coding exons (exons 2–9). The VDR gene could affect the expression of VDR (8). VDR genes' polymorphism has been associated with many autoimmune disorders such as autoimmune thyroid disease (9), idiopathic inflammatory myopathy (10), multiple sclerosis (11), type 1 diabetes mellitus (12), and systemic lupus erythematosus (13).

Our previous study found that VDR gene Tru9I (rs757343) polymorphism was associated with risk of MG in females older than 15 years (14). In this study, we undertook a case-control study to further explore the association between VDR gene polymorphisms and the susceptibility and severity of MG patients in a systematic way.

SUBJECTS AND METHODS

Subjects

Four hundred and eighty patients who were diagnosed and treated in the Affiliated Hospital of Qingdao University and Beijing Friendship Hospital, Capital Medical University and 487 randomly recruited healthy controls in the same area were included in this study. All MG patients met the following diagnostic criteria: typical symptoms of fluctuating muscle weakness, positive result of neostigmine test, and the presence of AChRAb or MuSK antibody and/or amplitude decrement response >10% in low frequency repetitive nerve stimulation test (15, 16). MG patients were followed up with at least twice a year, with additional follow-ups if symptoms worsened and within 2–3 months thereafter (15, 16). Maximal severity was acquired by the history of MG patients and follow-ups and qualified by Oosterhuis score (17). Chronic infections (with relevant tests to exclude suspected infection when possible) and commonly associated autoimmune diseases (by self-reported questionnaire and tests including anti-thyroid antibodies and ENA) were excluded in both MG patients and healthy controls. All MG patients and healthy controls were Han Chinese in origin and non-consanguineous. Informed consent was obtained from all adult participants and the guardians of juvenile MG patients. The study was approved by the ethical committees of the two hospitals.

Patients were classified by essential clinical characteristics such as gender, onset age (<15/15–50/>50 years) (1), thymoma (typical CT and/or pathology), AChRAb, onset involvement (ocular/generalized), and the maximal severity (modified Oosterhuis score 0–2/3–5). AChRAb was detected with ELISA kits (RSR Limited, Cardiff, UK) and MuSK antibodies were detected and measured with RIA method (RSR Limited, Cardiff, UK) in AChR antibody negative MG patients. A natural history study showed that 82% of MG patients reached maximum worsening within 2 years after onset (18); therefore, the maximum Oosterhuis score was analyzed only in patients with a clinical course of 2 years or more. Because of potential interactions among the essential clinical characteristics (16, 19), a comprehensive classification (**Figure 1**) (20) was also used in the analysis. The new classification used the combination of essential clinical characteristics of MG to classify biologically and clinically meaningful subgroups. It ensured that any MG patients with sufficient data were assigned into one subgroup and only to one subgroup.

Methods

SNP Selection and Genotyping

SNPs were selected systematically, including the functional loci [rs4516035 (5′ near gene), rs2228570 (exon 2), rs9729 (3′UTR)], hot SNPs [rs1544410 (9, 10, 12), rs731236 (9, 10, 12), rs7975232 (9, 10, 12), rs757343 (21), rs2238136 (22)], and tag SNPs (rs3847987, rs10875692, rs2107301, rs2239186, rs2853564, rs11574027, rs7136534, rs739837, and rs2239181). Nine tag SNPs were selected using the UCLA Association Study Design Server online software package (http://design.cs.ucla.edu), based on HapMap database (23). The minor allele frequency (MAF) of each SNP was more than 5% in the Han Chinese population (1,000 Genomes Project Phase 3). Genomic DNA was extracted using peripheral blood genomic DNA purification kit (Biochain, Newark, CA, USA). Rs1544410 and rs2107301 were genotyped by using improved multiplex ligation detection reaction (iMLDR) technique (Shanghai Genesky Biotechnologies Inc. China). The remaining fifteen SNPs were genotyped with SNPscan^{TM}

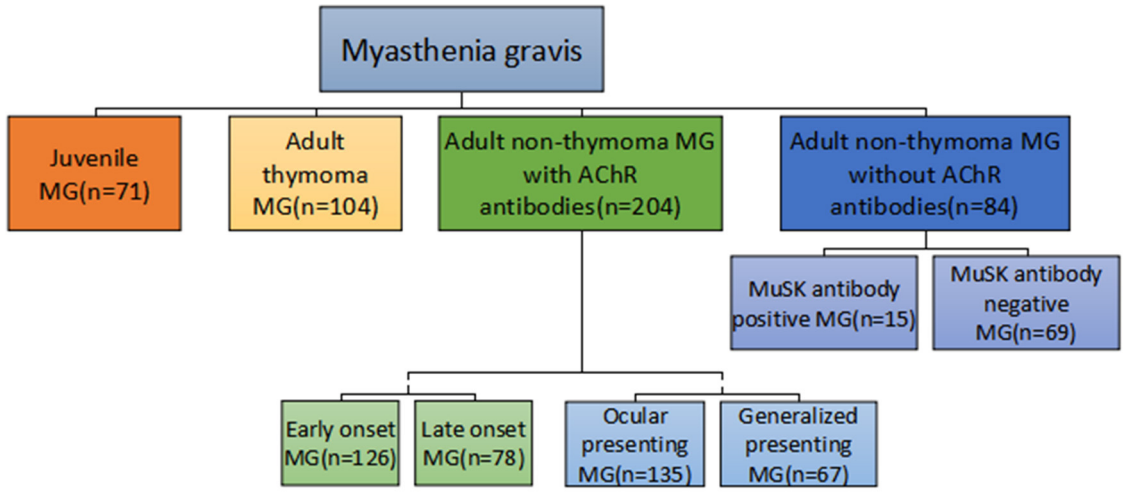

FIGURE 1 | MG comprehensive subgroup classification.

technique Kit (Cat#: G0104, Genesky Biotechnologies Inc. Shanghai, China). The primers and probes will be provided on request. Forty samples were randomly selected for double-blind quality control, and the results were consistent with the original genotyping results.

Statistical Analysis

The online SNPstats software (https://snpstats.net/start.htm) was used to test the Hardy-Weinberg equilibrium in the control group. Genotype frequencies were analyzed under codominant and additive inheritance models in SNPstats software. The χ^2 test or Fisher exact test was used to compare the allele frequencies between MG group and the control group, between each MG subgroup and the control group, and between each pair of MG subgroups (SPSS17.0). Bonferroni correction was applied for the multiple-testing. When there were significant differences in allele frequencies between MG subgroups and the control group or among MG subgroups, Logistic regression (SPSS 17.0) was used to adjust for potential confounding factors. The Haploview 4.2 software was used to calculate the linkage disequilibrium of SNPs and construct haplotype blocks. *Post-hoc* statistical power was calculated by Quanto program (version 1.2.4). $P \leq 0.05$ was considered as statistically significant.

RESULTS

General Characteristics

The successful genotyping rates of the seventeen SNPs were 96.07%∼99.79%. Among the 17 selected SNPs, frequencies of rs2853564 in the healthy controls ($P < 0.05$) were not consistent with the Hardy-Weinberg equilibrium and were excluded from further analysis (**Table 1**).

In MG patients, 189 were males and 291 were females. Onset age was 1–86 years old (median 40, interquartile range 32). The disease duration of MG ranged from 8 to 220 months (median 43, interquartile range 61). There were 107 patients with thymoma and 367 patients without thymoma. Three hundred and thirty eight patients were AChRAb positive and 124 patients were AChRAb negative. Three hundred and forty two patients were ocular presenting and 135 patients were generalized presenting at onset. The others had no relevant information. The maximum Oosterhuis score was available in 370 patients (77%). Two hundred and sixteen patients were classified into the mild subgroup (Oosterhuis score 0–2) and 154 patients into the severe subgroup (Oosterhuis score 3–5) (**Supplementary Table 1**).

Four hundred and eighty seven healthy controls, including 249 males and 238 females, were 14–78 years old (median 45, interquartile range 24).

Allele and Genotype Frequency Comparison in MG Group/Each MG Subgroup and the Control Group

There were no significant differences in allele frequencies between the MG group and the control group, and in genotype frequencies between the MG group and the control group under the codominant or additive inheritance model among the 16 SNPs. There were no significant differences in allele or genotype frequencies between each MG subgroup (gender, onset age, thymoma, AChRAb, onset involvement, and maximal severity) and the control group, or between each pair of MG subgroups (**Supplementary Table 1**).

Allele and Genotype Frequency Comparison in the Comprehensive Classified MG Subgroups and the Control Group

According to the comprehensive classification, 71 patients were juvenile MG (onset age <15) and 409 patients were adult MG. In adult MG patients, 104 patients had thymoma and 300 patients were without thymoma. In adult non-thymoma patients, 84 patients were AChRAb negative and 204 patients were AChRAb

TABLE 1 | General information of SNPs in MG patients and healthy controls.

SNPs	Function	Alleles	Allele frequencies		*P*-value	Genotypes	Genotype frequencies		*P*-value	
(Genotyping rate)			MG	HC			MG	HC	Codominant	Additive
rs4516035	5′near gene	T	929 (0.98)	939 (0.97)	0.280	TT	454 (0.96)	456 (0.94)	0.36	0.28
(99.17%)		C	21 (0.02)	29 (0.03)		TC	21 (0.04)	27 (0.06)		
						CC	0 (0)	1 (0)		
rs7136534	Intron	C	574 (0.61)	601 (0.62)	0.564	CC	178 (0.38)	184 (0.38)	0.61	0.57
(99.28%)		T	374 (0.39)	371 (0.38)		CT	218 (0.46)	233 (0.48)		
						TT	78 (0.16)	69 (0.14)		
rs11574027	Intron	C	829 (0.87)	852 (0.88)	0.796	AA	4 (0.01)	9 (0.02)	0.25	0.79
(99.38%)		A	121 (0.13)	120 (0.12)		CA	113 (0.24)	102 (0.21)		
						CC	358 (0.75)	375 (0.77)		
rs2238136	Intron	C	763 (0.8)	775 (0.8)	0.682	CC	304 (0.64)	314 (0.64)	0.27	0.69
(99.48%)		T	187 (0.2)	199 (0.2)		CT	155 (0.33)	147 (0.3)		
						TT	16 (0.03)	26 (0.05)		
rs2228570	Missense	G	531 (0.56)	529 (0.54)	0.422	AA	79 (0.17)	102 (0.21)	0.18	0.41
(99.07%)	Mutation	A	413 (0.44)	443 (0.46)		GA	255 (0.54)	239 (0.49)		
						GG	138 (0.29)	145 (0.3)		
rs2239186	Intron	A	484 (0.51)	490 (0.5)	0.637	AA	126 (0.27)	122 (0.25)	0.83	0.64
(98.86%)		G	456 (0.49)	482 (0.5)		AG	232 (0.49)	246 (0.51)		
						GG	112 (0.24)	118 (0.24)		
rs2239181	Intron	A	742 (0.78)	789 (0.81)	0.161	AA	289 (0.61)	324 (0.67)	0.16	0.16
(99.28%)		C	204 (0.22)	185 (0.19)		AC	164 (0.35)	141 (0.29)		
						CC	20 (0.04)	22 (0.05)		
rs2107301	Intron	A	665 (0.7)	681 (0.7)	0.864	AA	236 (0.49)	233 (0.48)	0.32	0.86
(99.79%)		G	291 (0.3)	293 (0.3)		AG	193 (0.4)	215 (0.44)		
						GG	49 (0.1)	39 (0.08)		
rs1544410	Intron	C	899 (0.94)	932 (0.96)	0.144	CC	422 (0.88)	445 (0.91)	0.13	
(99.69%)		T	55 (0.06)	42 (0.04)		CT	55 (0.12)	42 (0.09)		
rs757343	Intron	C	717 (0.78)	749 (0.79)	0.632	CC	278 (0.6)	295 (0.62)	0.87	0.63
(97.1%)		T	207 (0.22)	205 (0.21)		CT	161 (0.35)	159 (0.33)		
						TT	23 (0.05)	23 (0.05)		
rs10875692	Intron	C	796 (0.84)	798 (0.82)	0.279	CC	333 (0.7)	329 (0.68)	0.48	0.28
(99.48%)		T	154 (0.16)	176 (0.18)		CT	130 (0.27)	140 (0.29)		
						TT	12 (0.03)	18 (0.04)		
rs7975232	Intron	C	650 (0.71)	713 (0.74)	0.218	AA	36 (0.08)	39 (0.08)	0.22	0.22
(97.31%)		A	264 (0.29)	255 (0.26)		CA	192 (0.42)	177 (0.37)		
						CC	229 (0.5)	268 (0.55)		
rs731236	Synonymous	A	895 (0.94)	928 (0.95)	0.294	AA	422 (0.89)	442 (0.91)	0.56	0.3
(99.48%)	Mutation	G	55 (0.06)	46 (0.05)		AG	51 (0.11)	44 (0.09)		
						GG	2 (0)	1 (0)		
rs739837	3′UTR	G	640 (0.71)	704 (0.73)	0.253	GG	226 (0.5)	265 (0.55)	0.23	0.26
(96.07%)		T	260 (0.29)	254 (0.27)		GT	188 (0.42)	174 (0.36)		
						TT	36 (0.08)	40 (0.08)		
rs3847987	3′UTR	C	732 (0.77)	763 (0.78)	0.499	AA	24 (0.05)	24 (0.05)	0.73	0.5
(99.48%)		A	218 (0.23)	211 (0.22)		CA	170 (0.36)	163 (0.33)		
						CC	281 (0.59)	300 (0.62)		
rs9729	3′UTR	G	666 (0.7)	704 (0.73)	0.180	GG	235 (0.49)	256 (0.53)	0.4	0.18
(99.48%)		T	288 (0.3)	266 (0.27)		GT	196 (0.41)	192 (0.4)		
						TT	46 (0.1)	37 (0.08)		

positive. In adult non-thymoma AChRAb negative patients, 15 patients were MuSK antibody positive and 69 patients were MuSK antibody negative. In adult non-thymoma AChRAb positive patients, 126 patients were early onset MG (EOMG, onset age 15–50) and 78 patients were late onset MG (LOMG, onset age >50); 135 patients were ocular presenting and 67 patients were generalized presenting. The others had no relevant information (**Supplementary Table 2**).

The G allele frequency in rs731236 was significantly higher in the adult non-thymoma AChRAb negative MG subgroup than that in the control group ($P_{bon} = 0.032$, OR = 2.42) and in the adult non-thymoma AChRAb positive MG group ($P_{bon} = 0.032$, OR = 2.90) (**Table 2**). *Post-hoc* statistical power was 0.9791 and 0.9929 based on Log-additive inheritance mode for the two comparisons. There were significant differences in genotype frequencies between adult non-thymoma AChRAb negative MG and the control group ($P = 0.017$ and $P = 0.0044$) as well as between adult non-thymoma AChRAb negative MG and adult non-thymoma AChRAb positive MG ($P = 0.0092$ and $P = 0.003$) in rs731236 under the codominant and additive inheritance model (**Table 2**).

We further compared between the MuSK antibody positive and MuSK antibody negative (double negative) group within the AChRAb negative MG patients. The G allele frequency in rs731236 was significantly higher in the double negative group than those in the control group ($P_{bon} = 0.016$, OR = 2.65). There were no significant differences in allele frequency in rs731236 between the MuSK positive and the double negative group, as well as between the MuSK positive and the control group (**Supplementary Table 2**).

Adjustment of Potential Confounding Factors in Clinical Variable Based Subgroup Analysis

Logistic regression analysis was performed in adult non-thymoma MG patients with AChRAb (positive and negative) as a dependent variable, and with gender (male and female), onset age (15–50 and >50 years), muscle involvement at onset (ocular and generalized), and genotypes of rs731236 (codominant model) as independent variables. The genotype and muscle involvement at onset were found to be independent risk factors (**Table 3**).

Linkage Disequilibrium Analysis

Linkage disequilibrium analysis amongst 16 SNPs was shown in **Figure 2A**. Two haplotype blocks were constructed. Block 1 was constructed by rs9729, rs3847987, rs739837, rs731236, rs7975232, rs10875692, and rs757343, and block 2 was constructed by rs11574027 and rs7136534. There were no significant differences in haplotype frequencies between MG and the control group (**Table 4.1**). We also performed linkage disequilibrium analysis between the adult non-thymoma AChRAb negative MG subgroup and the control group (**Figure 2B**). Two haplotype blocks were constructed. Block 1 was constructed by rs9729, rs3847987, rs739837, rs731236, rs7975232, rs10875692, rs757343, and rs1544410, and block 2 was constructed by rs11574027 and rs7136534. There were also no significant differences in haplotype frequencies between the adult non-thymoma AChRAb negative MG subgroup and the control group (**Table 4.2**).

DISCUSSION

In our study, we found there were no significant differences between MG patients and healthy controls, between MG subgroups and healthy controls, or between each pair of MG subgroups in the analysis of subgroups classified by essential clinical features and the maximal severity. In subgroup analysis by the comprehensive classification, the frequencies of alleles and genotypes in rs731236 showed significant differences between the adult non-thymoma AChRAb negative MG subgroup and the control group, as well as the adult non-thymoma AChRAb positive MG group (**Table 2**). The statistical power of this association was high. We further compared between the MuSK antibody positive and the double negative group within the AChRAb antibody negative MG patients. There was significant difference between the double negative group and the control group. However, there were no significant differences between the MuSK antibody positive group and double negative group, as well as the control group. Although the comprehensive classification eliminates some of the confounding factors, other clinical variables (early or late onset, gender, and initial muscular involvement) might lead to confounding effects. Logistic analysis revealed that rs731236 was an independent risk factor in the adult non-thymoma AChRAb negative MG subgroup.

Rs731236 (also known as TaqI) is within exon 9. Its allele polymorphism yields a synonymous coding sequence. Nevertheless, it is located near the 3′UTR of VDR, which is known to be involved in the regulation of gene expression through the regulation of mRNA stability and protein translation efficiency (8). The rs731236 was found to be located in a CpG imposing a direct *cis* effect on site-specific and regional methylation (24). Children carrying the C allele for TaqI were more likely to develop asthma, and interleukin-10 levels were significantly low in asthmatics with the TC genotype for TaqI due to a decrease in expression of VDR (25). Therefore, it is presumed that rs731236 can affect the expression of VDR, and thus affect the expression of cytokines, thereby exerting immunomodulatory effects. rs731236 was also found to be associated with allergic diseases (8), autoimmune thyroid disease (9), and multiple sclerosis (26).

MG is a heterogeneous autoimmune disease with distinct immunogenetic characteristics in different MG subtypes (27). In AChRAb negative MG patients, antibodies against other NMJ proteins, such as MuSK and LRP4, are found (28). Moreover, some of the AChR antibody negative patients might be shown to be AChR antibody positive when more sensitive testing methods are used (29). In our study, there were no significant differences between the MuSK antibody positive and double negative group, as well as the control group. Hence, the double (AChR and MuSK antibodies) negative MG patients should be analyzed with further antibody testing.

TABLE 2 | Significant differences in subgroups and healthy controls.

	Number[a]	*P*-value[b]	P_{bon}	OR	95%CI	Codominant[c]	Additive
rs731236							
Adult thymoma (–) AChRAb (–) MG/control group	84/487	0.002	0.032	2.42	1.37–3.75	0.017	0.0044
Adult thymoma (–) AChRAb (–) MG/adult thymoma (–) AChRAb (+) MG	84/201	0.002	0.032	2.9	1.44–4.82	0.0092	0.003

[a] *successfully genotyped number with adequate clinical data in each group.*
[b] *P-value, P_{bon}, the OR, and 95%CI in allele frequency comparison between MG subgroup and the control group.*
[c] *P-value in genotype frequency comparison under codominant and additive inheritance models.*

TABLE 3 | Logistic regression analysis in subgroups.

Variables	Regression coefficient	Standard error	Wald x^2 value	*P*-value	OR
Onset age	0.261	0.272	0.924	0.337	1.298
Gender	−0.322	0.275	1.366	0.243	0.725
Muscle involvement at onset	−0.629	0.320	3.857	0.050	0.533
Rs731236	1.046	0.372	7.914	0.005	2.847

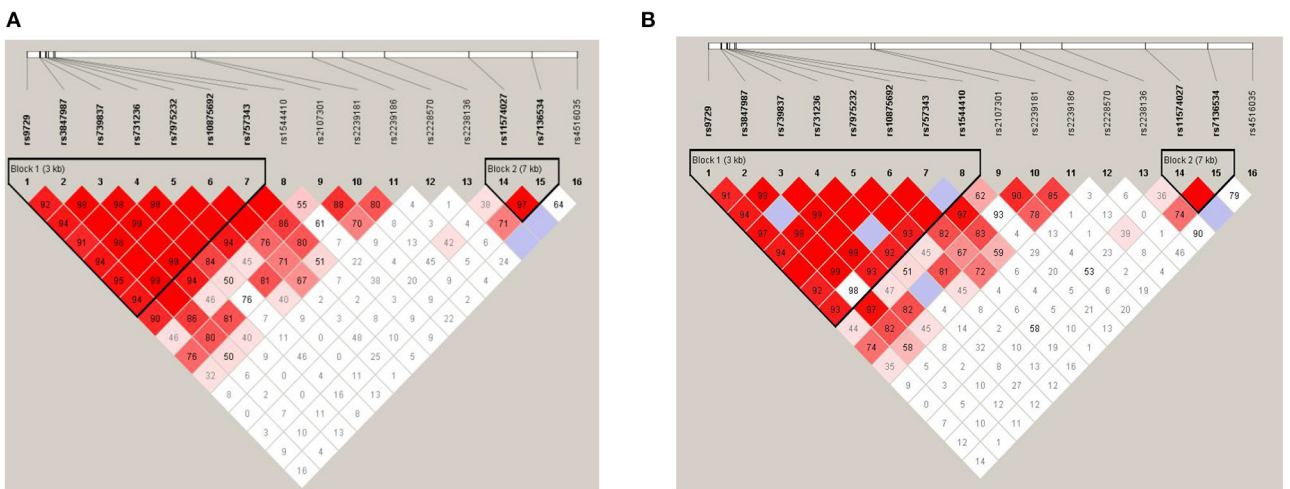

FIGURE 2 | Linkage disequilibrium (LD) plot and haplotype block construction. MG patients and healthy controls **(A)**; adult non-thymoma AChRAb negative MG patients and healthy controls **(B)**. The locations of each SNP were indicated by the straight lines. The D' value corresponding to each SNP pair was expressed as a percentage and shown within the respective square (D' = 1, not shown). Higher D' values were indicated in darker red.

Previous studies found that rs7975232, rs731236, and rs1544410 variants were in strong linkage disequilibrium (30), and we also performed haplotype analysis. We found that rs9729, rs3847987, rs739837, rs731236, rs7975232, rs10875692, and rs757343 were in high linkage disequilibrium, but there were no significant differences in haplotype frequencies between MG and the control group, which suggested that these haplotypes were not significantly related to the susceptibility of MG. There were also no significant differences in haplotype frequencies between the adult non-thymoma AChRAb negative MG subgroup and the control group.

Our previous research (14) found that VDR gene Tru9I (rs757343) polymorphism may be associated with risk of MG in females older than 15 years. However, the association was only found in a subgroup and the sample size was small. Moreover, AChR antibodies were not included in that study and no Logistic analysis was performed, which might lead to bias. Therefore, we recruited a new cohort with a larger sample size, selected SNPs of VDR gene in a systematic strategy, and performed Logistic analysis. In this study, we used both the subgroups classified by single clinical features and a new comprehensive scheme. We cannot confirm the previous association in this study.

TABLE 4.1 | Haplotypes of the VDR gene between MG and the control group.

Haplotypes[a]	MG freq.	Control freq.	Chi square	*P*-value
Block 1				
GCGACCC	0.526	0.540	0.359	0.549
TATAACT	0.214	0.208	0.132	0.7161
GCGACTC	0.159	0.179	1.287	0.2566
TCTGACC	0.053	0.047	0.308	0.5789
TCGACCC	0.014	0.016	0.106	0.7452
Block 2				
CC	0.480	0.496	0.473	0.4917
CT	0.392	0.381	0.279	0.5976
AC	0.126	0.123	0.04	0.8417

[a]*haplotypes with frequency < 1% were not listed.*
The SNP sequences are rs9729-rs3847987-rs739837-rs731236-rs7975232-rs10875692- rs757343 in block 1 and rs11574027-rs7136534 in block 2.

TABLE 4.2 | Haplotypes of the VDR gene between adult non-thymoma AChRAb negative MG and the control group.

Haplotypes[a]	MG freq.	Control freq.	Chi square	*P*-value
Block 1				
GTGATTTT	0.532	0.538	0.021	0.8846
CACAATCT	0.173	0.208	1.041	0.3076
GTGATCTT	0.147	0.182	1.073	0.3003
CTCGATTC	0.077	0.043	3.523	0.0605
CTGATTTT	0.019	0.018	0.017	0.8957
Block 2				
TT	0.476	0.495	0.212	0.6452
TC	0.373	0.381	0.037	0.8472
AT	0.151	0.123	0.943	0.3315

[a]*haplotypes with frequency < 1% were not listed.*
The SNP sequences are rs9729-rs3847987-rs739837-rs731236-rs7975232-rs10875692-rs757343-rs1544410 in block 1 and rs11574027-rs7136534 in block 2.

There are several limitations to our study. The first limitation is that we did not measure the serum vitamin D levels. Our study mainly explores the association between vitamin D receptor gene polymorphism and the susceptibility and maximal severity of MG. Although vitamin D levels may be related with severity of MG by its immune-modulating effects, the serum vitamin D levels on blood collecting did not reflect the vitamin D levels during the entire disease course and at the maximal severity, due to factors such as steroid use, dietary habits, and sun exposure. This study cannot exclude the effects of vitamin D level by its design. Another limitation is that severity is analyzed by Oosterhuis score. It is relatively crude but has been used in other association studies (17). However, our study analyzes the maximum severity during the whole disease course of the patients. Oosterhuis scores can be accessed from the medical history, while more accurate measurements of severity, such as QMG scores recommended by MGFA, require on-site measurement, and hence are unfit for collecting information of maximal severity. A final limitation is that the sample size of the MuSK antibody positive MG subgroup is small and other related antibodies were not examined.

In conclusion, in the Chinese Han population, rs731236 was found to be possibly associated with adult non-thymoma AChRAb negative MG patients. Our study is a preliminary study, which needs further confirmation.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found here: https://www.ncbi.nlm.nih.gov/SNP/snp_viewTable.cgi?handle=VDR-MG-CHINESE.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethical committees of Affiliated Hospital of Qingdao University, and Beijing Friendship Hospital, Capital Medical University. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

J-LH, Y-XY, and H-FL designed the study, interpreted the data, and wrote the manuscript. Y-XY and H-FL designed the experiment. J-LH and Y-XY performed statistical analysis. Y-CX and H-JH contributed to discussion. H-FL, Y-CX, and XG diagnosed and treated patients. XG, H-YL, X-LY, and JL maintained the database. R-SD and H-FL revised the manuscript. All authors approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2021.604052/full#supplementary-material

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Thymectomy in Juvenile Myasthenia Gravis Is Safe Regarding Long Term Immunological Effects

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Thymectomy is an established treatment in adult MG and also recommended for the treatment of post-pubertal onset juvenile MG. Whether the youngest children should be thymectomized is still debated. Signs of premature aging of the immune system have been shown in studies on early perioperative thymectomy in children with congenital heart defect. In this retrospective cohort study the objective was to investigate the long-term effects of treatment related thymectomy on T cell subsets and T cell receptor rearrangement excision circles (TRECs) in peripheral blood of juvenile myasthenia gravis (MG) patients, as well as clinical occurrence of autoimmune disorders, malignancies and infectious diseases. Forty-seven patients with onset of myasthenia gravis before the age of 19 years were included; 32 (68.1%) had been thymectomized and 15 (31.8%) had not. They were studied at varying times after thymectomy (7–26 years). We found a significant lower number of naïve helper T cells (CD4+CD45RA+) with an increased proportion of memory helper T cells (CD4+CD45RO+), and a significant lower number of naïve cytotoxic T cells (CD8+CD27+CD28+) in the thymectomized patients. In addition they showed a significant reduction in the number of TRECs and proportion of recent thymic emigrants (RTE) compared to non-thymectomized patients. In none of them an increased frequency of malignancies or infections was found. Our findings indicate a premature aging of the immune system after thymectomy in juvenile MG, but associated clinical consequences could not be verified.

Keywords: juvenile myasthenia gravis, thymectomy, TREC, T cells, polyautoimmunity, immunosenescence

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INTRODUCTION

Juvenile myasthenia gravis (MG) is a rare autoimmune disorder giving fatigable muscle weakness due to immunological destructions at the endplate of the neuromuscular junction. In the majority of cases these attacks are mediated through autoantibodies against the acetylcholine receptors (AChR) at the endplate (1). Although B cells produce these antibodies; the destructive process is T cell dependent and also involves complement activation (2).

It has long been established that the thymus plays an important role in the MG pathogenesis and the disorder is associated with thymic changes, both thymoma and thymus hyperplasia (3). Thymectomy has been used in the treatment of MG since 1941, but just recently the first randomized controlled trial on thymectomy in MG was conducted, and it showed benefit of thymectomy (4). The study did not disclose safety and efficacy findings in patients below the age of 18 years since these were excluded. Although there are no randomized controlled studies on thymectomy in juvenile MG, several prospective studies have shown positive effects (5, 6). In the latest international consensus guidelines for the management of MG, thymectomy is recommended for the treatment of postpubertal onset juvenile MG (7). It is still debated, however, whether the youngest children should be thymectomized. One of the questions raised is whether an early thymectomy has negative consequences for immune responses later in life. The weight of the thymus in proportion to the body is greatest just before birth. Atrophy and reduction of thymus activity start early in life, and its role after the initial T cell production is not clear (8). Studies on early perioperative thymectomy in children with congenital heart defects have shown signs of premature aging of the immune system, especially the T cell compartment (9–11). However, the clinical consequences are still not established.

Aging of the immune system is termed immunosenescence. T cell immunosenescence includes loss of thymus function, reduced number of recent thymic emigrants (RTE), proliferation of mature T cells and oligo clonal expansion of specific T cell subpopulations (12). Potential clinical implications are increased infection rates, reduced antibody response to vaccines and increased occurrence of autoimmunity and cancer.

We have in a previous retrospective study shown that thymectomy is efficacious in a Norwegian juvenile MG cohort including patients with prepubertal disease onset (13). The aim of the present study was to evaluate the long-term immunological consequences of thymectomy in juvenile MG especially focusing on association with age at thymectomy.

MATERIALS AND METHODS

Patients and Blood Samples

In this population-based study, patient identification was conducted nationwide from January 2012 to April 2016 using multiple strategies: (i) through neurological and/or pediatric departments at the 15 main hospitals in Norway, (ii) through the national AChR antibody database at Haukeland University Hospital and (iii) through the national adult MG database at Oslo University Hospital. All patients had disease onset before the age of 19 years. MG diagnosis was based on clinical, serological, electrophysiological and pharmacological criteria described in detail in a previous publication (14). Retrospective clinical data were collected from medical records. Updated data on comorbidity and immunosuppressive treatment were collected through interviews at the time of blood sample collection for the T cell subset analysis. Blood sample collection was conducted at Oslo University Hospital from May 2015 to April 2016. Peripheral blood was collected by venipuncture from 32 thymectomized and 15 non-thymectomized juvenile MG patients. One sample was collected from each patient. The blood samples were analyzed for lymphocyte subsets and T cell receptor rearrangement excision circles (TRECs).

Preparation and Quantification of T Cell Subsets

T cell subpopulations were analyzed by flow cytometry. Briefly EDTA-blood was incubated with optimally titrated antibodies for 15 min at room temperature, followed by erythrocyte lysis (BD FACS Lysing Solution, Becton Dickinson, San Jose, CA, USA). Data acquisition was performed on a Canto II flow cytometer (Becton Dickinson) and 100 000 cells was acquired when possible.

The following subpopulations were determined according to IPID (Immune phenotyping in Immunodeficiency), European Society of Immunodeficiencies.

T-cells were gated as CD3+ and further as naive CD4+ (CD4+ CD45RA+), recent thymic emigrants (CD4+ CD45RA+ CD31+), CD4+ memory (CD4+ CD45RO+), follicular like CD4+ (CD4+ CD45RO+ CCR5+), regulatory T-cells (CD4+ CD25+ CD127–), naive CD8+ (CD8+ CD27+ CD28+), CD8+ early effector memory (CD8+ CD27+ CD28–) and CD8+ late effector memory (CD8+ CD27–CD28–).

TREC Analysis

DNA extraction was done using BLOOD DNA kit (Omega-Biotek, USA). The extracts were analyzed by PCR as previously described (15). To assure adequate DNA extraction betaactin was used as housekeeping gene. All qPCR assays had as required R^2 values > 0.99 and similar slopes.

Statistical Analysis

Demographic and clinical data are presented as either proportions or median values with interquartile range (IQR). Differences in categorical variables between thymectomized and non-thymectomized patients were tested by chi square test or Fisher's exact test as appropriate. Person's correlation coefficient (r) was used to analyze the association between two continuous variables. Linear regression analysis was performed to investigate the relationships between thymectomized patients and non-thymectomized patients and the total counts of T cell subsets, and to adjust for the possible confounding effect of chronological age. A significance level of 5% was used. All statistical analyses were performed using IBM SPSS Statistics for Windows, version 23 (Armonk, NY, USA: IBMCorp.).

Ethics

The study was approved by the Norwegian Regional Committee for Medical and Health Research Ethics, South East Office. All patients, and also their parents when they were under 16 years old, gave written informed consent. Data were collected and registered in accordance with Norwegian guidelines.

TABLE 1 | Clinical characteristics of the included JMG cases, thymectomized vs. non-thymectomized.

	Thymectomized *n* = 32	Non-thymectomized *n* = 15
Female gender, *n* (%)	25 (78%)	13 (87%)
Prepubertal onset*	10 (31%)	8 (53%)
AChR antibody positive MG, *n* (%)	29 (91%)	7 (47%)
Generalized MG, *n* (%)	30 (94%)	12 (80%)
Age at thymectomy in years, median (range)	17 (2–33)	n.a.
Thymus hyperplasia**, *n* (%)	20 (63%)	n.a.
Time from onset to thymectomy in months, median (IQR)	21 (9–31)	n.a.
Complete stable remission	16 (50%)	6 (40%)
Age at sample collection in years, median (IQR)	28 (25–41)	24 (12-48)
Time from thymectomy to sample collection in years, median (IQR)	12 (7–26)	n.a.
Immunosuppressives***, *n* (%)	8 (25%)	4 (27%)
Other autoimmune disorder, *n* (%)	11 (34%)	6 (40%)
Malignancies, *n*	1 (skin)	0

IQR, interquartile range.
**Onset <12 years.*
***Histologically verified at time of thymectomy.*
****Steroids, azathioprine, or mycophenolate mofetil at time of blood sample collection/t-cell subset analysis.*
AChR, acetylcholine receptor; MG, myasthenia gravis; n.a, not applicable.

RESULTS

Clinical Data

Group characteristics and clinical data are presented in **Table 1**. The median follow up since thymectomy was 12 (IQR 7–26) years and median age at time of blood sample collection (chronological age) was 28 (IQR 25-41) years in the thymectomized (Tx) group and 24 (IQR 12-48) years in the non-thymectomized (non-Tx) group. At the time of the T-cell subset analysis, 12 patients received immunosuppressive treatment, 8 (25%) in the Tx and 4 (27%) in the non-Tx group. Autoimmune comorbidity was present in 11 (34%) of the Tx patients and in 6 (40%) of the non-Tx patients while malignancy only was reported in one patient, who was thymectomized. No patients reported increased frequency of infectious diseases.

Reduced Number of Lymphocytes in Tx Patients

A reduced number of lymphocytes, both T cells (CD3+) (1066 vs. 1727 $\times$ 10^6/L; $P < 0.0001$) and B cells (CD19+) (188 vs. 359 $\times$ 10^6/L; $P = 0.008$), were found in the thymectomized patients compared to the non-thymectomized. Natural killer (NK) cells were unaffected. See **Figures 1A–C**.

Reduction in Naïve CD4+ T Cell Numbers and Naïve CD8+ T Cell Numbers in Tx Patients

Looking more in detail at the T cell subsets, there were a decrease in the number of both CD4+ helper T cells and cytotoxic CD8+ T cells. See **Figures 1D,G**. The naïve helper T cell count (CD4+CD45RA+) was lower in the Tx group than in the non-Tx group, whereas the memory helper T cells (CD4+CD45RO+) were unaffected. However, the latter T cell subset count showed a proportional increase since the CD4+ helper T cell number was low. See **Figures 1E,F**. The number of naïve cytotoxic T cells (CD8+CD27+CD28+) was also lower in Tx cases, whereas the effector/memory cytotoxic T cells (early effector/memory: CD8+CD27+CD28-, and late effector/memory: CD8+CD27-CD28-) were unaffected by thymectomy, as were the regulatory T cells (CD4+CD25+CD127-). See **Figures 1H–J**. These associations were still present after adjusting for chronological age. We found no correlation of these T cell subsets with age at thymectomy (**Table 2**).

Reduction in TREC Numbers and RTE in Tx Cases

Thymic activity was assessed by measuring TREC levels and recent thymic emigrants (RTE) by the surface marker CD4+ CD45RA+CD31+. TRECs (222 vs. 1868/200µl; $p = 0.001$) and the proportion of RTE (51 vs. 64% in CD4+CD45RA+ T cells; $p = 0.012$) were both lower in Tx patients (**Figures 1K,L**). The TREC and RTE values were negatively correlated with chronological age, $r = -0.65$ ($p < 0.001$) and $r = -0.65$ ($p < 0.001$), respectively. After adjusting for chronological age in patients, TRECs and RTE were still significantly lower in Tx group compared to the non-Tx group. There was no correlation of either TREC or RTE with age at thymectomy.

DISCUSSION

In this study we find that thymectomy in juvenile MG patients results in significant alterations in the peripheral T cell subsets, especially in the CD4+ subset with a decrease in naïve helper T cell with a relative increase in memory helper T cells, and a decrease in naïve cytotoxic T cells. These changes in the T cell compartment resemble findings characterizing the normal aging of the immune system (12), and they have been shown in other studies on children thymectomized while undergoing cardiac surgery (9–11). However, in an adult MG population neither lower total T cells, nor lower naïve T cells were found (16). Although the evidence of early T cell immunosenescence, there has been no clinical report of immunodeficiency following thymectomy in children during cardiac surgery, but the long-term clinical consequences are still incompletely revealed (9, 17).

Autoimmune comorbidity is a known feature in patients with myasthenia gravis, also in those with juvenile onset (13, 18, 19). Among our juvenile MG patients an autoimmune comorbidity was not more common in the Tx group than the non-Tx

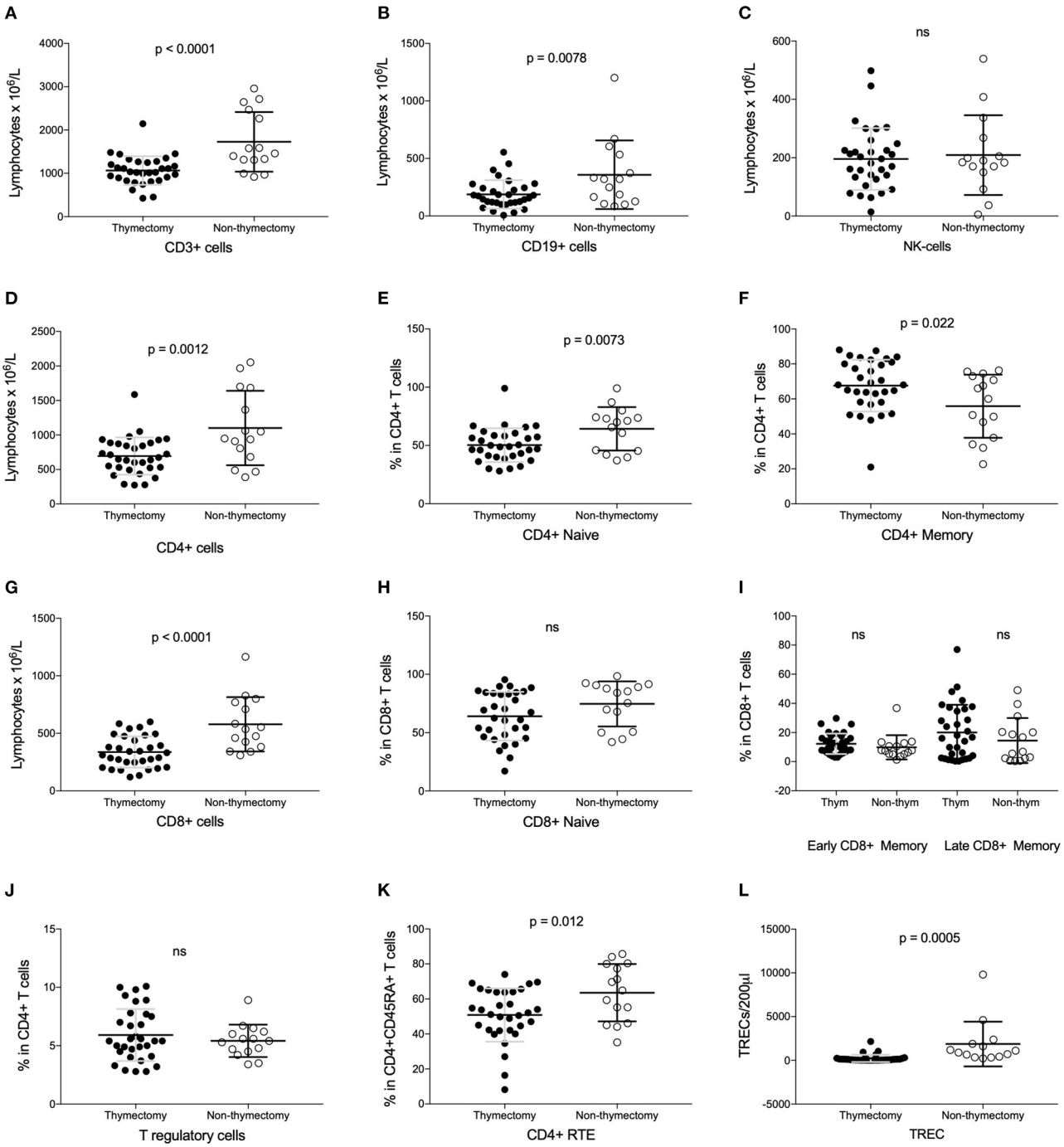

FIGURE 1 | Analysis of lymphocyte subsets in thymectomized vs. non-thymectomized juvenile myasthenia gravis patients. **(A–D,G)** Number of CD3+, CD19+, NK, CD4+ and CD8+ cells. **(E,F)** Value of naïve and memory CD4+ cells as proportions. **(H,I)** Value of naïve, early/late memory CD8+ cells as proportions. **(J)** T regulatory cells as proportion of CD4+ cells. **(K)** Recent thymic emigrants (RTE) as proportion of naïve CD4+ cells. **(L)** TRECs per 200 µl. *ns,* not significant.

group, and in neither were malignancies nor infections frequently occurring (**Table 1**).

We could not show any effect on the T reg cells when comparing the Tx and non-Tx juvenile MG patients. Although a reduction of T reg cells have been shown after thymectomy in children undergoing cardiac surgery, several studies on adult MG patients have shown that T reg cells are not affected by thymectomy alone (20–22). Changes in T reg cells in MG

TABLE 2 | Correlations of the T cell subsets of thymectomized patients with age at thymectomy.

	r	*p*
CD3	0.06	0.73
CD4+	0.05	0.77
Naive CD4+	0.01	0.96
Memory CD4+	0.07	0.69
RTE	0.08	0.64
CD8+	0.04	0.82
TRECs	0.01	0.96

r, Person's correlation coefficient.
RTE, recent thymic emigrants; TREC, T cell receptor rearrangement excision circles.

patients are thought to be an effect of immunosuppressive treatment (21, 22).

TRECs and RTE were both lower in the Tx patients compared to the non-Tx patients. This illustrates the expected reduced thymic activity after thymectomy. This finding differs from an earlier study on thymectomy in adult MG patients where no difference in TREC numbers was found when Tx patients were compared with non-Tx patients. However, TRECs in both Tx and non-Tx patients were decreased compared to normal controls, suggesting an accelerated thymic atrophy in MG patients also independent of thymectomy (16).

Being a retrospective cohort study there are some limitations due to variability within the study population. The thymectomies were done at different ages, and the T cell subsets and TREC levels were measured at various time intervals after thymectomy. Earlier studies have shown a restoration of the T cell compartments with time after thymectomy, hypothesized to be due to thymic regeneration, and the regenerating capacity is speculated to be dependent upon age at thymectomy (23, 24). However, we found no correlation with age at thymectomy in our material. The AChR antibody positivity rate was higher in the Tx group as could be expected since thymectomy is more often advised in seropositive patients. Does this mean that the two subgroups are immunologically different? This question is beyond the scope of this study, but a longitudinal study on an adult MG population found no significant difference in levels of T cell subset after thymectomy in seronegative compared to seropositive MG patients (22). Approximately one fourth of the juvenile MG cases were on immunosuppressive medication. This was similar in both groups, and Sempowski et al. found that neither prednisolone nor immunosuppressive drugs affected TREC levels in their MG population (16).

CONCLUSION

All though indications for a premature immunosenescence in the T cell compartment in thymectomized juvenile MG patients, we could not show any clinical consequences in our population at last follow up. The change in immunosenescence markers was not related to age at thymectomy. Thus, our study could not confirm any increased risk of thymectomy in prepubertal juvenile MG compared to postpubertal juvenile MG. The retrospective methodology, small sample size and variability within the study population however, are limitations of the study. Additional prospective studies including healthy controls are necessary to elucidate the effect of thymectomy in juvenile MG further.

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 human participants were reviewed and approved by REC South East. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

TP: study design, patient inclusion, analyses and interpretation, and manuscript writing. KG and LO: analyses, revision, and approval the manuscript. CB: analyses, revision, and approval the manuscript. RO, TA, and EK: study concept, data interpretation, revision, and approval the manuscript. All authors contributed to the article and approved the submitted version.

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