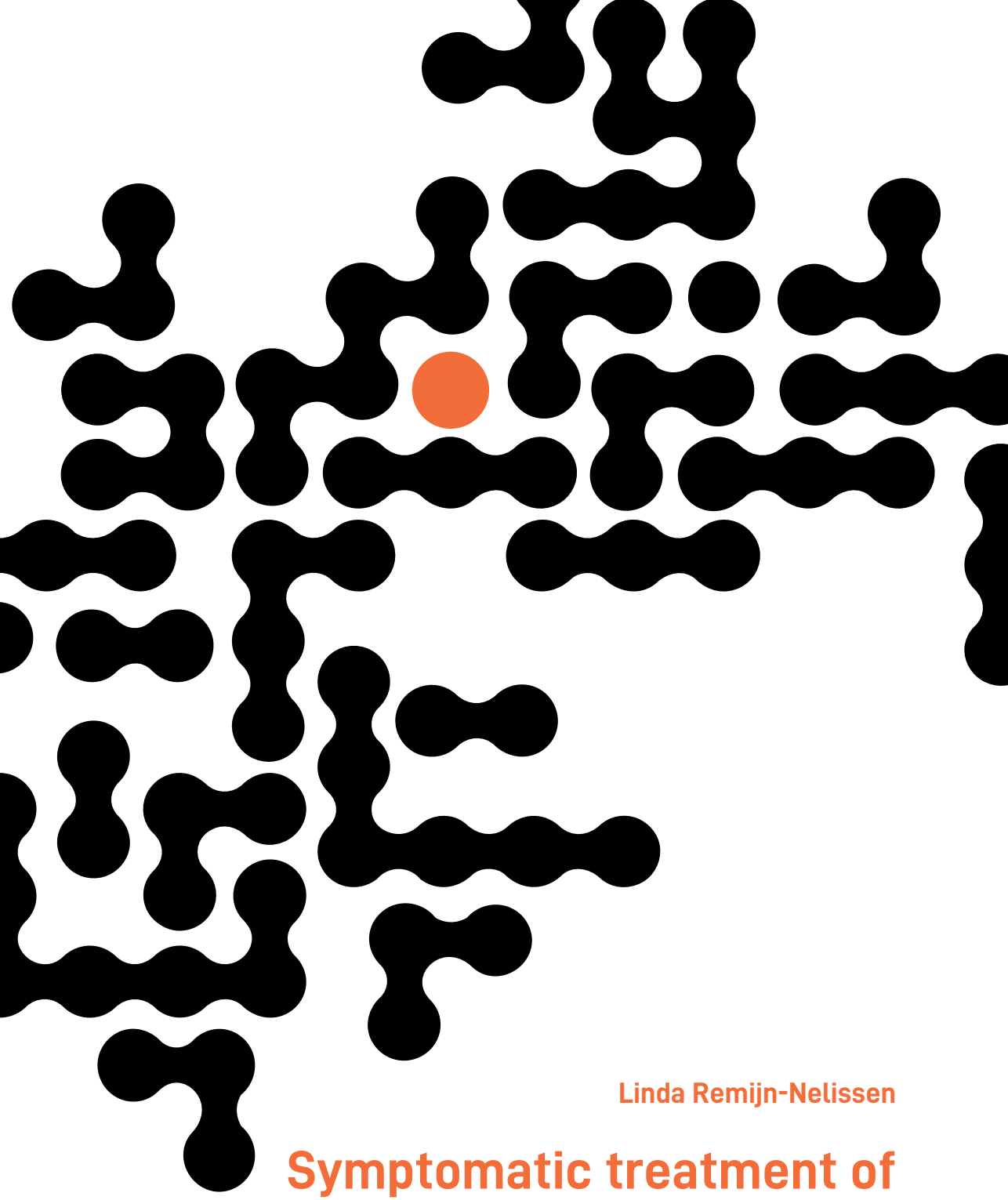
Symptomatic treatment of myasthenia gravis and its role in the era of emerging immunomodulatory treatments

Remijn-Nelissen; L.



Linda Remijn-Nelissen

Symptomatic treatment of myasthenia gravis

and its role in the era of emerging immunomodulatory treatments

Stellingen behorende bij het proefschrift getiteld

Symptomatic treatment of myasthenia gravis

and its role in the era of emerging immunomodulatory treatments

1. Pyridostigmine demonstrates benefit over placebo in patients with acetylcholine receptor positive myasthenia gravis chronically using pyridostigmine. *(This thesis)*

2. Although adverse effects are common during pyridostigmine, discontinuation is more often the result of lack of efficacy. *(This thesis)*

3. Pyridostigmine has a very high probability of being cost-effective, both on societal and healthcare costs. *(This thesis)*

4. Amifampridine should not be added to treatment with pyridostigmine in acetylcholine receptor positive myasthenia gravis. *(This thesis)*

5. Within the first ten years of disease onset, more than a third of patients continue to depend on corticosteroids, indicating a considerable unmet medical need within the current treatment landscape. *(This thesis)*

6. Efgartigimod is effective in a clinically relevant subset of patients with refractory myasthenia gravis, but requires frequent dosing, with administration at weekly, biweekly, or triweekly intervals. *(This thesis)*

7. Although therapeutic advances may reduce the need for symptomatic treatment in the future, it remains a key component of care in current myasthenia gravis management.

8. Determining the optimal treatment regimen in myasthenia gravis is complicated by disease heterogeneity and the lack of reliable biomarkers of disease activity.

9. Transparency of health outcomes and utility data is essential to enable robust cost-effectiveness analyses and inform optimal treatment strategies.

10. Although early intervention with complement inhibitors and FcRn blockers represents a promising approach to minimize corticosteroid exposure, current evidence is lacking, and its widespread use requires a substantial reduction in costs.

11. The principle that strengthening a message is more effective than repeating it when reception is impaired applies not only to myasthenia gravis, but also to communication in clinical practice.

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The research presented in this thesis was performed at the Department of Neurology of the Leiden University Medical Center.

Coverdesign & lay-out: Wendy Schoneveld, www.wenzid.nl
Printing: ProefschriftMaken, www.proefschriftmaken.nl
ISBN: 978-94-6534-541-3

Financial support: The research projects described in chapters 3 and 4 were (partially) supported by unrestricted funding from NMD Pharma. The work in chapter 4 was also supported by a grant from the Netherlands Organisation for Health Research and Development (ZonMw, The Hague, The Netherlands; grant number 10140022110010, granted to Dr. M.R. Tannemaat, paid to institution). The project described in chapter 6 was partially supported by unrestricted funding from UCB Pharma. The funders had no role in the study design, data collection, data analysis, data interpretation, the writing of the manuscripts, or the decision to publish.

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Proefschrift

ter verkrijging van
de graad van doctor aan de Universiteit Leiden,
op gezag van rector magnificus prof.dr. S. de Rijcke,
volgens besluit van het college voor promoties
te verdedigen op donderdag 3 september 2026
klokke 11.30 uur

door

Linda Remijn-Nelissen

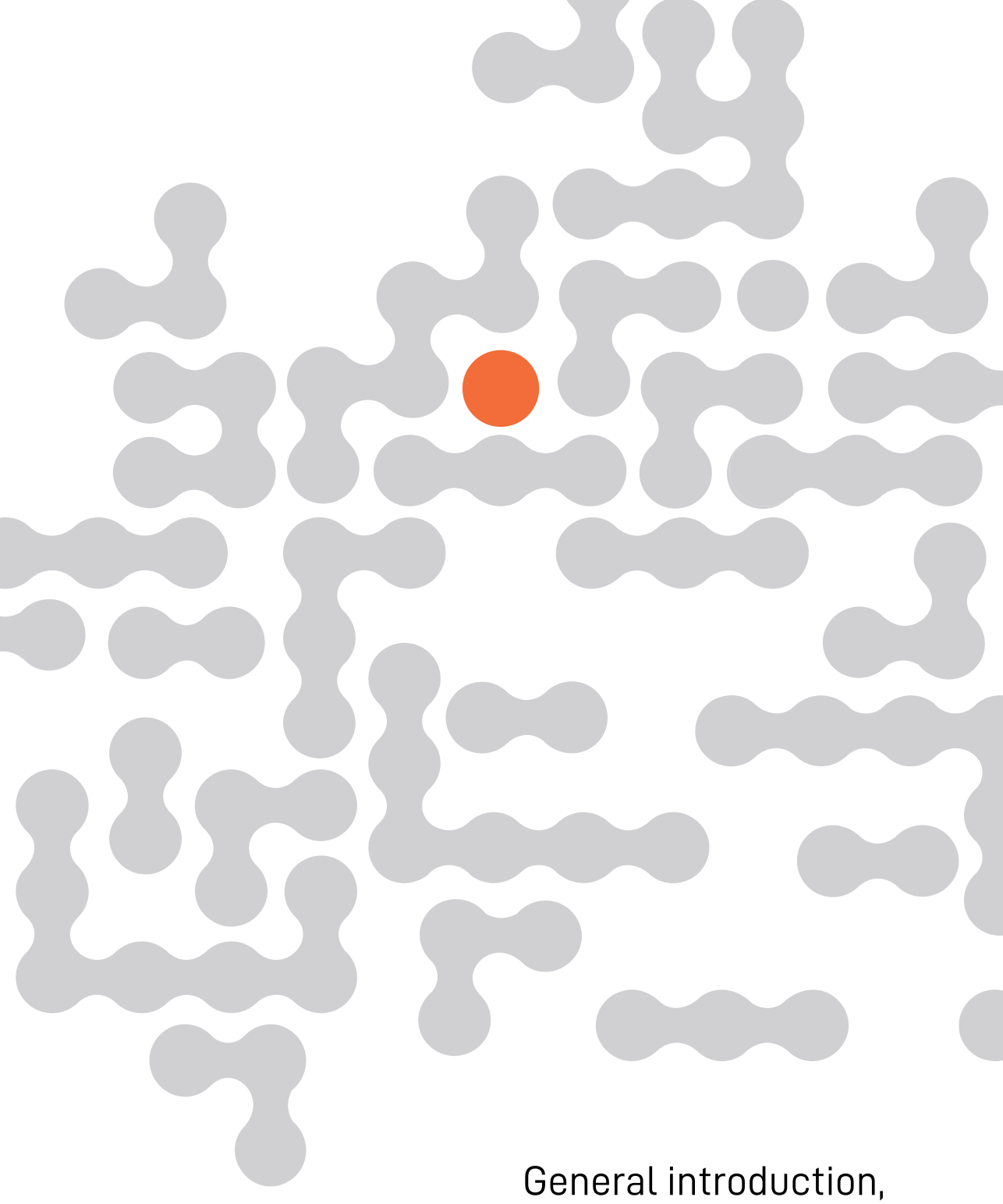
geboren te Heesch
in 1988

Promotores	Prof. dr. J.J.G.M. Verschuuren Prof. dr. T. van Gelder
Copromotor	Dr. M.R. Tannemaat
Leden promotiecommissie	Prof. dr. ir. S.M. van der Maarel Prof. dr. H.J. Guchelaar Dr. C. Barnett-Tapia (University of Toronto) Dr. A.M. Dekker (Universitair Medisch Centrum Utrecht)

Table of Contents

CHAPTER

1



General introduction, aims and thesis outline

Background and clinical characteristics

Myasthenia gravis (MG) is a well-characterized autoimmune disease affecting the neuromuscular junction (NMJ).[1] It is a rare disease with an annual incidence of 10-29 cases per one million people, and a prevalence between 100 to 350 cases per one million people.[2] There are two age-related incidence peaks. The first occurs around the age of 30, predominantly affecting women, and is commonly associated with thymic hyperplasia and the HLA haplotype HLA-A1-B8-DR3. The second peak occurs after the age of 50, more frequently affecting men, and is characterized by thymic atrophy and an association with the HLA-DRB1*15:01 allele.[3]

MG is caused by autoantibodies targeting functionally important proteins on the postsynaptic membrane leading to impairment of neuromuscular transmission. Most commonly antibodies are directed against the acetylcholine receptor (AChR), and less commonly against other components of the NMJ such as muscle-specific tyrosine kinase (MuSK), or lipoprotein receptor-related protein 4 (LRP4).[4] Patients without detectable serum antibodies are classified as seronegative MG.

The clinical hallmark of MG is skeletal muscle weakness that typically worsens with repetitive movement.[2] Muscle weakness primarily affects ocular, bulbar, respiratory and proximal trunk and limb muscles. Most patients initially present with ocular symptoms, specifically diplopia and ptosis.[5] Approximately 15% of patients have solely ocular symptoms, while 85% have more generalized symptoms, including weakness of non-ocular muscles.[1] Muscle weakness can fluctuate in severity both throughout the day, with symptoms worsening as the day progresses, and over weeks to months.

Severe weakness may require hospitalization, and involvement of the respiratory muscles can result in myasthenic crisis requiring mechanical ventilation. Around 15% of patients experience myasthenic crisis, predominantly in the early stages of the disease.[2] Continuous progressive deterioration is not observed in MG.[1] MG is often associated with thymic abnormalities, including thymoma and thymic hyperplasia, which contribute to disease pathogenesis.[6] 10-20% of patients with AChR MG have a thymoma, and over 80% of patients with early-onset AChR MG show follicular hyperplasia in the thymus.[1]

Several MG subtypes may be classified based on clinical presentation (ocular or generalized MG) antibody type (AChR, MuSK, LRP4, seronegative), age at disease onset (juvenile-onset, early-onset and late-onset MG), or thymic pathology (thymoma or non-thymoma).[2]

Pathogenesis of MG

Neuromuscular transmission

Figure 1.1 shows key steps in the process of neuromuscular transmission. Acetylcholine is synthesized from acetyl coenzyme A and choline by the enzyme choline acetyltransferase (ChAT), and is then packed into synaptic vesicles via the vesicular acetylcholine transporter (VAChT).[7] These synaptic vesicles accumulate in the presynaptic terminal near specialized release sites, named active zones.[7] A presynaptic action potential depolarizes the nerve terminal, leading to the opening of voltage-gated calcium channels. The resulting calcium influx triggers the fusion of acetylcholine-filled vesicles with the plasma membrane. This process, mediated by the Soluble N-ethylmaleimide-sensitive factor Attachment protein Receptor (SNARE) complex, leads to the release of multiple acetylcholine quanta into the synaptic cleft[7,8].

Acetylcholine then diffuses across the synaptic cleft and binds to densely packed postsynaptic AChRs, inducing ion channel opening and subsequent cation influx. The resulting depolarization propagates across the postsynaptic membrane, ultimately initiating muscle fiber contraction. To restore the presynaptic membrane potential and terminate calcium influx, voltage-gated potassium channels open and the subsequent efflux of potassium ions repolarizes the nerve terminal.[9] In addition, acetylcholinesterase rapidly hydrolyzes acetylcholine in the synaptic cleft, effectively terminating synaptic transmission.[6]

The efficiency of this system is maintained by a safety factor, ensuring that each nerve impulse generates a suprathreshold response in the muscle fiber. Under physiological conditions, sustained or repetitive contractions, lead to decreasing quantal content.[8] In AChR MG, where fewer AChRs are available at the postsynaptic membrane, the combination of a reduced safety factor and acetylcholine rundown during sustained activity leads to transmission failure, contributing to the hallmark clinical presentation of fluctuating muscle weakness and fatigability.[6]

Effective neuromuscular transmission depends on multiple factors. A critical aspect is the clustering of AChRs on the postsynaptic membrane, directly opposite the active zones of the presynaptic terminal. In healthy neuromuscular junctions the structure is dynamically maintained, through a constant balance exists between mechanisms that promote AChR-clustering and those that are involved with cluster dispersal.[10] Receptor clustering is promoted by the agrin-LRP4-MuSK signaling pathway.[6,7,11] MuSK is located at the postsynaptic membrane and plays a central role in stabilizing postsynaptic AChR clusters.[6] LRP4 interacts with MuSK to form an inactive complex.[7] Agrin, released from the motor nerve terminal, binds to LRP4, inducing a conformational rearrangement that facilitates MuSK dimerization and subsequent autophosphorylation.[11] This activation is further amplified by DOK7

(downstream of tyrosine kinase 7), leading to the clustering of AChRs at the neuromuscular junction via the protein rapsyn. The mechanism by which the signal is transmitted from MuSK to rapsyn remains unclear.[7]

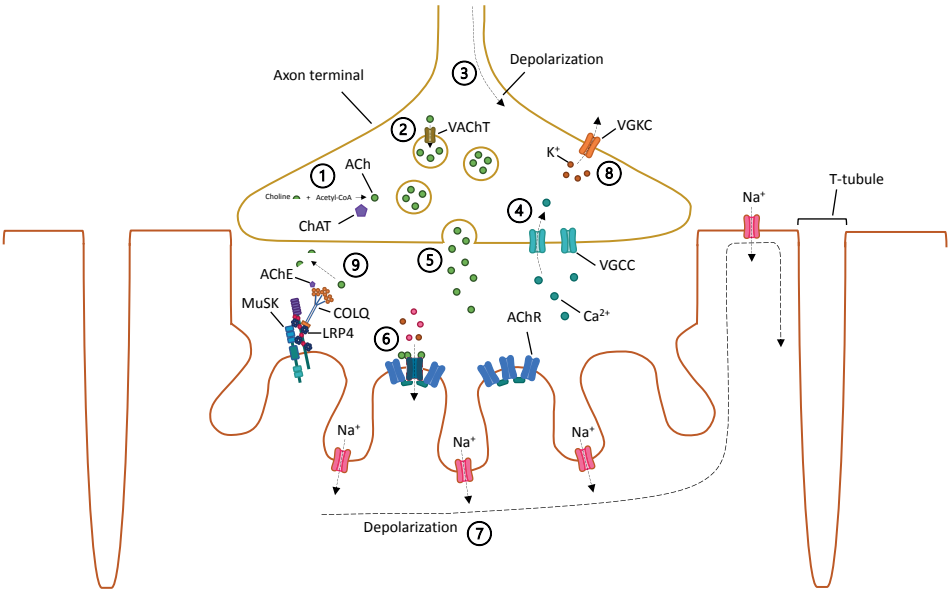


Figure 1.1 Normal neuromuscular transmission
1. Choline acetyltransferase catalyzes the synthesis of acetylcholine from acetyl coenzyme A and choline. 2. Acetylcholine is packed into synaptic vesicles by the vesicular acetylcholine transporter. 3. Depolarization of the nerve terminal by an action potential. 4. Opening of voltage-gated calcium channels, causing calcium influx. 5. Release of acetylcholine quanta into the synaptic cleft. 6. Acetylcholine binds to post-synaptic acetylcholine receptors, triggering cation influx. 7. Postsynaptic depolarization propagates across the muscle membrane. 8. Voltage-gated potassium channels open, repolarizing the presynaptic membrane and stopping calcium influx. 9. Acetylcholine is hydrolyzed, terminating synaptic transmission.
Abbreviations: ACh, acetylcholine; AChE, acetylcholinesterase; AChR, acetylcholine receptor; ChAT, Choline acetyltransferase; VAChT, vesicular acetylcholine transporter; VGCC, voltage-gated calcium channel; VGKC, voltage-gated potassium channel.

The ACh-CDK5-calpain pathway is one of the key mechanisms that negatively regulates AChR clustering at the neuromuscular junction.[7] This signaling cascade is initiated by acetylcholine binding to AChRs, which leads to the activation of cyclin-dependent kinase 5 (CDK5). CDK5 subsequently activates the calcium-dependent protease calpain, resulting in the degradation of proteins such as rapsyn. This degradation destabilizes the postsynaptic membrane and promotes the dispersal of AChR clusters.[7] In parallel, a second acetylcholine-driven mechanism acts in opposition to the agrin-LRP4-MuSK signaling pathway.[6] Here, acetylcholine binding to its receptors initiates a modest calcium influx that is amplified

through IP receptor-mediated calcium release.[6] This elevated intracellular calcium triggers caspase-3 activation, which disrupts MuSK signaling integrity and thereby weakens the stabilization of AChR clusters.[6]

Effector mechanisms of autoantibodies

In AChR MG, three primary mechanisms contribute to the reduction in AChR density and impaired receptor function at the postsynaptic membrane (**Figure 1.2**).[6] First, AChR autoantibodies, which are predominantly of the IgG1 and IgG3 subtypes, activate the classical complement pathway, leading to the formation of the membrane attack complex.[6] This disrupts the postsynaptic membrane, reduces AChR density, and results in a loss of voltage-gated sodium channels.[10] Second, due to their bivalent nature, IgG1 and IgG3 autoantibodies can induce AChR crosslinking, triggering receptor internalization and accelerated lysosomal degradation, thereby decreasing receptor density at the NMJ.[6,10] Third, autoantibodies can directly interfere with acetylcholine binding, either by occupying the binding site itself or by binding in close proximity, thereby preventing acetylcholine from accessing its receptor due to spatial constraints.[12] AChR antibodies are polyclonal and can bind to multiple receptor subunits.[13,14] In individual patients, these antibodies may simultaneously mediate different pathogenic mechanisms,[12] and even an individual autoreactive clone can contribute to one or more of these mechanisms.[14]

MuSK antibodies, in contrast to AChR antibodies, are predominantly of the IgG4 subclass. These IgG4 antibodies are anti-inflammatory and do not activate the complement system.[6] IgG4 MuSK antibodies bind to the first immunoglobulin-like domain of MuSK, thereby blocking its interaction with LRP4 and inhibiting agrin-induced MuSK phosphorylation.[15] Furthermore, evidence suggests that the retrograde signaling of LRP4 is impaired in MuSK MG,[16] thereby preventing the upregulation of quantal content, unlike in AChR MG. This is supported by the observation that repetitive nerve stimulation in MuSK MG produces a decremental response pattern similar to that seen in Lambert-Eaton Myasthenic Syndrome (LEMS), consistent with a presynaptic dysfunction.[17] Additionally, MuSK antibodies are thought to interfere with the binding of collagen Q (ColQ) to MuSK.[18] Since acetylcholinesterase is anchored to the synaptic basal lamina via ColQ, this mechanism may underlie the hypersensitivity to acetylcholinesterase inhibitor therapy observed in some patients with MuSK MG.[6]

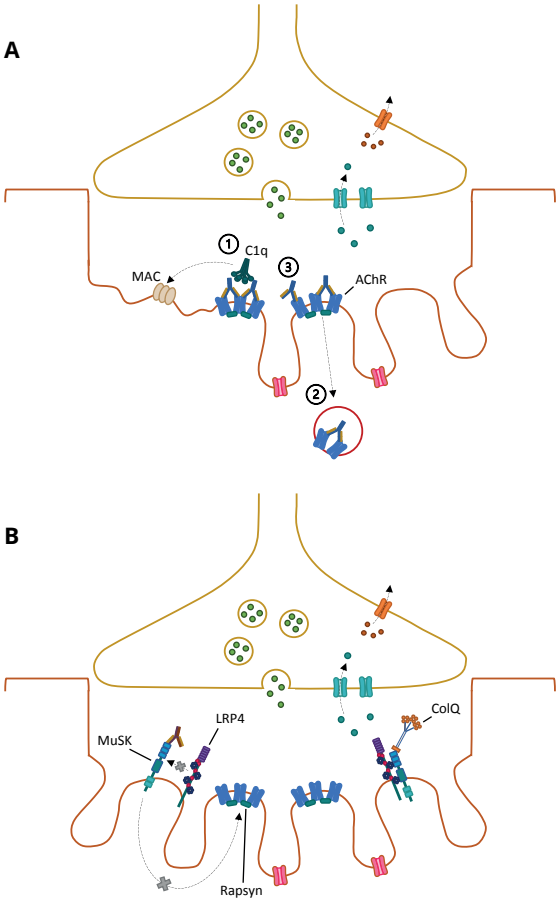


Figure 1.2 Effector mechanisms of AChR and MuSK autoantibodies
A.1. Complement activation. 2. Enhanced internalization of the receptor leading to accelerated lysosomal degradation. 3. Direct antagonism of the receptor. B. MuSK antibodies block LRP4 from activating MuSK leading to reduced acetylcholine receptor clustering.
Abbrevations: AChR, acetylcholine receptor; C1q, complement component 1q; ColQ; collagen Q; LRP4, low density lipoprotein receptor-related protein 4; MAC, membrane attack complex; MuSK, muscle-specific kinase;

Thymic changes

The thymus is the primary site for T cell maturation and central immune tolerance by eliminating self-reactive T cells.[19,20] Under normal conditions, thymic epithelial cells present peripheral tissue proteins, including self-antigens, to developing T cells in the thymus. The main factor responsible for self-antigen expression is the autoimmune regulator (AIRE),[21] a transcription factor that promotes the expression of these proteins in the thymic medulla.[6] T cells that strongly recognize self-antigens undergo apoptosis.[1] Some self-reactive T cells

escape thymic deletion and enter the peripheral circulation. In the periphery, mechanisms like apoptosis, functional inactivation, or suppression by regulatory T cells help maintain tolerance by controlling these self-reactive cells.[1]

In AChR MG, B cells play a central pathogenic role, but their activation and autoantibody production depend on T cell help.[19] Increasing evidence points to T cell dysregulation as a key factor maintaining autoreactive B cells.[19,20] In early-onset AChR MG, thymic regulatory T cells have impaired suppressive function,[19] leading to increased expression of AChR and pro-inflammatory signals by thymic epithelial cells.[19] These changes support the expansion of autoreactive T cells and the recruitment of B cells into the thymus, where ectopic germinal centers can form.[6] Within these structures, B cells undergo affinity maturation and differentiate into long-lived plasma cells producing AChR antibodies.[19] In thymoma-associated MG, the autoimmune regulator AIRE is absent in nearly all thymomas.[6,19] These tumors abnormally express muscle protein fragments, promoting the development of autoantibodies not only against AChR, but also against titin, the ryanodine receptor, and neurofilament proteins.[19]

This facilitates the development of autoreactive T cells within the thymoma, which, only in patients with MG, then migrate to the periphery to support B cells targeting these antigens.[6] In contrast, in MuSK MG thymic abnormalities are typically absent,[6,22] and thymectomy in these patients is ineffective. [23,24]

Current treatment guidelines

Symptomatic treatment

According to national and international guidelines, the first-line treatment for MG is symptomatic therapy, most commonly pyridostigmine.[25-28] Pyridostigmine is a reversible acetylcholinesterase inhibitor that increases the level and duration of action of acetylcholine in the synaptic cleft by inhibiting its breakdown. Other acetylcholinesterase inhibitors that act on the peripheral nervous system include physostigmine, neostigmine, edrophonium, and ambenonium.[5,29] These agents differ in their route of administration (oral or parenteral), and duration of action. Based on years of clinical experience, pyridostigmine is considered to have a more favorable balance between efficacy, duration of action, and side effect profile compared to the other acetylcholinesterase inhibitors. However, data on its efficacy and side effects are limited, and current understanding is based only on (small) observational studies.[30]

In addition to acetylcholinesterase inhibitors, there are currently only limited alternatives for the symptomatic treatment of MG. Ephedrine may be effective in AChR MG. Although a small series of n-of-1 trials showed a statistically significant reduction of symptoms, the

effect size was smaller than the predefined minimal clinically important difference.[31] Therefore, its clinical use is limited, particularly in light of its restricted availability. Another symptomatic treatment is amifampridine, a potassium channel blocker. Observational studies and a small crossover trial suggest that amifampridine may be effective in patients with MuSK MG.[32-34] Its use in AChR, however, is supported only by anecdotal evidence.

Thymectomy

The first case description of thymectomy in MG dates back to 1911,[35,36] and its efficacy was confirmed in the MGTX trial published in 2016.[37] This trial evaluated the efficacy of extended transsternal thymectomy combined with alternate-day prednisone versus alternate-day prednisone alone in 126 nonthymomatous MG patients aged 18 to 65 years.[37] Over a three-year follow-up, thymectomy resulted in a lower average Quantitative Myasthenia Gravis (QMG) score compared to prednisone monotherapy. Additionally, patients in the thymectomy group required a lower average dose of alternate-day prednisone, fewer needed adjunctive immunosuppression with azathioprine, and hospitalization rates due to disease exacerbations were reduced relative to the prednisone-only group.[37] The 2-year extension study further substantiated the benefits of thymectomy, demonstrating that after 5 years, patients treated with thymectomy plus prednisone had significantly lower mean QMG scores and reduced mean alternate-day prednisone doses compared to those receiving prednisone alone.[38]

Immunosuppressive therapies

For patients with insufficient response to symptomatic treatment, the next treatment step is the initiation of immunosuppressive therapies such as prednisolone and steroid sparing therapies such as azathioprine, mycophenolate mofetil, methotrexate, rituximab, ciclosporin, or tacrolimus.

Corticosteroid treatment is generally accepted as the first line immunosuppressive therapy.[39] It acts via the glucocorticoid receptor to regulate gene transcription and immune responses, including inhibition of dendritic cells and Th1/Th17 cells, and promotion of regulatory T cells.[40] The clinical efficacy of corticosteroids in MG is established through decades of use, expert consensus, and limited trial data.[41] Generally, patients start with high daily doses of prednisolone for several weeks, followed by gradual tapering, over months, to the lowest effective dose.[39] Both short- and long-term use of corticosteroids are associated with significant adverse effects, including osteoporosis, diabetes mellitus, glaucoma, weight gain, hypertension, and increased risk of cardiovascular disease and infections.[42] Consequently, corticosteroids should be tapered as much as possible, and combined with other immunosuppressive (corticosteroid-sparing) therapies at an early stage.

Azathioprine, mycophenolate mofetil, and methotrexate act by inhibiting the proliferation of T- and B-cells, while the calcineurin inhibitors cyclosporine and tacrolimus primarily block T-cell activation.[43]

Due to the limited number of comparative studies, the choice of a specific immunosuppressive agent is currently primarily based on expert opinion, resulting in substantial variability among countries in the use of immunosuppressive agents.[39] Drug choice is often influenced not only by clinician preference but also by factors such as availability and cost, the latter being particularly relevant in countries with limited financial resources.

Azathioprine is the most commonly used first-line non-steroidal immunosuppressant, and its use is supported by evidence from a randomized clinical trial demonstrating a significant steroid-sparing effect,[44] and expert consensus.[25] Mycophenolate is widely used and expert consensus holds that it is effective for the treatment of MG, despite two negative trials whose outcomes were likely influenced by methodological limitations.[45-47] The effect of methotrexate was assessed in two trials with conflicting results, likely due to differences in patient selection and trial duration.[48,49] Two recent studies have provided further insight into the choice of the initial immunosuppressive therapy.[50,51] A prospective cohort study demonstrated comparable clinical efficacy and improved quality of life with both azathioprine and mycophenolate; however, azathioprine was associated with more adverse events (most frequently hepatotoxicity), while mycophenolate had fewer side effects, but included a higher teratogenic risk.[51] A cross-sectional study further showed that azathioprine was more frequently discontinued due to side effects than mycophenolate or methotrexate, with liver dysfunction, diarrhea, and fatigue being the most common reported side effects for each drug respectively.[50] Over the long-term, skin problems were the primary reason for discontinuing azathioprine after 10 years.[50] In addition, although extensive long-term data for mycophenolate are limited, it appears to carry a lower risk of squamous cell carcinoma compared to azathioprine in transplant recipients.[52] These findings have led to a shift in first-line non-steroidal immunosuppressive therapy in the recently updated Dutch national MG guideline, which now recommends mycophenolate over azathioprine, except for women of childbearing age, for whom azathioprine should be considered as the first-choice treatment.[28]

Immunoglobulin therapy and plasma exchange

Due to the rapid onset of action, both immunoglobulin therapy, either intravenously (IVIg) or subcutaneously (SCIg), and plasma exchange have been used as rescue medication.[25] Immunoglobulin therapy has immunomodulatory effects by saturation of Fc receptors, inhibiting complement activation and neutralizing cytokines and autoantibodies, while plasma exchange reduces the levels of circulating pathogenic factors, including antibodies, complement, cytokines, and adhesion molecules.[1] There are several situations in which treatment with immunoglobulins or plasma exchange as short-term therapies should be considered:[25]

- In patients presenting with life-threatening symptoms, such as respiratory failure or severe dysphagia
- Preoperatively in patients with marked bulbar muscle weakness
- When a rapid clinical improvement is required
- As a preventive measure to avoid exacerbation when initiating corticosteroids

Plasma exchange may provide greater short-term symptom relief in MG compared to IVIg.[53,54] However, IVIg is associated with shorter hospital stays and a lower risk of complications.[53] For that reason, the choice between these treatments should be guided by individual patient and disease characteristics.

In addition to the use of immunoglobulin therapy and plasma exchange in acute indications, both therapies can be considered as maintenance therapy in patients with refractory MG, or in patients in whom immunosuppressive therapies are relatively contraindicated.[25] However, the scientific evidence on this topic is contradictory. A randomized controlled trial showed that IVIg infusions in adult patients with MG did not increase the proportion of patients achieving a ≥50% reduction in corticosteroid dose compared to placebo.[55] A second study found no difference in the primary outcome, although several efficacy parameters showed numerically greater improvements in the IVIg group than in the placebo group. This study was small and may have been underpowered to detect significant differences.[56]

Novel immunomodulatory therapies

Advances in the understanding of the pathophysiology of MG have led to the development of several novel therapeutic options in recent years.

Complement inhibitors

As outlined above, complement plays a crucial role in the pathogenesis of AChR-positive MG, and has therefore emerged as a promising therapeutic target to prevent destruction of the postsynaptic membrane at the neuromuscular junction.

Eculizumab is a humanized monoclonal antibody that binds to the complement protein C5, thereby inhibiting its cleavage into C5a and C5b and preventing the formation of the C5b-induced membrane attack complex. Treatment requires biweekly intravenous administration. Its efficacy has been demonstrated in patients with refractory AChR MG.[57] Although the primary endpoint of the phase 3 REGAIN trial did not reach statistical significance,[57] multiple secondary outcomes and long-term data demonstrated sustained clinical benefit and good tolerability.[57,58]

Ravulizumab is a modified version of eculizumab designed for prolonged complement inhibition. This allows for less frequent dosing, with intravenous administrations once every eight weeks.[59] In the CHAMPION MG trial, ravulizumab demonstrated significant efficacy on MG-ADL and QMG scores, with clinical responses typically observed within 2-4 weeks, and showed a favorable safety profile.[59]

A third complement inhibitor is zilucoplan, a small macrocyclic peptide that binds C5 with high affinity and specificity. This binding prevents cleavage into C5a and C5b and also inhibits C6 binding by targeting the region of C5 that forms C5b. Zilucoplan is administered as a once-daily subcutaneous injection, which can be self-administered by the patient. The RAISE study demonstrated a significant improvement in MG-ADL scores at week 12 compared to baseline, and zilucoplan was well tolerated.[60]

FcRn blockers

The neonatal Fc receptor (FcRn) was originally identified in neonatal intestinal epithelial cells, hence its name, but is now known to be widely expressed throughout the body, including in endothelial cells.[61] These cells internalize circulating IgG, which binds to FcRn in acidic endosomes. FcRn protects IgG from degradation by recycling it back to the circulation, thereby prolonging its half-life. FcRn antagonists block this interaction, leading to lysosomal degradation of unbound IgG and a marked decrease in circulating IgG levels.[61] This mechanism is analogous to the effect of plasma exchange, as both therapies reduce serum IgG concentrations.[61]

Efgartigimod is a human IgG1-derived Fc fragment administered intravenously and was the first FcRn-blocker evaluated in a phase 3 trial for generalized MG. In the ADAPT study, efgartigimod demonstrated significant clinical improvement, with 68% of patients classified as MG-ADL responders, compared to 30% in the placebo group.[62] Treatment consisted of weekly infusions over 4 weeks, followed by observation. It was generally well tolerated with the most common adverse events being upper respiratory infections, headache, and urinary tract infections. Long-term efficacy and safety were confirmed in an open-label extension with repeated treatment cycles.[63]

Rozanolixizumab is a humanized IgG4 anti-FcRn monoclonal antibody administered subcutaneously.

In the MycarinG study, rozanolixizumab demonstrated significant improvements in MG-ADL scores compared to placebo in patients with generalized myasthenia gravis.[64] Treatment-emergent adverse events were more common during rozanolixizumab treatment, mainly headache, but serious adverse events were infrequent and comparable across groups.

The efficacy of a third FcRn inhibitor, nipocalimab (a deglycosylated IgG1 anti-FcRn monoclonal antibody) has been demonstrated in the Vivacity-MG3 study.[65] Nipocalimab significantly improved MG-ADL scores over 24 weeks compared to placebo. Adverse event rates were comparable between groups, with infections and headaches being most common.

Aims and outline of this thesis

The aim of this thesis is to provide a comprehensive overview of the therapeutic landscape for patients with MG, with a special emphasis on existing and novel symptomatic treatment strategies (Part I), and on immunomodulatory therapies and their clinical implementation (Part II).

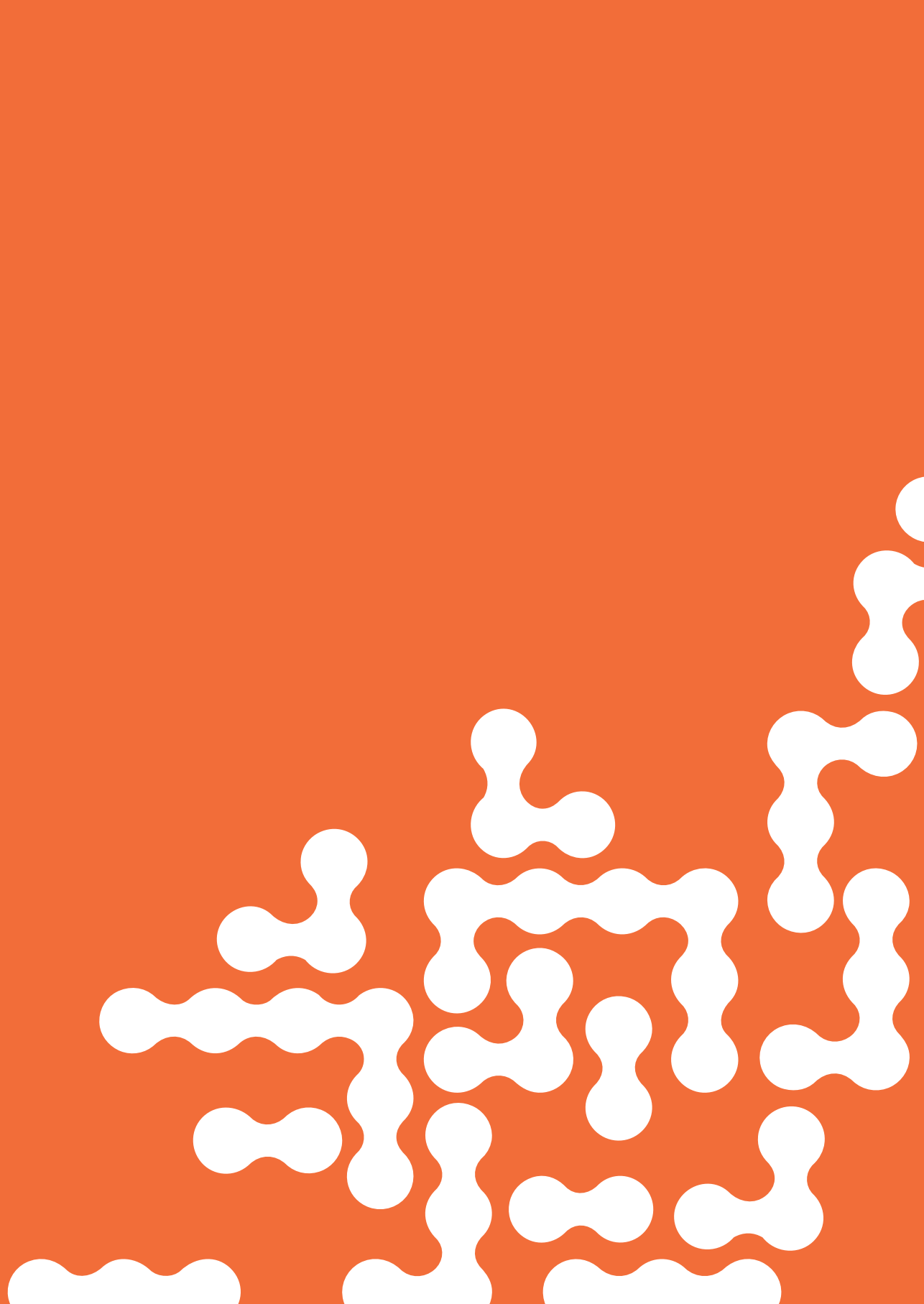
Part I: Symptomatic treatment

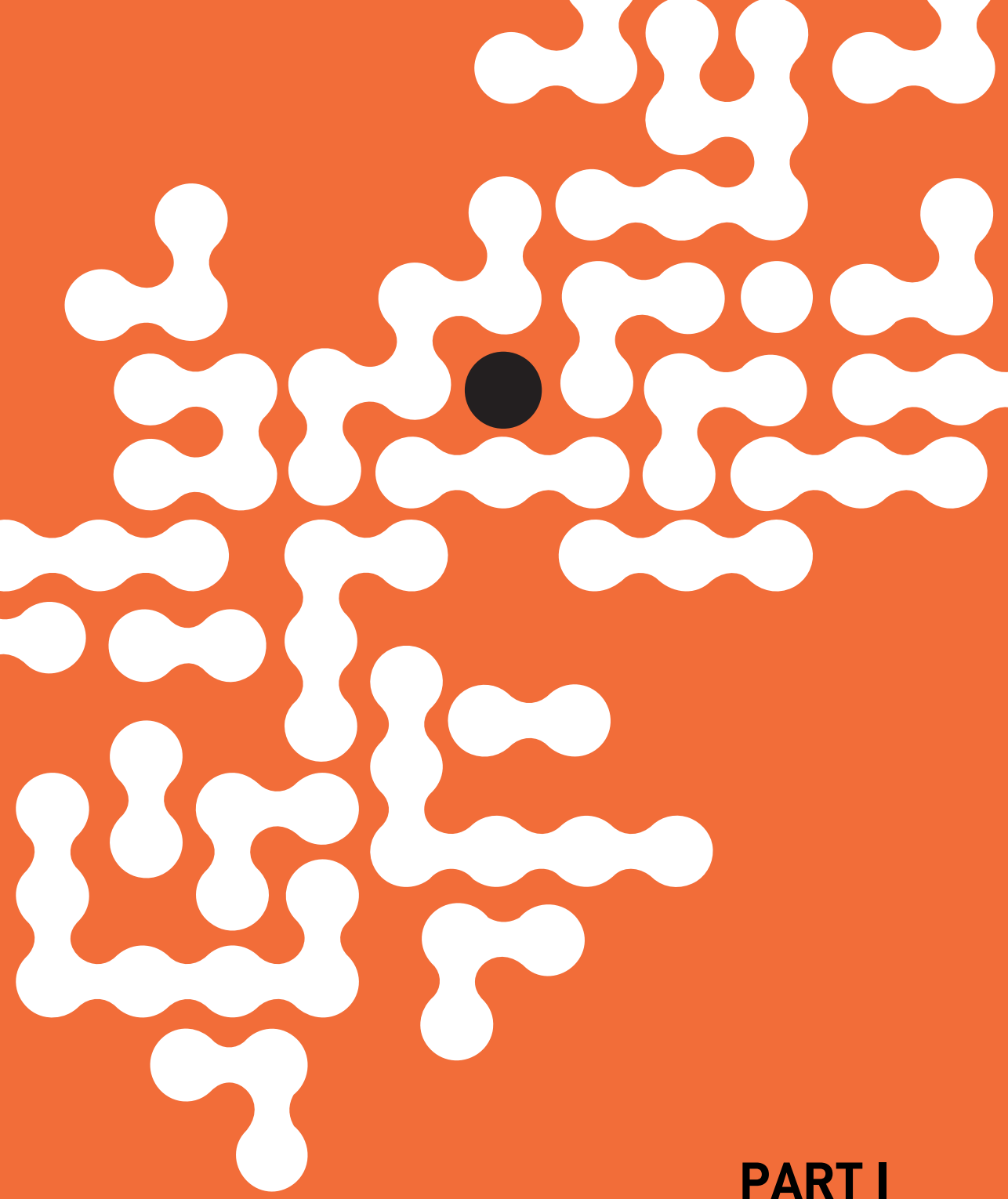
Chapter 2 describes a review of the literature on currently available and emerging symptomatic treatment options for MG, highlighting ongoing challenges in their clinical application and the continued need for clinical trials to optimize symptom control. In **Chapter 3** we describe results of a cross-sectional survey in a large cohort of MG patients, quantifying effectiveness, side effects and net benefit of pyridostigmine use in clinical practice. **Chapters 4 and 5** report on the findings of IMPACT-MG, a randomized, double-blind, placebo-controlled crossover study. In **Chapter 4**, we investigated the efficacy and cost-utility of pyridostigmine in AChR-positive MG patients, while **Chapter 5** describes the effect of adding amifampridine in patients where symptom control with pyridostigmine alone was insufficient.

Part II: Immunomodulatory therapies

In **Chapter 6**, an overview of longitudinal treatment patterns of currently available treatment options including pyridostigmine, corticosteroids and immunosuppressive and immunomodulatory therapies, in different subgroups of MG patients is given. **Chapter 7** describes the clinical implementation of efgartigimod, a neonatal Fc receptor blocker, in patients with refractory MG.

Finally, **Chapter 8** provides a summary and general discussion of this thesis, reviewing the results and discussing future perspectives on symptomatic and immunomodulatory therapies in MG.





PART I

Symptomatic treatment

CHAPTER

2

Symptomatic pharmacological treatment

Symptomatic, pharmacological treatment of myasthenia gravis

RRNMF Neuromuscular Journal, 2023, 4(3)

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Abstract

Myasthenia gravis (MG) is a chronic antibody-mediated autoimmune disease. The most frequent form is MG with antibodies directed against the acetylcholine receptor on the postsynaptic membrane. The first step in the treatment of autoimmune myasthenia gravis consists of symptomatic therapy. If this is insufficiently effective, the next step is to start immunosuppressive treatment with corticosteroids, usually prednisolone. A corticoid-sparing agent is often added because of the long long-term side effects of high doses of corticosteroids. Emerging immunomodulatory therapies targeting B and T cells, the complement cascade, the neonatal Fc receptor and cytokines associated with antibody production, are currently expected to be limited to patients with severe refractory MG. It is therefore likely that symptomatic treatment will remain the cornerstone in the management of patients with MG in the foreseeable future. In this review, we provide an overview of currently available symptomatic treatments and recent advances in this field.
Pyridostigmine, an acetylcholinesterase inhibitor, is the most commonly used symptomatic drug for MG. Acetylcholinesterase inhibitors prolong and enhance the effect of acetylcholine on the muscarinic and nicotinic receptors. In addition, there is evidence that pyridostigmine may also have an anti-inflammatory effect. Pyridostigmine is moderately effective and side effects are frequently reported by patients. Other therapies include amifampridine and sympathomimetics such as ephedrine, salbutamol and terbutaline. At present, there is insufficient evidence for the use of amifampridine as monotherapy or as add-on therapy to pyridostigmine. The addition of β2-adrenergic agonists to pyridostigmine may possibly be beneficial in some patients, however, well-designed randomized trials are needed to establish their efficacy. Emerging symptomatic therapies include ClC-channel blockers, fast-skeletal muscle troponin activators and antisense oligodeoxynucleotides. These therapies appear to be promising, with fewer side effects than pyridostigmine. However, phase III clinical trials are needed to assess their effectiveness and determine their place in the symptomatic treatment of MG patients.

Introduction

Myasthenia gravis (MG) is an autoimmune disease of the neuromuscular junction in which autoantibodies bind to the acetylcholine receptor (AChR) or associated structures on the postsynaptic membrane, resulting in impairment of neuromuscular transmission.[5] Clinical features are fluctuating weakness in ocular, bulbar, limb and respiratory muscles. Typically patients experience an increase in weakness with exercise and an improvement after rest of the involved muscles.[3,66] Antibodies are found against the AChR in approximately 80% of the patients with generalized MG.[3] Less commonly, antibodies against muscle specific kinase (MuSK) or low-density lipoprotein receptor-related protein 4 (LRP4) are formed,[2] resulting in different clinical features including an altered response to pharmacologic treatment.[67]
First line pharmacological treatment consists of symptomatic treatment.[25] Patients who do not meet treatment goals with symptomatic drugs, are advised to start corticosteroids often in combination with nonsteroidal immunosuppressive drugs. In recent years, advances in the understanding of the pathophysiology of MG have led to the development of new immunomodulatory therapies, acting at many different sites of the immune system, including IL-6, CD19, CD20, CD38, CD40, CTLA-4, FcRn and the complement pathway.[39]
Although these novel therapies appear to be effective in reducing MG related muscle weakness, there are some drawbacks: they are associated with high costs and most of them require intravenous administration, for which hospitalization or infusion in day care setting is often necessary. Furthermore, little is known about their long-term safety and treatment therefore requires more intensive monitoring. In contrast, the long term risks associated with symptomatic drugs are probably negligible, they are relatively cheap and can be used "as needed" allowing the patient a greater degree of control over the management of their disease.
It is therefore likely that symptomatic treatment will remain one of the cornerstones in the treatment of patients with MG. However, despite this fact, only limited high-quality data is available regarding their efficacy and safety. In this review, we aim to provide an overview of currently available symptomatic treatments and recent advances in this field (**Figure 2.1**).

Existing therapies

Acetylcholinesterase inhibitors

Acetylcholinesterase inhibitors increase the amount of acetylcholine in the synaptic cleft by blocking the enzyme acetylcholinesterase, resulting in enhanced neuromuscular transmission. Their beneficial effects in patients with autoimmune MG have long been recognized; the first application of an acetylcholinesterase inhibitor dates from April 1934

when dr. Mary Broadfoot Walker treated a patient with physostigmine with dramatic results.[68] A year later, neostigmine was introduced and this remained the primary drug for the treatment of MG until the first case reports with pyridostigmine were published in 1947.[69-72] Neostigmine was known to have significant response fluctuations due to a short half-life, which led to patients taking neostigmine frequently throughout the day resulting in high cumulative doses. Furthermore, neostigmine had pronounced side effects, both muscarinic (such as gastrointestinal symptoms, increased salivation and a marked increase in bronchial secretions) and nicotinic (such as skeletal muscle cramps). These side effects remained and were difficult to control, even with the use of atropine. Pyridostigmine, which had a longer duration of action and had fewer side effects, was developed by Hoffmann-La Roche as a superior alternative.[69-73] Since then, pyridostigmine has been the first choice in the symptomatic treatment of myasthenia gravis.[25] Other acetylcholinesterase inhibitors include ambenonium chloride, which is used less frequently than pyridostigmine due to a less favorable side effect profile, and hydrophonium or edrophonium, which is only used in the diagnosis of seronegative MG due to a brief duration of action.[74]

Mouse models suggest that long-term anticholinesterase therapy may have an adverse effect on neuromuscular transmission and motor end-plate structure,[75,76] resulting in a potential decrease in efficacy over time and an increased risk of cholinergic side effects.[77] Neurotransmission itself has a dispersal effect on AChR clusters and postsynaptic structures, which is counteracted by the agrin/muscle-specific kinase pathway (i.e. the AChR-clustering pathway). An increase of neurotransmission, through a pharmacological intervention such as acetylcholinesterase inhibitors, will therefore lead to an amplification of the disruption of the postsynaptic structures, especially in diseases in which the counteracting AChR agrin/muscle-specific kinase pathway is affected.[78] However, in patients with MG, no correlation has been found between the perceived efficacy and age or diseases duration, nor between the number and severity of side effects and age or disease duration.[79]
In addition to the direct effect of acetylcholinesterase inhibitors on the neuromuscular junction, there is evidence that pyridostigmine may also have an anti-inflammatory effect,[80,81] through the cholinergic anti-inflammatory pathway. This pathway can modulate the activity of immune cells through activation of nicotinic acetylcholine receptors on macrophages, dendritic cells, monocytes and T and B lymphocytes. Furthermore, it can inhibit cell proliferation and differentiation, as well as suppress cytokine release.[82] The exact role of acetylcholinesterase inhibitors and their interaction with the cholinergic pathway and inflammatory reactions in MG has not been established.

Pyridostigmine
All international guidelines recommend pyridostigmine as the first step in the pharmacological treatment of MG.[25,83-85] In a recent study involving participants of the Dutch national

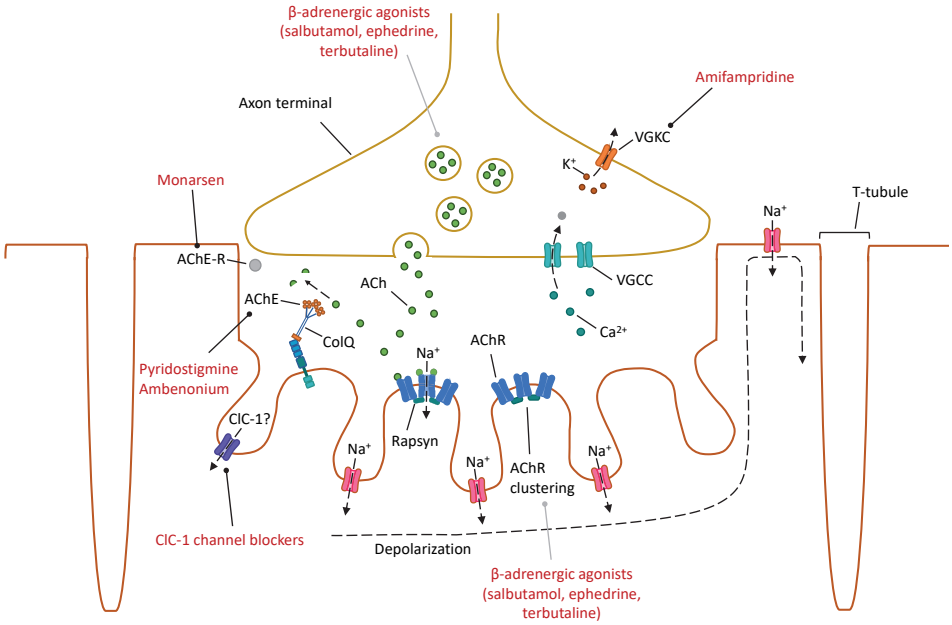

Figure 2.1 (Assumed) mechanism of action of different drugs in the symptomatic treatment of patients with myasthenia gravis

Pyridostigmine and ambenonium, both acetylcholinesterase inhibitors, block the enzyme acetylcholinesterase and thereby increase the amount of acetylcholine in the synaptic cleft. Amifampridine blocks potassium efflux which results in a prolonged action potential of the presynaptic nerve terminal and thereby enhances the release of acetylcholine into the synaptic cleft. The mechanism of action of β-adrenergic agonists (salbutamol, ephedrine and terbutaline) is not fully understood. There is evidence that β-adrenergic agonists affect post-synaptic AChR clustering. Furthermore, it is hypothesized that β-adrenergic agonists play a role in the regulation of quantal acetylcholine content. ClC-1 channel blockers reduce the inhibitory currents that counteract neuromuscular transmission. The precise localization of this channels at the neuromuscular junction is unknown. Monarsen is an antisense oligodeoxynucleotide which inhibits the expression of AChE-R, a isoform of AChE mainly found in patients with myasthenia gravis. Not shown: Fast-skeletal muscle troponin activators (tirasemtiv, reldesemtiv). Abbreviations: ACh Acetylcholine. AChE Acetylcholinesterase. VGCC Voltage-gated calcium channel. VGKC Voltage-gated potassium channel.

MG registry, 74% reported using pyridostigmine.[86] Unfortunately, no precise data are available on its effect, as no randomized controlled studies have ever been performed since the first published case-studies.[30] However, in a large-scale cross-sectional study on 410 MG patients; 61% reported that they currently used pyridostigmine, 36% had discontinued pyridostigmine and 2% reported to never have used pyridostigmine. On a scale of 0 (no effect at all) to 100 (maximum effect), patients currently using pyridostigmine reported a median effectiveness of 60 (IQR 28-78) and net benefit of 65 (IQR 45-84). In the group of patients who discontinued pyridostigmine, side effects were the reason for discontinuation in 26%. Pyridostigmine monotherapy is used in 22-66% percent of all patients,[86-88] suggesting that it is sufficiently effective to prevent the use of immune suppressant medication in

patients with relatively mild symptoms. In 2018, the first randomized controlled trial started recruiting patients to evaluate the effect of pyridostigmine in two groups: 1) newly diagnosed, treatment-naïve patients treated with 60 mg pyridostigmine administered twice in four hours and 2) patients with MG on stable anti-myasthenic medication treated with the patient's usual dosage also administered twice in four hours (NCT03510546). In 2023, in our center a randomized controlled withdrawal trial will start, comparing the efficacy of pyridostigmine versus placebo over a 5-day period.

Very few studies have reported on the side effects of pyridostigmine. Current knowledge is mainly based on years of clinical experience. Side effects are due to overstimulation of the muscarinic receptor causing symptoms such as abdominal cramping, diarrhoea, hyperhidrosis, increased salivation, sweating, lacrimation and bradycardia. Nicotinic side effects have also been reported due to overdosage of pyridostigmine and include muscle cramps, fasciculations and muscle weakness.[89] Side effects are frequently reported by patients using pyridostigmine.[77,79] Most frequently reported side effects are gastro-intestinal symptoms (flatulence, diarrhea and abdominal cramps), urinary urgency, muscle cramps, blurred vision, hyperhidrosis, increased salivation, light-headedness and flu-like symptoms. Diarrhea, abdominal cramps and muscle twitching are the most frequently cited reasons to discontinue pyridostigmine.[79] Cumulative side-effects after long-term treatment have not been reported [3]. Many patients with MuSK-MG respond poorly to acetylcholinesterase inhibitors with less effect and frequent side effects compared to patients with anti-AChR antibodies.[90]

Muscarinic side effects of pyridostigmine can be controlled by the addition of muscarinic antagonists such as atropine,[3] glycopyrronium bromide,[3] propantheline,[91] or hyoscyamine.[92] Loperamide can be used to treat persistent diarrhea.[3] There have been no studies comparing these agents in patients with MG. Their use in clinical practice is therefore based on single case reports, personal experience and expert opinion. In 2021, a phase II trial started to evaluate the effect of combined therapy of pyridostigmine and ondansetron, an anti-emetic drug which selectively blocks the serotonin 5-HT3-receptor, in patients experiencing pyridostigmine-related gastrointestinal adverse events (NCT04226170).

Ambenonium chloride

The first use of ambenonium in patients with MG was reported in 1955. Out of fifty patients treated with oral ambenonium, 41 patients experienced more benefit from it than from neostigmine or pyridostigmine with the main advantages being its longer duration of action and fewer side effects.[93] In a later study, patients experienced more side effects with ambenonium than with pyridostigmine, although the duration of action of ambenonium was longer.[94] Ambenonium has an unpredictable pattern of bioavailability in MG patients, with a greater risk of accumulation and overdosage, possibly because pharmacokinetics showed no correlation between the daily dose and the area under the curve.[95] In current

clinical practice, ambenonium is rarely used, although it may be a good alternative for patients for whom pyridostigmine is contra-indicated.

Amifampridine

Amifampridine is a well-known treatment for other diseases of the neuromuscular junction such as Lambert Eaton Myasthenic Syndrome and congenital myasthenic syndromes. It is a short-acting potassium channel blocker, which blocks potassium efflux presynaptically. This results in a prolonged action potential of the presynaptic nerve terminal, which enhances the release of acetylcholine into the synaptic cleft by an increase in calcium influx into the nerve terminal.[96]

A preliminary report of a double-blind, placebo-controlled crossover study noted an improvement of at least 3 points on the QMG scale in two of eight MG patients on amifampridine. However, only a limited description of methods and results of this study is available.[97] Another randomized controlled crossover trial showed an improvement of 6.9 points on the QMG scale and 5.7 points on the MG-ADL in 7 patients treated with amifampridine monotherapy with MuSK-MG.[32]

Amifampridine and pyridostigmine act on different parts of the neuromuscular junction and it is hypothesized that they work in synergy to enhance neuromuscular transmission. Indeed, in a study on LEMS patients, the combination of amifampridine and pyridostigmine did have an effect on some pharmacokinetic parameters: pharmacokinetics of amifampridine were not significantly affected by cotreatment with pyridostigmine, whereas amifampridine caused an increase in the average pyridostigmine serum concentration. However, the average plasma concentrations of pyridostigmine corresponded with clinically therapeutic levels in both the co-treatment and the stand-alone treatment arm.[98] The combined use of pyridostigmine and amifampridine in clinical practice is not uncommon in patients with LEMS; 71% of all patients in the Dutch LEMS registry reported using a combination of pyridostigmine and amifampridine.[86] The efficacy and tolerability in patient with MG in clinical practice has only been described in a limited number of studies. Two small case studies and one case report provide anecdotal evidence that patients may benefit from the use of amifampridine as add-on in MG.[33,34,99]

A phase II trial provided evidence that amifampridine phosphate was effective in patients with MuSK-MG.[32] However, results were not replicated in a phase III trial evaluating the efficacy and long term safety of amifampridine in 55 patients with MuSK MG and 15 patients with AChR MG (NCT03304054). Results have yet to be published.

Sympathomimetics

The effects of sympathomimetics on the (myasthenic) neuromuscular junction are complex and insufficiently understood. Therapies acting on the sympathetic nervous system, such

as the β2-adrenergic agonist salbutamol and the α- and β-adrenergic agonist ephedrine, have a well-documented effect in subtypes of congenital myasthenic syndromes; a diverse range of genetic disorders in which neuromuscular transmission is impaired at the motor endplate.[100] However, the molecular mechanisms underlying the therapeutic effect of sympathomimetics are not understood. It is hypothesized that β2-adrenergic agonists directly influence synaptic organization and the therapeutic effect may therefore be through morphological restoration of the neuromuscular junction.[101] In vitro studies have shown that β-adrenergic agonists affect postsynaptic AChR clustering.[102] This hypothesis is supported by observations in clinical practice that β-adrenergic agonists are particularly beneficial in disorders in which the endplate structure is disrupted such as DOK7 and COLQ CMS.[103] Furthermore, there is evidence that sympathomimetics regulate quantal acetylcholine content and influence the probability of quantal release at the neuromuscular junction [104]. Pharmacological stimulation of adrenoceptors, as well as sympathectomy, can affect acetylcholine release from motor nerve terminals.[105] Notably, it is uncertain whether the observed effects in these animal studies are relevant for the understanding of the observed effect in clinical practice since the concentrations of the adrenergic agonists used in animal studies were much higher than those reached in patients. Considerable ambiguities therefore remain regarding the mode of action of sympathomimetics.
In addition to their action at the neuromuscular junction, catecholamines play a role in several immune parameters: they affect lymphocyte proliferation and modulate cytokine production and the functional activity of different lymphoid cells.[106,107] However, the role of sympathomimetics on the immune system in MG patients is currently not fully understood.

Ephedrine

Ephedrine is a sympathomimetic drug with a stimulating effect on both α- and β-adrenergic receptors.[108] The use of ephedrine in patients with MG was first described in 1930 by Edgeworth, who serendipitously found that the ephedrine she was taking for menstrual cramps was effective for her myasthenic symptoms.[109] In a small series of randomized controlled n-of-1 trials with ephedrine as add-on treatment to pyridostigmine or prednisone in MG patients, a small reduction of MG symptoms was seen in both the primary outcome measure (QMG) and the secondary outcome measures (MG-ADL, MGC and VAS-score). This effect was statistically significant, but below the previously defined cut-off value for a clinically relevant difference.[31]

Salbutamol

Salbutamol is a selective β2-adrenergic agonist and is mainly used in patients with CMS. As described above, pyridostigmine may have a long-term adverse effect on the motor end-plate structure and thus on neuromuscular transmission. The addition of salbutamol to

pyridostigmine might be beneficial to counteract the long term adverse effects of pyridostigmine use because of the supposed mechanism of β2-adrenergic agonists to morphologically restore the neuromuscular junction. The functional effect of this combination therapy was explored in acetylcholine receptor deficiency syndrome, the most common form of CMS, in a small long-term cohort of CMS patients and in a mouse model.[110] In the cohort of eleven patients with severe AChR deficiency, a sustained response on QMG score was seen: after 4 years of combination therapy with pyridostigmine plus salbutamol, the mean QMG score improved from 17.7 (95% CI 13.25-22.2) at baseline to 12.3 (95% CI 9.1-15.6), although this effect did not reach statistical significance. Mouse models showed improvement of muscle fatigue which became apparent shortly after starting salbutamol addition. Furthermore, improved neuromuscular transmission and improved synaptic structure was seen. Whether the addition of salbutamol is useful in patients with MG as well is investigated in an ongoing randomized, controlled cross-over trial to study the efficacy and tolerability of oral salbutamol as adjuvant therapy in patients with MG. This study started in 2019 (NCT03914638).

Terbutaline

Like salbutamol, terbutaline is a β2-adrenergic agonist. In a mouse model, clinical symptoms were suppressed after treatment with terbutaline and electrophysiological studies showed a significantly larger first compound muscle action potential.[111] In a phase II cross over study in eight patients with generalized MG, treated with terbutaline or placebo during two weeks, a significant improvement was seen on the QMG score. Five out of eight patients (63%) had a clinically relevant improvement on the QMG score of 3.0 or greater. Pyridostigmine was withheld for at least eight hours before each visit. Terbutaline was well-tolerated in all patients.[112]

Emerging therapies

CIC-1 channel blockers

In MG, due to the loss of AChR, the excitatory endplate currents are reduced. Skeletal muscle-specific ClC-1 chloride ion channels carry the inhibitory currents that counteract neuromuscular transmission. Inhibition of ClC-1 has been shown to reduce the inhibitory current, increasing muscle membrane excitability and strengthening of neuromuscular transmission.[113] In 2020, a phase I/II randomized, controlled trial was initiated to assess safety and tolerability of NMD670, an inhibitor of the ClC-1 channel (NL8692). Results have yet to be published, although the company announced positive results in a press release.[114]

Fast-skeletal muscle troponin activators

Tirasemtiv is a highly selective activator of the troponin complex of fast skeletal muscles. It was developed to increase muscle strength in neuromuscular disease by amplifying the response of the muscle when neuromuscular input is diminished. Binding of tirasemtiv to the troponin complex slows the rate of calcium release from fast skeletal troponin and consequently sensitizes muscle fibers to calcium.[115] In a phase II study, the efficacy, safety and tolerability of single doses of tirasemtiv in patients with AChR MG was investigated. This study showed small dose-related improvements in QMG with tirasemtiv. Furthermore, twice as many patients had clinically significant improvements in QMG (>3 points) at six hours after the 500 mg dose compared to placebo, but this difference did not reach significance due to the small sample size. Two single doses of tirasemtiv were well tolerated and no serious adverse events occurred.[116] A phase III clinical trial in patients with amyotrophic lateral sclerosis did not meet primary or secondary endpoints. Poor tolerability after 48 weeks of double blind treatment may have contributed to this result: 34.2% of all patients treated with tirasemtiv stopped treatment before week 24 vs. 12.2% in the placebo group. Dizziness, fatigue, nausea, weight loss, and insomnia occurred more frequently on tirasemtiv.[117] As a result, further development of tirasemtiv has ceased and the focus will be on reldesemtiv, a next-generation fast skeletal muscle troponin activator. Reldesemtiv advanced into clinical development for its potential to demonstrate increased efficacy relative to tirasemtiv as well as improved tolerability and less potential for drug-drug interactions.[118] A phase III trial is initiated in patients with ALS in 2021 (NCT04944784). To our knowledge, no trials in MG are currently planned.

Antisense oligodeoxynucleotides

MG is associated with the production of a rare isoform of acetylcholinesterase which is referred to as the "read-through" transcript (AChE-R).[119,120] This isoform is found in half of all patients with myasthenia gravis and not in healthy subjects.[121] Monarsen (formerly EN101) is an antisense drug which inhibits the expression of AChE-R, potentially resulting in an increase of acetylcholine levels. In a phase II cross-over trial designed to compare three doses of monarsen, a decrease in QMG scores was found compared to baseline. All doses appeared to be effective, but no statistically significant difference between the three doses was found and the study did not include a placebo control group. Only preliminary results have been published.[122] Currently, no plans have been announced to further develop monarsen.

Conclusion

Despite years of experience with symptomatic drugs in the treatment of MG patients, much remains unknown. This makes it challenging to make individually tailored treatment decisions. In the past decades, only 6% of all clinical trials have focused on the symptomatic treatment of MG,[39] while almost all patients initially start with symptomatic treatment and approximately two thirds of all patients continue using it throughout their disease.[79]

Based on the available evidence, it is clear that pyridostigmine should remain the cornerstone of symptomatic treatment of MG patients. Many patients report a moderate effectiveness and a substantial number can be treated with pyridostigmine monotherapy without need for immunosuppressants. Nonetheless, pyridostigmine may cause considerable side effects, which can have substantial impact on quality of life. The addition of specific muscarinic antagonists such as atropine may alleviate side effects, but in our experience, the effect of atropine is often insufficient and it can be difficult to find a balance between adequate treatment of muscarinic side effects and inducing signs of overdosage of atropine.

The place of amifampridine and sympathomimetics such as ephedrine, salbutamol and terbutaline in the treatment of MG remains unclear. The addition of β2-adrenergic agonists to pyridostigmine may possibly be beneficial in some patients, however well-designed randomized trials are needed to establish the efficacy of these agents. At present, there is insufficient evidence for the addition of amifampridine to the standard symptomatic treatment with pyridostigmine. New emerging symptomatic therapies, especially the ClC-channel blockers and antisense oligodeoxynucleotides, appear to be promising therapies with fewer side effects than the current standard. Hopefully, phase III trials can shed more light on their effectiveness and determine their place in the symptomatic treatment of MG patients.

In the future, MG patients would greatly benefit from properly designed trials on symptomatic drugs for MG, as they are likely to remain an important element in achieving symptom relief for a large number of MG patients. Therefore, exciting developments involving drugs targeting the immune system should not overshadow efforts to improve the quality of life of MG patients by optimizing existing symptomatic treatment.

CHAPTER

3

The effectiveness and side effects of pyridostigmine

The effectiveness and side effects of pyridostigmine in the treatment of myasthenia gravis: a cross-sectional study

Neuromuscular disorders, 2022, 32(10), 790-799

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Abstract

Pyridostigmine is the most commonly used drug in the symptomatic treatment of myasthenia gravis (MG); however, research into its effectiveness and side effects is scarce. The aim of this study was to assess the effectiveness, prevalence of side effects and net benefit of pyridostigmine. All MG patients participating in the Dutch-Belgian myasthenia patient registry were included. A dynamic online questionnaire was developed to assess the effectiveness, side effects and net benefit of pyridostigmine. Out of 642 invited patients, 410 patients (64%) fully completed the questionnaire; 61% reported that they currently used pyridostigmine, 36% had discontinued pyridostigmine and 2% reported to never have used pyridostigmine. Patients reported a median effectiveness of 60, IQR 28-78 and net benefit of 65, IQR 45- 84. Of all patients currently using pyridostigmine, 91% reported side effects (vs. 55% in the control group). Most frequently reported side effects were flatulence, urinary urgency, muscle cramps, blurred vision and hyperhidrosis. In the group of patients who discontinued pyridostigmine, side effects were the reason for discontinuation in 26%. Diarrhea, abdominal cramps and muscle twitching were the most frequently cited reasons to discontinue pyridostigmine. These results can be used to guide shared decision making prior to starting symptomatic treatment for MG.

Introduction

Myasthenia gravis (MG) is the most common neuromuscular junction disorder and is characterized by fluctuating muscle weakness in ocular, bulbar, limb and respiratory muscles. Pyridostigmine, an acetylcholinesterase inhibitor, is recommended as the initial treatment of myasthenia gravis in most patients.[25] The use of acetylcholinesterase inhibitors as a treatment for MG was first described in 1934 by dr. Mary Broadfoot Walker. She identified physostigmine, a partial antagonist of curare, as an effective treatment for MG, based on the observations that clinical symptoms of patients with MG were similar to the symptoms seen in patients with curare poisoning.[68] A year later in 1935, Prostigmin (generic name neostigmine), was shown to have a similar effect.[123] Until 1947, neostigmine was the primary drug for treatment of MG, but due to a short half-life, high daily doses were needed and patients experienced pronounced and difficult to control side effects. Pyridostigmine was first synthesized by Hoffmann-La Roche Laboratories in Switzerland in 1945.[35] The first published case-studies comparing the effectiveness of neostigmine to pyridostigmine showed that patients experienced a longer duration of action with pyridostigmine, with fewer fluctuations of symptoms during the day and fewer side effects.[69-73] Since then, pyridostigmine has remained the preferred drug for symptomatic treatment of myasthenia gravis, even though no randomized controlled trials evaluating the efficacy of pyridostigmine have been performed.[30] Importantly, no studies have reported on the perceived effectiveness and net benefit of pyridostigmine from the patient's perspective either. Over the years, very few studies have reported on the frequency of side effects during the use of pyridostigmine.[77,124] One prospective study, with a limited number of patients (n=22), reported that 64% of patients experienced daily muscarinic side effects, most commonly gastro-intestinal symptoms. A total of 36% experienced additional nicotinic side effects, including muscle fasciculations and fatigue.[77] A retrospective study published in 1997 on 100 patients reported side effects in 34% of patients using acetylcholinesterase inhibitors, most commonly gastro-intestinal in nature (30%). Only one patient in this study had to stop taking pyridostigmine because of stomach complaints.[124]

The remarkable absence of detailed data may have negative consequences for the treatment of MG patients. Patients do not know the frequency or magnitude of potential side effects and no objective data are available to inform them what to expect after starting pyridostigmine. Additionally, the development of alternative symptomatic treatments requires the establishment of the net effect of the current gold standard (i.e. pyridostigmine), against which future treatment regimens can be compared.

We therefore aimed to provide a comprehensive overview of all relevant aspects of pyridostigmine use in current clinical practice, by formulating the following primary objectives: 1) to quantify the current use of pyridostigmine in a representative cohort of the

MG population, 2) to assess patients' perceived effectiveness and its net benefit and 3) to assess the prevalence and characteristics of its side effects. The secondary objective of this study was to identify predictors of pyridostigmine discontinuation.

Methods

Patient selection and selection procedure

All MG patients participating in the Dutch-Belgian myasthenia patient registry were invited to participate in this study. The registry is an initiative of the Dutch patient advocacy organization for neuromuscular diseases, "Spierziekten Nederland" and the Leiden University Medical Center (LUMC).[86] Only Dutch MG patients were included in the current study, because of the small number of Belgian patients in the registry (n=10). Patients received a study invitation by email containing an information letter and a personal link for participation. Upon request, a printed questionnaire was available. Baseline information on sex, age, age at diagnosis and antibody status was obtained from previously completed forms already available from the registry.

The study protocol was reviewed by the Medical Ethics Review Committee of the Leiden University Medical Center; the need for formal approval was waived due to the non-invasive nature of the study. All patients provided (digital) informed consent before study participation.

Survey design

A study-specific online questionnaire was developed in a conditional format tailored to individual responses to minimize the burden for the patient; responses determined the subsequent questions asked, e.g. if a patient indicated to have never used pyridostigmine, no further questions regarding its effectiveness were asked.

Data was collected on MG (history of medication use, thymectomy, plasmapheresis or intravenous immunoglobulin) and, if applicable, on the use of pyridostigmine (frequency, dose, dose alterations). Patients were subsequently divided into 3 groups by asking the question "Have you ever used pyridostigmine?". Patients were attributed to "the currently using group" when they answered the question with "Yes, I currently use pyridostigmine"; to the "discontinued group" when they answered the question with "Yes, I have used pyridostigmine in the past, but I discontinued it"; and to the "never group" when they answered the question with "No, I have never used pyridostigmine". The currently using group was further divided by asking the question "Have you used a higher dose in the past?" If applicable, reasons for lowering the dose of pyridostigmine (in the currently using group) or stopping pyridostigmine (in the discontinued group) were identified. Patients in the never

group were asked about the reason why they never used pyridostigmine. A schematic overview of the survey structure is shown in **Supplement 3.1**.

Effectiveness

The effectiveness of pyridostigmine was established on a VAS-scale with a range from 0 ("no effect") to 100 ("complete resolution of all symptoms") and on individual MG symptoms (ptosis, diplopia, hand weakness, arm weakness, leg weakness, neck weakness, facial weakness, dysarthria, difficulty chewing, dysphagia, dyspnea, fatigue) on a 3-point scale. In addition, patients were asked whether the effectiveness of pyridostigmine changed over the course of time.

Side effects

A list of 30 potential side effects of pyridostigmine was established. The list was adapted from the Dutch Summary of Product Characteristics of pyridostigmine with the accompanying patient information leaflet,[125] and included: abdominal cramps, stomach ache, nausea, vomiting, diarrhea, flatulence, heartburn, excessive belching, urinary urgency, headache, increased salivation, increased lacrimation, hyperhidrosis, blurred vision, increased bronchial secretion, irregular heartbeat, slow heartbeat, chest pain, light-headedness, fainting, fatigue, blushing, hot flashes, flu-like symptoms (chills, runny nose, coughing up phlegm), tremor, muscle cramps, muscle weakness, muscle twitching, skin rash and hives. Additionally, patients were given the option to select "other" and specify in a text box.

For each symptom, the presence of the symptom was recorded dichotomously (yes/no); when patients selected "yes", the tolerability of the symptom was subsequently established on a 5-point scale (ranging from "not annoying" to "extremely annoying") and finally patients were asked whether this symptom had been a reason to lower/discontinue pyridostigmine.

All patients, regardless of current pyridostigmine use, were asked to fill out the list of potential side effects with respect to the past 7 days (with the discontinued group and the never group serving as control groups). In addition, the discontinued group were asked to fill out the list over the period they used the highest dose of pyridostigmine. If patients in the current group had used a higher dose in the past, they were asked to fill out the same list over that period as well.

An advisory board consisting of five MG patients assessed the content of the questionnaire and its comprehensibility before it was sent to participating patients. A copy of the questionnaire is available in Dutch upon reasonable request by a qualified researcher.

Net benefit

The net benefit was assessed by asking patients whether the positive effects of pyridostigmine outweigh the negative effects by means of a VAS scale ranging from 0 ("I felt much worse with pyridostigmine") to 100 ("I felt much better with pyridostigmine").

Statistical analysis

Only patients who fully completed the questionnaire were included in the analysis. Continuous data are summarized with median (interquartile range) or mean (standard deviation) where appropriate. Comparisons were based on either student's T-test/one-way ANOVA (normal distribution) or Mann-Whitney/Kruskal Wallis (non-normal distribution). Categorical data are presented as proportions (%) and comparisons were assessed by either the Chi-Square test or Fisher's exact test. P values were considered significant when <0.05. Bonferroni correction was applied to the analysis of the side effects. To determine the correlation between the dose of pyridostigmine and the number of reported side effects, Spearman's rank test was applied. Multivariate logistic regression was performed with currently using/discontinued as dependent variable and age at diagnosis, sex, autoantibody class and clinical symptoms at onset as covariates. Missing data of age at diagnosis, autoantibody class and clinical symptoms were imputed using multiple imputation (10 imputations) with the imputation model including all covariates and the outcome variable. Data was assumed to be missing at random. Data were independently analyzed in the multivariate logistic regression analyses, each with missing values imputed. Subsequently, data were pooled to give a single mean estimate and adjusted standard errors. Post-hoc analyses were performed to explore potential explanations for males discontinuing pyridostigmine more often than females. All statistical analyses were performed with IBM SPSS Statistics (version 25.0).

Results

Baseline characteristics

Out of 642 invited patients, 410 (64%) fully completed the survey. Nineteen (3%) patients partially completed the survey and were excluded from further analyses. Baseline data on the 232 non-responders were present in the registry; baseline characteristics did not differ between the responding and non-responding group. At the time of the survey, 61% of patients reported that they currently used pyridostigmine (the currently using group), 37% had discontinued pyridostigmine (the discontinued group) and 2% reported to never have used pyridostigmine (the never group).

Male patients discontinued pyridostigmine more often than female patients (44% vs. 30%, p=0.001). Patients with purely ocular MG symptoms at disease onset were more likely to discontinue pyridostigmine than patients with generalized symptoms (52% vs. 35%, p=0.024). Patients in the discontinued group used prednisone (p=0.005) and "other medication" (p=0.040) more often than patients in the currently using group. In both the currently using group and the discontinued group, five percent of the patients reported the use of

distigmine. There was no significant difference between the currently using, discontinued and never groups for age at diagnosis, myasthenia gravis duration, previous thymectomy and autoantibody class (**Table 3.1**).

The current dose of pyridostigmine was higher in the currently using group (median 300, IQR 180-360) than the highest dose in the discontinued group (median 220, IQR 90-360) (p=0.011).

In the currently using group, 165 patients (65%) reported to have used a higher dose in the past. The most frequently reported reason for lowering the dose was that the higher dose of pyridostigmine was no longer required (43%). Twenty-five percent of all patients lowered the dose due to side effects and 17% of patients responded that the higher dose was not effective. The most common reason to discontinue pyridostigmine was that pyridostigmine had no effect (38%) or patients no longer needed it due to the start of other medication (34%). Twenty-six percent of all patients reported side effects as the main reason for ceasing pyridostigmine. Medication to control side effects was used in 20 percent of all patients, of whom 24% used diarrhea inhibitors such as loperamide, 56% used atropine, 18% used "other medication" and 13% responded "I don't know". Out of 14 patients that responded to have used "other medication", proton pump inhibitors were most frequently reported (n=7). The third group of "never used" was too small for detailed and meaningful analysis and therefore the data are only presented in **Table 3.1**.

Effectiveness

Overall, patients reported a median effectiveness of 60 (IQR 28-78). Patients in the currently using group perceived a better effect of pyridostigmine (median 69.5, IQR 49-81.75) than patients in the discontinued group (median 35, IQR 4.5-66) (p<0.001).

Figure 3.1 shows the number of patients in the currently using and discontinued groups who reported that they experienced a positive effect of pyridostigmine for each individual MG symptom. Fatigue appeared to be less responsive to pyridostigmine than other symptoms, in both the currently using and discontinued groups. Patients in the discontinued group experienced less response on generalized MG symptoms than patients in the currently using group.

Fifty-five percent of patients in the currently using group and 21% of the patients in the discontinued group reported no change of the effect of pyridostigmine over the course of time. In the discontinued group, 23% stated that the initial effect was good, but diminished over time; 12% described that the initial effect was good, but increasing doses were required to accomplish the same effect.

Table 3.1 Baseline characteristics

	Currently using (n=252)	Discontinued (n=149)	Never (n=9)	Overall (n=410)	Non-responders (n=232)§
Age at survey (years)	59.8 (± 13.5)	62.7 (± 12.6)	71.0 (± 14.0)	61.1 (± 13.3)	63.7 (± 16.8)
Age at diagnosis (years)†	48.0 (± 16.3)	51.0 (± 16.0)	60.1 (± 16.8)	49.3 (± 16.3)	50.7 (± 19.7)
Sex					
Male	99 (39)	84 (56)	7 (78)	190 (46)	113 (49)
Female	153 (61)	65 (44)	2 (22)	220 (54)	119 (51)
Myasthenia gravis duration (years)†	9 (5-16)	10 (4-15)	10 (8-11)	9 (4-16)	9 (5-16)
Previous thymectomy‡	103 (41)	51 (34)	1 (11)	155 (38)	70 (35)
Autoantibody class					
AChR	154 (61)	91 (61)	5 (56)	250 (61)	130 (56)
MuSK	3 (1)	7 (5)	0	10 (2)	8 (3)
LRP4	1 (0)	0	0	1 (0)	1 (0)
Seronegative	36 (14)	18 (12)	1 (11)	55 (13)	33 (14)
Unknown	58 (23)	33 (22)	3 (33)	94 (23)	60 (26)
Current and previous MG treatments					
Distigmine	12 (5)	7 (5)	0	19 (5)	-
Corticosteroids	151 (60)	110 (74)	5 (56)	266 (65)	-
Azathioprine	135 (54)	93 (62)	3 (33)	231 (56)	-
Rituximab	14 (6)	7 (5)	0	21 (5)	-
IVIg	65 (26)	48 (32)	2 (22)	115 (28)	-
Plasmapheresis	44 (17)	22 (15)	1 (11)	67 (16)	-
Other	69 (27)	27 (18)	4 (44)	100 (24)	-
No other medication	55 (22)	21 (14)	1 (11)	77 (19)	-
Dose	300 (180-360)	220 (90-360)	N.A.	240 (180-360)	N.A.
Clinical symptoms at onset					
Ocular	27 (11)	29 (20)	2 (22)	58 (14)	30 (13)
Generalized	208 (83)	112 (75)	7 (78)	327 (80)	173 (75)
Unknown	17 (7)	8 (5)	0	25 (6)	29 (13)

Categorical data are presented as n (%). Continuous data are presented as mean (±SD) for normal distributed data (age at survey and age at diagnosis) and median (IQR) for non-normal distributed data (myasthenia gravis duration and dose). The "non-responders" group consists of participants of the MG registry who did not complete the questionnaires for the current study. Percentages may not sum to 100 because of rounding. §Data obtained from the Dutch-Belgian myasthenia patient registry.
†Missing values: responders (n = 16), non-responders (n = 13) ‡ Missing values: non-responders (n = 32).
Abbreviations: IVIg, intravenous immunoglobulin therapy

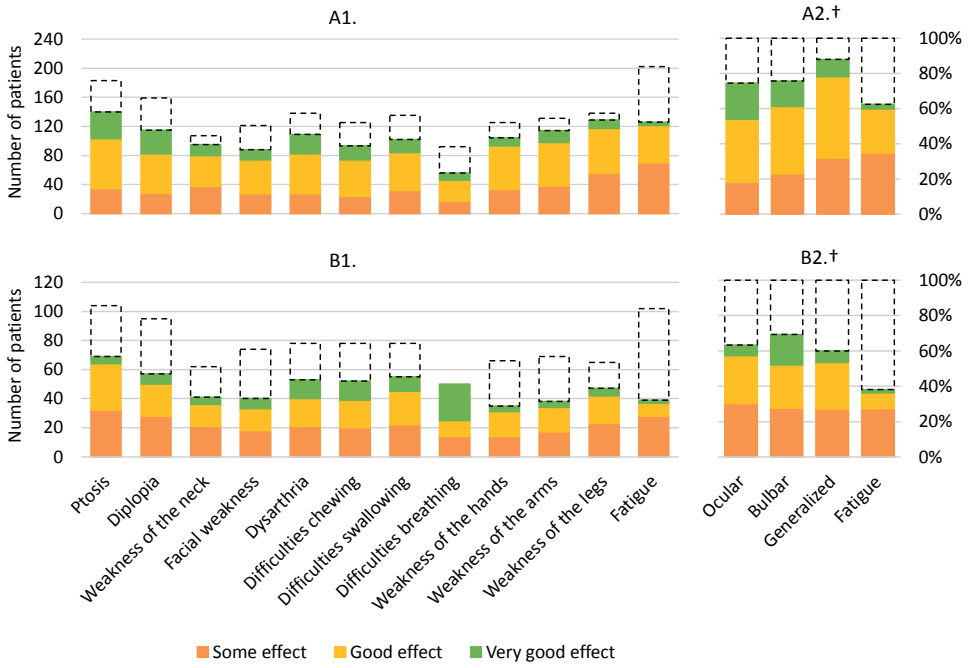


Figure 3.1 Perceived effectiveness
A1. Number of patients in the currently using group who reported to have effect of pyridostigmine per individual MG symptom
A2. Percentage of patients in the currently using group who reported to have effect of pyridostigmine in subgroups: ocular, bulbar, generalized, fatigue.
B1. Number of patients in the discontinued group who reported to have effect of pyridostigmine per individual MG symptom.
B2. Percentage of patients in the discontinued group who reported to have effect of pyridostigmine in subgroups: ocular, bulbar, generalized, fatigue.
The dotted line represents the number of patients who reported in the Dutch-Belgian Myasthenia Patient Registry to have the MG symptom in the first 6 months of the disease.
†Ocular (ptosis + diplopia), bulbar (weakness of the neck, dysarthria, difficulties chewing/ swallowing/breathing), generalized (weakness of the hands/arms/legs) and fatigue.

Side effects

Ninety-one percent of the patients in the currently using group reported one or more side effects in the past seven days, compared with 54% of the patients in the control group (consisting of the discontinued and never groups). Patients in the currently using group reported a higher number of side effects over the past seven days (median 5, IQR 2-8) than patients in the control group (median 1, IQR 0-4) ($p<0.001$). In the currently using group, the number of side effects was correlated with the pyridostigmine dose (Spearman's R=0.196, p=0.002), but not with disease duration (p=0.312) or age at diagnosis (p=0.530). In the discontinued group, younger age was correlated with more side effects (Spearman's R=-0.217, p=0.010).

Figure 3.2 shows the percentage of patients reporting one or more side effects in the currently using group compared to the control group. Flatulence, urinary urgency, muscle cramps, blurred vision, hyperhidrosis, diarrhea, abdominal cramps, increased salivation, light-headedness and flu-like symptoms were reported significantly more frequently than in the control group. ”Other” side effects that were reported more than once by patients were a runny nose (n=2), insomnia (n=2) and dyspnea (n=2).
Patients in the discontinued group reported fatigue, flatulence, blurred vision, muscle weakness, urinary urgency, increased lacrimation and excessive belching more frequently than the currently using group (**Supplement 3.2**).
Diarrhea was the most frequent cause for discontinuation or lowering of the dose of pyridostigmine (n=42); 32 patients discontinued or lowered the dose due to abdominal cramps; 24 patients discontinued or lowered the dose due to muscle cramps and muscle twitching (**Figure 3.3**). In the group of patients who stopped pyridostigmine because of side effects, diarrhea, abdominal cramps and muscle twitching were most frequently considered a reason to discontinue.

Figure 3.4 shows the severity of side effects in the currently using and discontinued group. Abdominal cramps and muscle cramps were considered to be more severe (moderately, very and extremely annoying) in the discontinued group than in the currently using group (p=0.008 and p=0.003 resp.).

Net benefit
Taken into account the perceived benefit and the burden of the side effects, patients reported a median net benefit of pyridostigmine of 65 on a VAS scale of 0 (“I felt much worse”) to 100 (“I felt much better”) (IQR 45-84). Patients in the currently using group perceived a better net benefit (median 73, IQR 87-53.25) than patients in the discontinued group (median 49, IQR 72.5-8.5) (p<0.001), as shown in **Figure 3.5**.

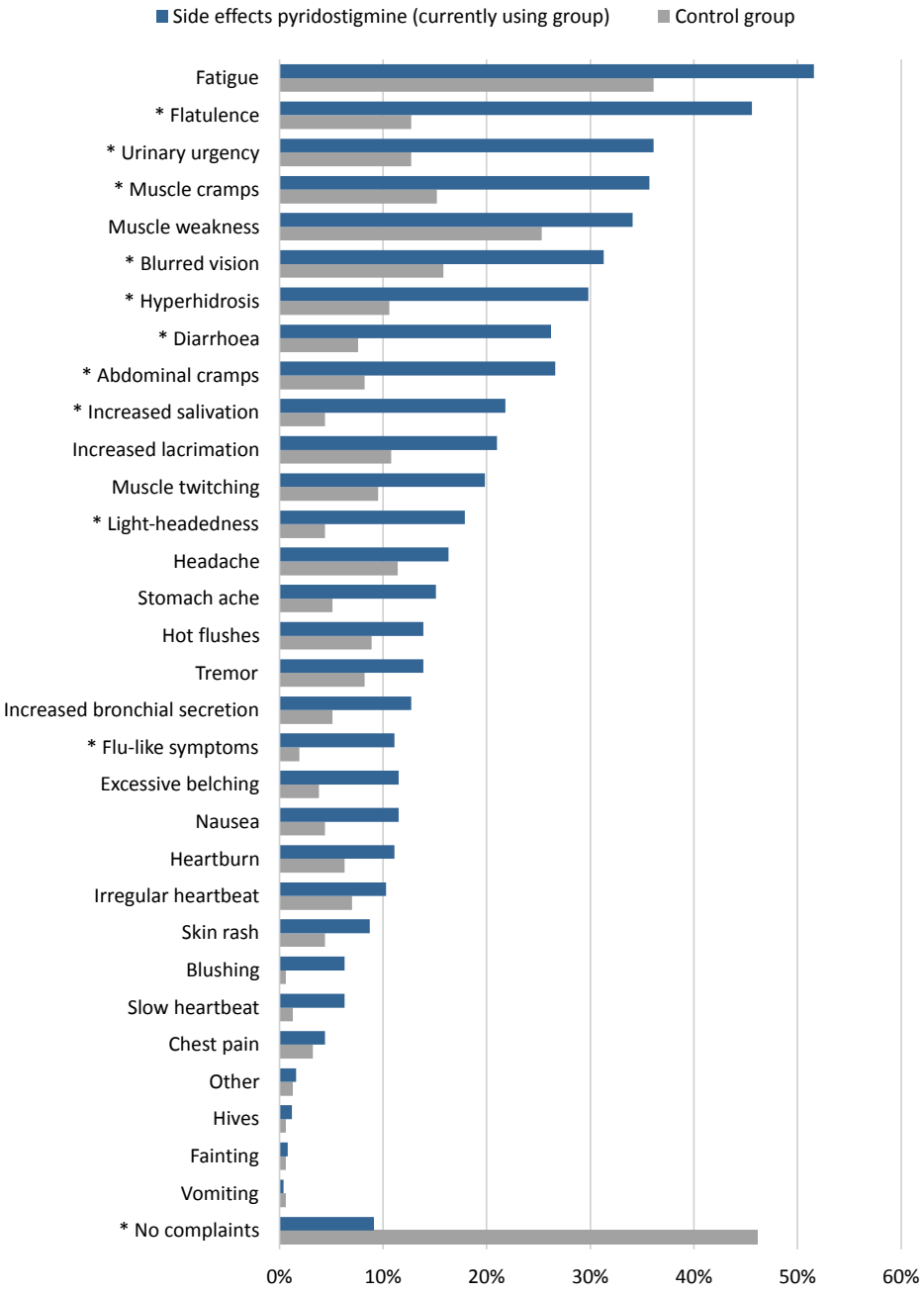


Figure 3.2 Percentage side effects in the currently using group vs. controls
**indicates a significant difference (p < 0.0017, Fisher's Exact test) between the currently using group and control group. The control group consists of patients from the discontinued and never group answering the question "Have you experienced one of the following symptoms over the last 7 days?".*

Factors associated with discontinuing pyridostigmine

Table 3.2 shows the results of the multivariate logistic regression analysis with current use/ discontinued as dependent variable. Discontinuation of pyridostigmine was more likely in male patients (OR 1.781, 95% CI 1.125-2.819) and patients with MuSK antibodies (OR 4.127, 95% CI 1.194-14.269).

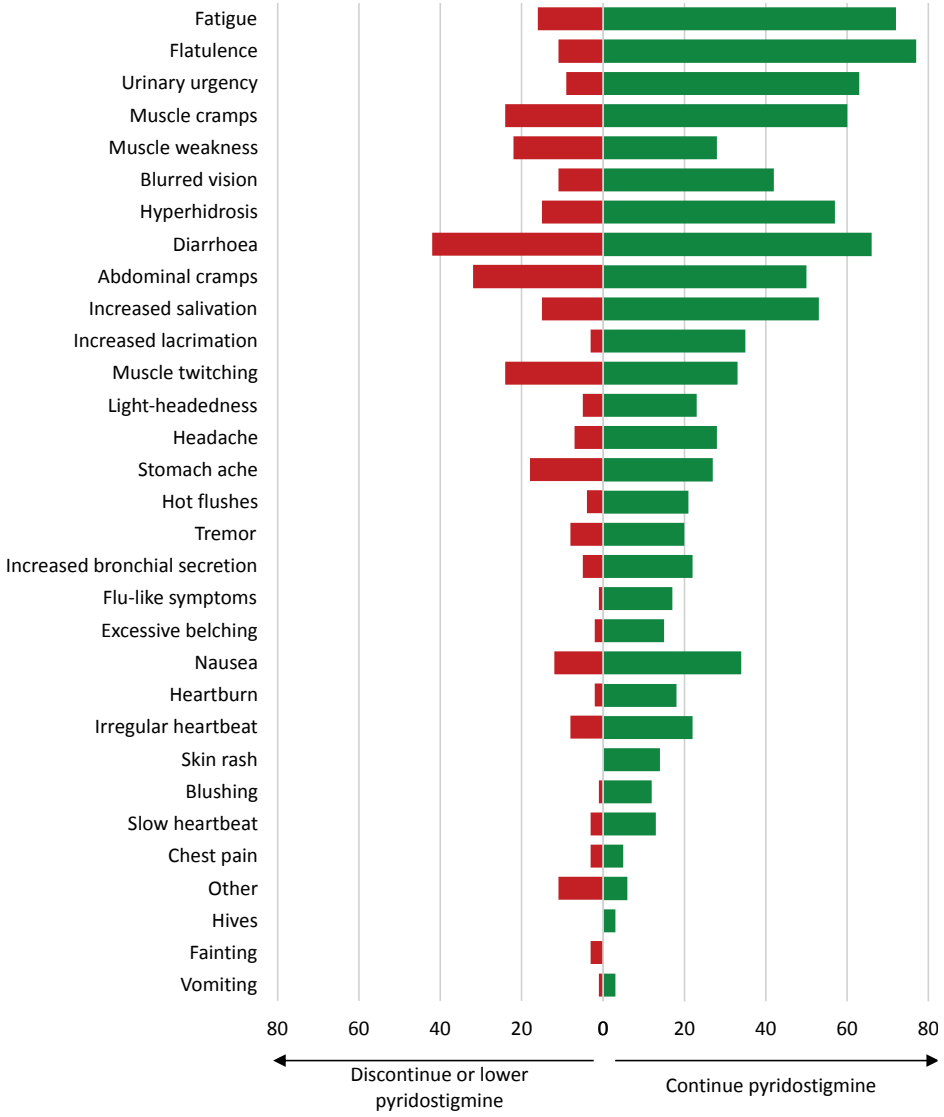


Figure 3.3 Number of patients considering the side effect a reason to discontinue or lower the dose of pyridostigmine

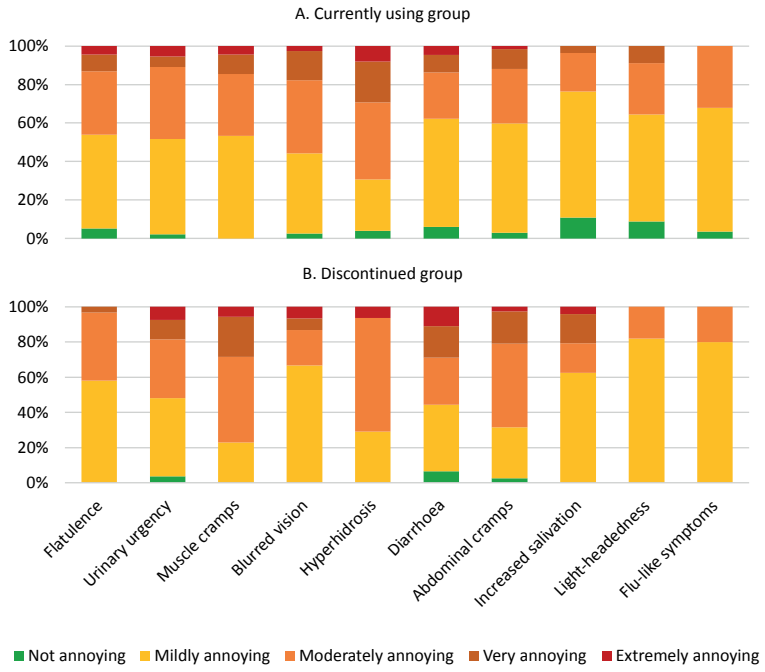


Figure 3.4 Severity of side effects in A. the currently using group and B. the discontinued group
Only the side effects occurring significantly more often in the currently using group than in the control group as depicted in Figure 3.2, are shown.

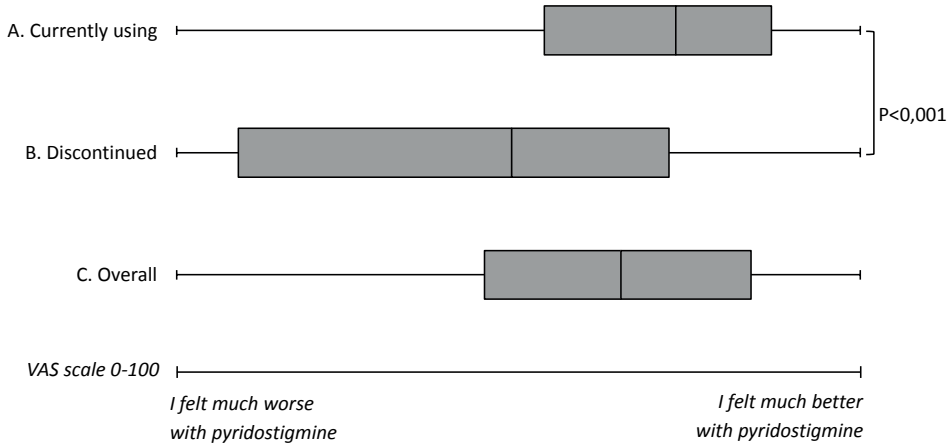


Figure 3.5 Perceived net benefit of pyridostigmine on VAS-scale (0-100) for A. currently using, B. discontinued and C. overall group
Box plot indicates 25th and 75th percentile, middle vertical line represents the median response. The perceived net benefit was higher in the currently using group than in the discontinued group (P < 0.001, Mann-Whitney U test).

Table 3.2 Factors potentially associated with discontinuation of pyridostigmine†

Parameter	B	Std. Error	Sig.	Exp (B)	95% confidence interval Exp (B)	
					Lower	Upper
Age at diagnosis	0.004	0.007	0.590	1.004	0.990	1.018
Sex						
Male	0.577	0.234	0.014	1.771	1.125	2.819
Female‡	-	-	-	-		
Autoantibody class						
AChR	0.124	0.300	0.680	1.132	0.628	2.041
MuSK	1.417	0.630	0.025	4.127	1.194	14.269
Seronegative‡	-	-	-	-		
Clinical symptoms at onset						
Ocular	0.461	0.321	0.151	1.586	0.845	2.976
Generalized‡	-	-	-	-		

† Pooled estimates of multivariate logistic regression after multiple imputation
‡ Reference group for statistical comparison

Post-hoc analyses of sex differences

After finding that male patients were more likely to stop using pyridostigmine, we performed a post-hoc comparison between men and women. Female patients were younger than male patients at diagnosis (mean age 43.3 vs. 56.2 years, p<0.001) and at the time of the survey (mean age 57.4 vs. 65.5 years, p<0.001). Furthermore, females had a longer disease duration (median 12 years vs. 7 years, p<0.001), had undergone thymectomy more often (48.6% vs. 25.2%, p<0.001) and presented more frequently with generalized symptoms at disease onset (91.9% vs. 76.4%, p<0.001) than males.

There was no significant difference in autoantibody class, other medication use and pyridostigmine dose between male and female patients. In the entire study population, the experienced net benefit was the same in male (median 63, IQR 46.5-83) and female patients (median 66, IQR 43.25-84.75). Furthermore, there was no significant difference between male and female patients for the perceived effectiveness (males: median 55, IQR 21-80.5, females: 62, IQR 39.25-78).

With regards to side effects, male patients reported more frequently to have “no complaints” compared to female patients (p<0.0017). Fatigue, hyperhidrosis, abdominal cramps, hot flushes and an irregular heartbeat were more frequently reported by females than by males (p<0.0017) (**Figure 3.6**).

No significant differences were found between male and female patients in reasons for discontinuing pyridostigmine. In the discontinued group, 47 (42%) male patients responded that pyridostigmine was no longer needed due to the start of other medication, compared to 26 (32%) female patients (p=0.069). Thirty-two percent of female patients reported side

effects as the main reason for discontinuing pyridostigmine, compared to 23% of male patients (p=0.224).

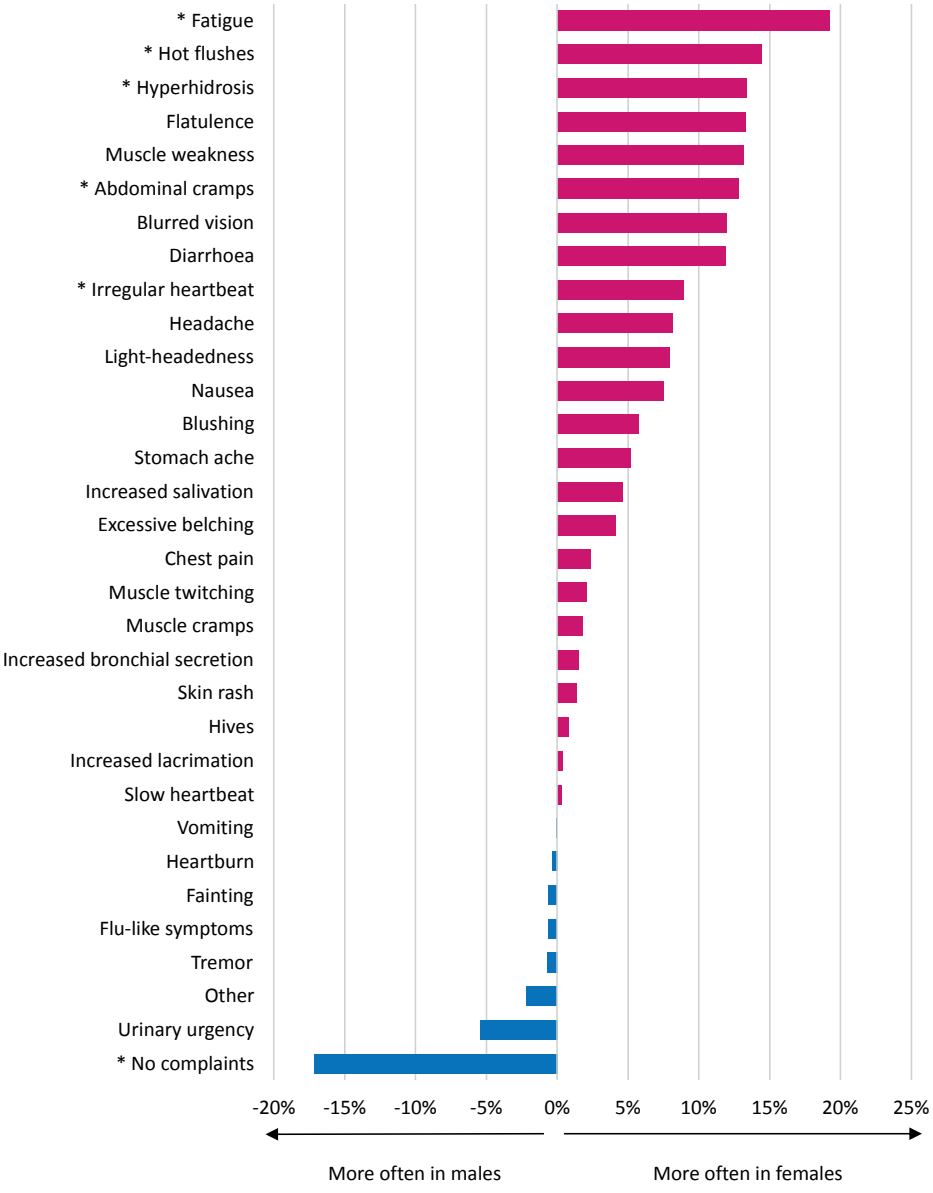


Figure 3.6 Difference in occurrence of side effects between male and female patients (%)
**indicates a significant difference (p < 0.0017, Fisher's exact test) between males and females.*

Discussion

This study reports a comprehensive assessment of pyridostigmine use, its effect on symptoms, side effects and its net benefit, in a very large, well-defined and representative sample of the Dutch MG population. We show that virtually all MG patients have used pyridostigmine at some point in their disease, and that approximately two thirds (62%) continue to use it.

The net benefit and effectiveness are moderate: only a small number of patients reported that pyridostigmine had a very good effect on their symptoms. Side effects are frequent: 91% of all patients currently using pyridostigmine reported at least one side effect (vs. 54% in the control group). Most frequently reported side effects were gastro-intestinal symptoms (flatulence, diarrhea and abdominal cramps), urinary urgency, muscle cramps, blurred vision, hyperhidrosis, increased salivation, light-headedness and flu-like symptoms. The reasons for discontinuing pyridostigmine were varied: 38% stated it was not effective (anymore) and 34% reported that it was no longer needed due to the start of other medication. Twenty-six percent reported side effects as the main reason for discontinuation. Within the latter group, diarrhea, abdominal cramps and muscle twitching were the most frequently cited reasons to discontinue pyridostigmine.

Patients with MuSK antibodies were more likely to discontinue pyridostigmine. This finding is in line with previous studies showing that acetylcholinesterase inhibitors are less effective in patients with MuSK antibodies and that these patients experience more side effects.[126-128] Surprisingly, male patients were also more likely to stop using pyridostigmine, which may be explained by the fact that female patients more frequently have severe or refractory MG,[129,130] and male patients appear to respond better to standard care than female patients.[131] This is supported by our post-hoc analysis showing that male patients were more likely than females to report "it was no longer needed due to the start of other medication" as the main reason for discontinuing pyridostigmine, although this difference did not reach statistical significance (probably due to small numbers). Female patients reported side effects more frequently than males. This is in line with literature reporting that female patients are 50 to 75 percent more likely to experience an adverse drug reaction than males,[132] probably due to sex-based differences in pharmacokinetics and pharmacodynamics.[133]

One of the main strengths of our study is the large patient sample (n=642) with a relatively high response rate of 64%. This response rate demonstrates the importance of this topic to MG patients. The digital format of the questionnaire allowed for standardized data collection, minimizing missing data. The burden of participation was minimized by the dynamic design of the questionnaire, so that patients were only required to fill out questions applicable to their own situation.

A limitation of this study is its observational design. First, the sampling method, consisting of an email invitation to all MG patients in the Dutch-Belgian MG registry, may have led to a selection bias. However, given the absence of differences in baseline characteristics between patients who filled out the survey and patients who did not respond, this limitation appears to be minimal. Second, this study does not provide information on the causality of the reported side effects and the use of pyridostigmine. Third, recall bias may have affected results, especially for patients who were asked to report side effects for medication that was discontinued several years ago. Finally, all collected data on net benefit, effectiveness and side effects were patient reported and therefore cannot be related to commonly used quantitative outcome measures such as the QMG and the MG-ADL. Although the observational design brings about some limitations, it is the most common way of obtaining evidence on effectiveness and side effects for medication that has gained market approval.

Another limitation in this study is that patients were actively queried about a list of 30 potential side effects. It has been shown that patients report more side effects when asked to check off a list, than when they are asked to spontaneously name them.[134] We tried to minimize this effect by comparing the side effect profile in the currently using group to the reported symptoms in a control group, which consisted of patients not using pyridostigmine in the period queried (the past seven days).

It may have been challenging for patients (and physicians) to distinguish whether their muscle weakness or fatigue were caused by MG or by pyridostigmine. However, as our results show that the reported frequency of fatigue and muscle weakness did not differ significantly between the group currently using pyridostigmine and the group not using pyridostigmine, we believe that the potential inability to distinguish the causes of these symptoms did not affect the main conclusions of this paper.

In this cross-sectional questionnaire study, longitudinal data on comorbidity and comedication was not available. It is possible that patients who stopped using pyridostigmine were more susceptible to side effects due to certain comorbidities or comedications.

Remarkably, only a small number of patients experienced a very good effect of pyridostigmine on their symptoms. This is surprising since 19% report using pyridostigmine as monotherapy and are apparently satisfied with its effect.

The use of distigmine was somewhat more common than expected. It appears to have been prescribed by a single neurologist in the Netherlands (now retired) and used mainly as an add-on to pyridostigmine.

Medication to treat side effects was used in 20 percent of all patients. The Dutch national myasthenia gravis consensus guideline contains the following recommendation: "consider starting atropine in patients with muscarinic side effects such as abdominal cramps and diarrhea and hypersalivation", leaving considerable leeway for different traditions and habits among treating neurologists in the approach towards side effects. This may have influenced

the prevalence and severity of the observed side effects. There is no influence of reimbursements on the prescription of these medications since all medications are fully reimbursed in the Netherlands. Fatigue is a frequent and disabling symptom of MG.[135] Immunomodulatory drugs might improve fatigue, as suggested by a small number of studies. In our study, pyridostigmine resulted in only a limited improvement of fatigue. This suggests that the contribution of neuromuscular transmission disturbances to the development of fatigue is limited. Indeed, previous studies have suggested that the pathophysiology of fatigue is likely multifactorial.[136]

In contrast to two previous small studies,[137,138] and expert opinion,[139-141] we found no difference on the patient' reported effectiveness on ptosis and diplopia compared to generalized weakness.

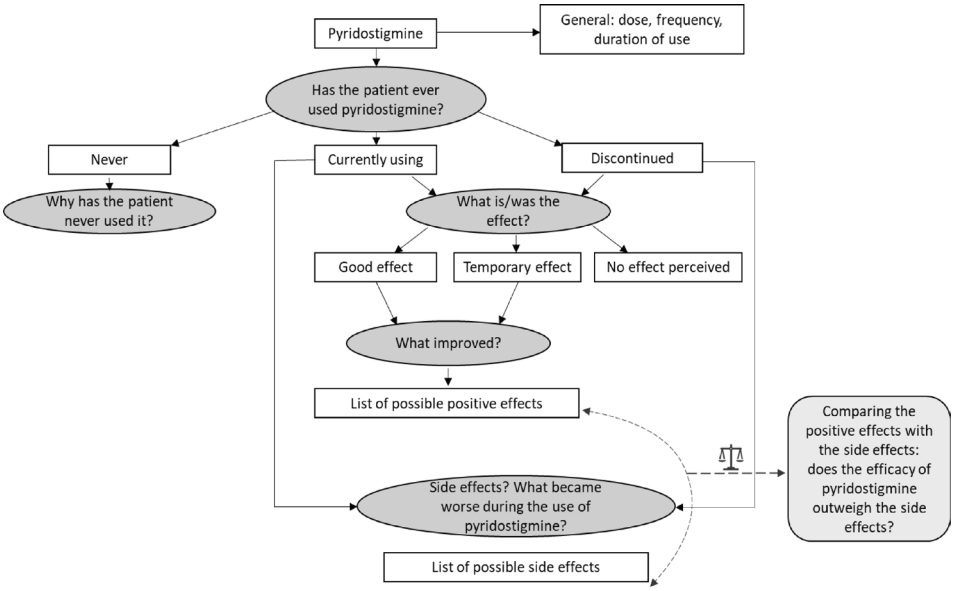
Twenty-six percent of all patients reported side effects as the main reason for lowering or discontinuing pyridostigmine, which is higher than reported in previous studies.[77,124]

It has been hypothesized that the risk of cholinergic side effects increases after prolonged treatment with pyridostigmine or with increasing age.[77] In our study, we found no significant correlation between the number and severity of side effects and age or disease duration. Moreover, in the discontinued group, younger patients reported more side effects than older patients. We therefore recommend that pyridostigmine be used in all patients regardless of age or disease duration.

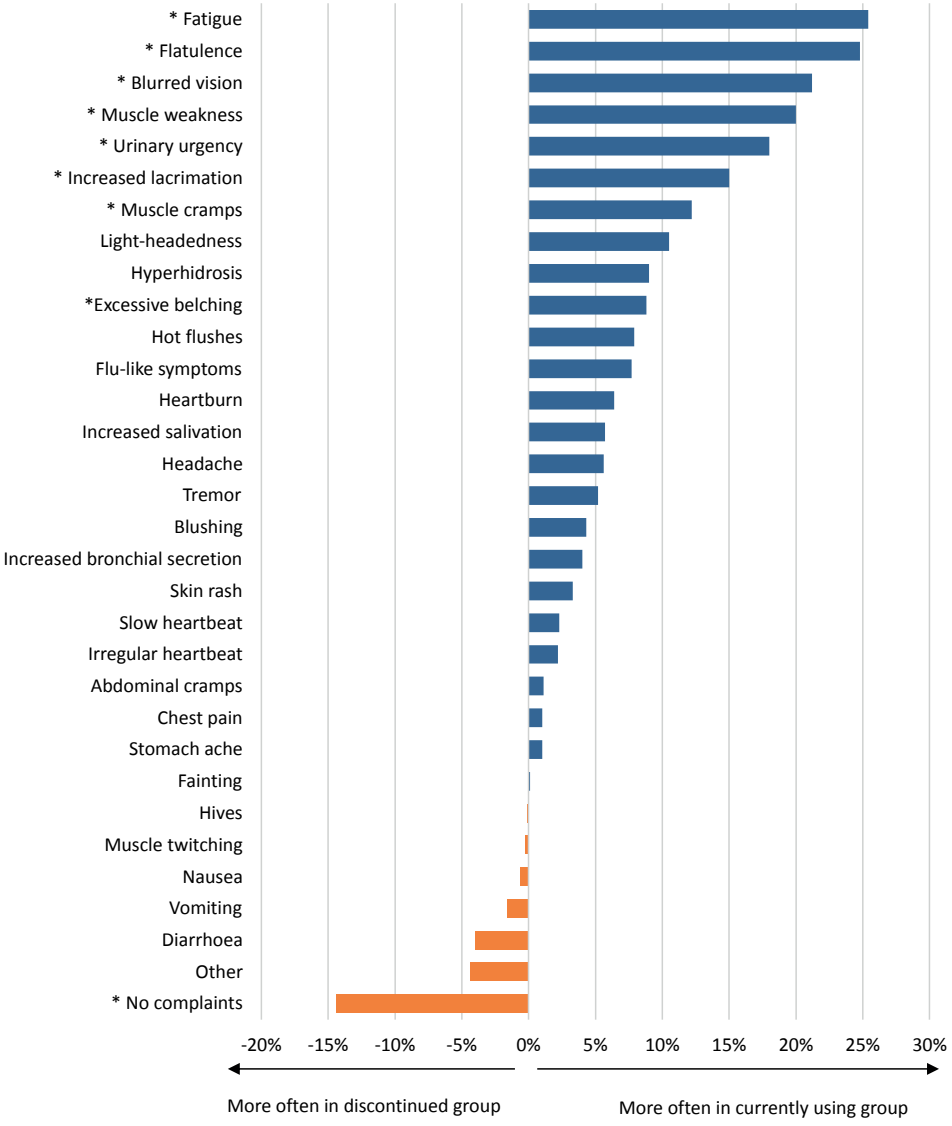
Conclusions

Almost all international guidelines recommend pyridostigmine as the first step in the treatment of MG.[25,83,85] Our data do not support a change in these guidelines, although the observed effectiveness was relatively modest and side effects occurred frequently. Nonetheless, pyridostigmine remains a drug that is readily available at low cost and has a very favorable long term safety profile.[39] In addition, the majority of patients do experience a positive effect on their symptoms, and potential side effects are generally mild and reversible. However, our results can be used to guide shared decision making, prior to starting symptomatic treatment for MG, as they provide a nuanced quantification of its expected effectiveness and side effects. To further aid patient education, we have summarized the main results of this study in an easy-to-read patient leaflet that can be handed out with the prescription (**Supplement 3.3**).

Supplements



Supplement 3.1 Survey design



Supplement 3.2 Difference in occurrence of side effects between currently using and discontinued group (%)

** indicates a significant difference (p<0.0017, Fisher's Exact test) between the currently using and discontinued group*

PYRIDOSTIGMINE

FOR THE TREATMENT OF MYASTHENIA GRAVIS

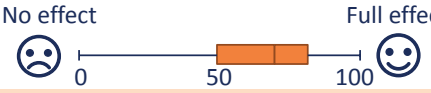
What to expect when starting?

TWO THIRDS of all patients continue using pyridostigmine

ONE THIRD of all patients discontinue pyridostigmine

EFFECTIVENESS

Most patients currently using pyridostigmine rate the effect with a score between **49 AND 81.**



FATIGUE appears to be less responsive to pyridostigmine than other MG symptoms

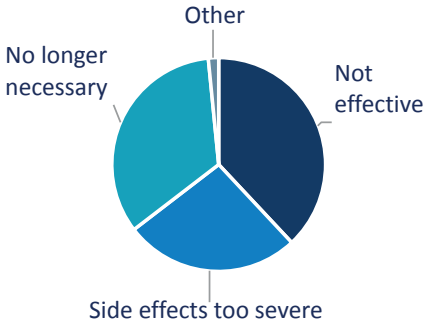
MOST COMMON SIDE EFFECTS

- Flatulence
- Diarrhea
- Abdominal cramps
- Urinary urgency
- Muscle cramps
- Blurred vision
- Excessive sweating
- Increased salivation
- Light-headedness
- Flu-like symptoms

DOSE

- Most patients use a dose between **180 AND 360 mg** per day.
- **95%** of all patients use a dose **LESS THAN 660 mg** per day.

REASONS FOR DISCONTINUING



ALWAYS CONSULT YOUR NEUROLOGIST BEFORE MAKING ANY CHANGES TO YOUR MEDICATION.

Data are based on a cross-sectional study conducted in 410 patients with MG participating in the Dutch-Belgian MG Patient Registry
L. Remijn-Nelissen, J.J.G.M. Verschuuren, M.R. Tannemaat

Supplement 3.3 Patient leaflet to inform patients what to expect when starting with pyridostigmine

CHAPTER

4

Efficacy and cost-utility of pyridostigmine

Efficacy of pyridostigmine in myasthenia gravis:
a randomized, double-Blind, placebo-controlled crossover trial

Neurology, 2026, 106(8)

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Abstract

Background and objectives

Pyridostigmine, an acetylcholinesterase inhibitor, is a symptomatic drug approved for the treatment of myasthenia gravis (MG), but no randomized controlled trials substantiate its widespread use. We aimed to assess the efficacy and cost-utility of pyridostigmine in patients with anti-acetylcholine receptor positive MG (AChR MG).

Methods

A randomized, double-blind, placebo-controlled crossover trial was conducted at Leiden University Medical Center, a tertiary center for the treatment of MG in the Netherlands. Main eligibility criteria were current use of pyridostigmine, and a stable dose of other MG treatments. Participants were assigned to a sequence of two treatment periods for five days separated by a two-day washout, in which patients either first received placebo and then pyridostigmine, or vice versa. Pyridostigmine dosing matched each participant's pre-study regimen. The primary outcome was change in Myasthenia Gravis Impairment Index (MGII) score. Secondary efficacy outcome measures included the Myasthenia Gravis-Activities of Daily Living (MG-ADL) scale, the Quantitative Myasthenia Gravis (QMG) score and the revised 15-item Myasthenia Gravis Quality of Life (MG-QOL15r) questionnaire. For the post hoc cost-utility analysis, a mathematical model was developed to translate the observed study results into long-term annual impact on societal costs and quality-adjusted life-years (QALYs).

Results

Nineteen patients (median age 59 years, 58% female) were recruited between March 23, 2023 and February 21, 2024. The estimated mean difference between pyridostigmine and placebo was 5.3 for MGII score (95% confidence interval [CI] 1.9-8.7, p=0.004). Secondary efficacy outcome measures showed estimated mean differences of 1.4 (95% CI 0.5-2.3) for QMG-score, 1.2 (95% CI 0.5-1.8) for MG-ADL-score, and 2.0 (95% CI 0.03-3.91) for MG-QoL15r-score. The post hoc cost-utility analysis showed lower annual societal costs (€6565, 95% CI €328-€15945), and annual improved QALYs (0.106, 95% CI 0.019-0.210) for patients using pyridostigmine.

Discussion

This trial showed that in patients with AChR MG chronically using pyridostigmine, pyridostigmine demonstrated benefit over placebo across all efficacy outcome measures and substantially reduced societal costs.

Introduction

Myasthenia gravis (MG) is a chronic autoimmune disease of the neuromuscular junction in which autoantibodies bind to the acetylcholine receptor (AChR) or other target proteins on the postsynaptic membrane, resulting in impairment of neuromuscular transmission. The main clinical features are weakness and fatigability in ocular, bulbar, limb and respiratory muscles.

Pyridostigmine is considered the first drug of choice in the treatment of MG and remains the cornerstone in the management of MG since its first introduction almost eighty years ago,[25,142] despite the recent introduction of new immunomodulatory drugs.[39] Pyridostigmine blocks the degradation of the neurotransmitter acetylcholine by inhibiting the enzyme acetylcholinesterase (AChE), which is responsible for hydrolyzing acetylcholine. As a result, pyridostigmine prolongs the duration of activity of acetylcholine in the synaptic cleft, thereby enhancing neuromuscular transmission.[143]

Formal recommendations on treatment with pyridostigmine suffer from a lack of evidence from randomized trials and rely solely on anecdotal data from case reports, case series, and clinical experience.[26,142] A 2014 Cochrane review concluded that, despite the absence of adequate randomized controlled trials, the clear effect of AChE inhibitors found in observational studies support their widespread use in the treatment of MG. However, these observational studies primarily assessed short-term effects of not only pyridostigmine, but also of neostigmine or edrophonium, which have a more rapid onset. Moreover, these studies were conducted prior to the advent of immunosuppressive therapies such as corticosteroids and azathioprine. Consequently, there is a lack of data on the long-term efficacy of AChE inhibitors, especially when used alongside modern immunosuppressive treatments. Given that over 60% of MG patients use pyridostigmine,[79] usually in combination with one of the many immunosuppressive agents, a critical evaluation of its role is warranted.

In addition to assessing the efficacy of pyridostigmine, it is essential to evaluate its cost-utility. In recent years, the advent of novel, but very costly immunomodulatory drugs has brought renewed focus on the cost-effectiveness of treatments for MG. Limited data on the cost-effectiveness of these emerging therapies are available,[144-146] and these studies mostly estimate utilities using proxy measures, such as QMG scores, rather than directly observed values. This limitation hampers the development of rational and cost-effective treatment strategies.

In IMPACT-MG, a randomized, double-blind, placebo-controlled crossover study, we aimed to investigate the efficacy, tolerability, and cost-utility of pyridostigmine in patients with AChR MG. The primary research question being addressed in this study was whether pyridostigmine improves MG symptoms compared with placebo, in patients on stable standard of care therapy and currently using pyridostigmine.

Methods

Study design and participants

IMPACT-MG was a two-part, investigator-initiated, prospective, randomized, double-blind, placebo-controlled crossover study of the efficacy, tolerability and cost-utility of pyridostigmine (part one) and pyridostigmine plus amifampridine combination therapy (part two). The results of part two will be published separately. The study was performed at Leiden University Medical Center, a tertiary center for the treatment of MG in the Netherlands.

Patients were eligible for the study if they were at least 18 years of age and had a documented diagnosis of ocular or generalized MG with evidence of elevated AChR antibodies. A maximum of five patients with purely ocular MG were included, to ensure that the study population accurately reflected the clinical population. Eligibility required a Myasthenia Gravis Foundation of America (MGFA) clinical classification of class I to IV at screening,[147] current use of pyridostigmine, and a stable dose of other MG treatments. Stability was defined as follows: at least one month on a stable steroid regimen; at least three months since initiating azathioprine, mycophenolate mofetil, cyclosporine or other nonsteroid immunosuppressive agents with a stable dose for at least one month; at least six months since starting rituximab, complement inhibitors or Fc receptor inhibitors with a stable dose for at least three months for the latter two. Patients were excluded from the trial if they had a history of thymectomy within the six months before screening, or thymectomy (expected) to take place during the trial, had used intravenous immunoglobulin (IVIg) or undergone plasma exchange (PLEX) in the last four weeks preceding the trial or planned to do so during the study, or were currently using AChE inhibitors other than pyridostigmine, including pyridostigmine slow-release. A full list of inclusion and exclusion criteria is provided in the supplementary material.

Potential patients were recruited from the investigators' practice, through the Dutch neuromuscular patient organization "Spierziekten Nederland", or through the existing newsletter for participants in the Dutch MG registry.

Randomization and masking

Eligible participants were randomly assigned (1:1) to one of two treatment groups, in which patients either first received placebo and then received pyridostigmine, or vice versa. Randomization was done with the Castor Electronic Data Capturing System (Castor EDC; Amsterdam, the Netherlands) using variable block sizes (two, four and six patients), without any stratification. The pyridostigmine 10 mg and placebo tablets appeared identical to maintain the double-blinding and were dispensed by the hospital pharmacy and distributed in identical blister packs. Only tablets of 10 mg were used, since the 60 mg tablets have a coating color that could not be identically reproduced for the placebo. All study personnel

(except the study pharmacist) and all participants remained masked to the allocation sequence until after the study database lock. The efficacy of blinding was assessed by asking the investigator and the patient which treatment they believed the patient had received in the past week.

Study procedures

After consent was obtained, screening assessments were performed and patients were instructed to stop their regular use of pyridostigmine for a two-day washout period. Patients who were unable to complete this two-day washout period were excluded from participating in part one of the study. Patients were randomly assigned to a sequence of two treatment periods for five days separated by a second two-day washout. The dose of pyridostigmine was set at the therapeutic dose of pyridostigmine as determined before inclusion in clinical routine care based on patient response and tolerance. The dosing schedule of placebo was the same as that of the active treatment. Patients were allowed to prematurely discontinue treatment and resume their prior pyridostigmine regimen if they experienced a compelling need to alleviate MG symptoms. Before initiation of rescue pyridostigmine treatment, primary, secondary and tolerability parameters were assessed by the investigator.

Throughout the trial, patients were required to maintain the same dosage and treatment schedule for oral corticosteroids, and other immunosuppressive treatments as before inclusion in the trial.

For evaluation of efficacy, we assessed the following validated outcome measures: the MGII [148], the Myasthenia Gravis-Activities of Daily Living (MG-ADL) scale,[149] the Quantitative Myasthenia Gravis (QMG) score,[150] and the revised 15-item Myasthenia Gravis Quality of Life (MG-QOL15r) questionnaire.[151] Treatment satisfaction was evaluated using the 9-item Treatment Satisfaction Questionnaire for Medication (TSQM-9).[152] MGII was assessed at screening, baseline (day 1, pre-treatment), day 5 and day 12. MG-ADL, QMG, and MG-QOL15r were scheduled at baseline, day 5 and day 12. TSQM-9 was assessed at day 5 and day 12.

Patients were actively asked for adverse events during each visit and the severity of these events was assessed. To assess tolerability, side effects were evaluated using a questionnaire that was previously developed to assess the side effects of pyridostigmine in a cross-sectional study.[79] A full list of the side effects queried is shown in the supplementary material.

Laboratory testing at baseline and day 12 included a blood cell count, assessment of electrolytes, renal function, liver enzymes, creatine kinase and AChR antibody titers. In addition, serum concentrations of pyridostigmine were collected at each visit.

For the economic evaluation, to measure health-related quality of life, the 5-level EuroQol 5 Dimensions (EQ-5D-5L) and the EuroQol's visual analogue scale (EQ-VAS) were obtained at baseline, day 5 and day 12.[153] Prior healthcare use and productivity were assessed at baseline using the Medical Technology Assessment (iMTA) Medical Consumption

Questionnaire (iMCQ),[154] and the iMTA Productivity Cost Questionnaire (iPCQ).[155] The iMCQ was modified by omitting the modules related to medication and travel, and the recall periods for the iMCQ and iPCQ were set to 12 and 3 months, respectively.

Outcome measures

The primary efficacy outcome was a mean clinically meaningful difference in MGII total score while using pyridostigmine compared to placebo. A change of ≥8 is considered a clinically meaningful improvement.[156] The MGII was chosen as the primary outcome measure because it provides a broader range of scores and has less floor effect compared to other commonly used outcome measures.[148]

Prespecified secondary efficacy endpoints were mean clinically meaningful changes on the MG-ADL scale, the QMG score, the MG-QOL15r questionnaire, the MGII ocular and generalized subscores, and the TSQM-9. A clinically meaningful improvement was defined as a 2-point change in the MG-ADL score,[157] and a 3-point change in the QMG score.[150] Tolerability and safety outcomes were incidences of adverse events, side effects, and vital signs. Treatment adherence was assessed by use of a diary and via pill counts of returned study medication.

Economic evaluation

The economic evaluation consisted of a cost-utility analysis from a societal perspective. Main outcomes were the differences in costs, quality-adjusted life years (QALYs) and net benefit between the policies with and without pyridostigmine.

Utilities were calculated from the EQ-5D-5L responses using the Dutch tariff.[158] Utilities reflect the value of quality of life on a scale anchored at 0 (= as bad as death) and 1 (= full health). EQ-VAS scores were transformed to a utility scale using the power transformation 1 - (1 - EQ-VAS/100)^1.61.[159] All healthcare costs were calculated as reported in the iMCQ and valued according to Dutch guidelines for economic healthcare evaluations, using Dutch unit reference prices and including travel costs.[160] Standard prices for medication were obtained from the database provided by the National Health Care Institute.[161] Productivity costs were estimated from the number of workdays lost due to absenteeism and work cutback (presenteeism) as reported in the iPCQ questionnaire. Productivity losses were valued using the human-capital method, at €43.44 per hour for paid labor and €20.48 for unpaid labor.[160] Societal costs were calculated as the sum of healthcare costs, including medication, and productivity costs. All costs are reported in Euros and all prices were indexed to 2024 using the Dutch consumer price index.

Since the time horizon of the current clinical trial was five days for each treatment period, a mathematical model was needed to extrapolate the observed short-term trial results to the impact on societal costs and QALYs for a long-term one-year period. This impact was

predicted from correlations in the baseline measurements. First, linear regression and logistic regression models on the baseline data were used to estimate (1) the impact of corticosteroid use on EQ-5D and EQ-VAS utility scores using a linear regression model, (2) the probability of long-term corticosteroid use based on the MGII using a logistic regression model, and (3) the impact of MGII, utility according to the EQ-5D and EQ-VAS, and corticosteroid use on overall costs using a linear regression model.
Next, these regression models were used to translate the observed short-term impact on MGII and utility scores to long-term annual outcome. The modelled total long-term impact on QALYs consisted of the directly observed short-term difference in EQ-5D utility scores, minus the long-term probability of corticosteroid use (as predicted by the logistic regression) times the disutility due to corticosteroid use (as predicted by the linear regression model for utility). The modelled total long-term impact on societal costs consisted of the medication costs, plus other healthcare and non-healthcare costs (as predicted by the linear regression models for costs and the logistic regression for corticosteroid use). Finally, the differences in QALYs and costs were combined using net-benefit analysis. The willingness-to-pay (WTP) among MG patients was based on the recommendations of the National Health Care Institute with a value of €20,000 per QALY for conditions with limited burden of disease.[162] Cost-effectiveness acceptability curves (CEACs) were used to estimate the probability that pyridostigmine is cost-effective compared to placebo. Overall sampling uncertainty was incorporated using non-parametric bootstrapping, taking 1000 resamples and applying the entire process of regression models and calculations to each sample. We used a bias-corrected and accelerated approach to calculate confidence intervals. Two sensitivity analyses were performed: (1) applying a healthcare instead of societal perspective and (2) using QALYs estimated from the EQ-VAS instead of the EQ-5D (thus taking the patients' perspective on health instead of the societal perspective).

Statistical analysis

Based on previous data with similar populations of MG patients,[156,163,164] a sample size of 17 participants was expected to have 90% power to detect a difference of 8 points on the MGII with a two-sided p value of 0.05. We assumed the standard deviation of repeated measurements of MGII between patients to be 13 and the intraparticipant correlation to be 0.7.[156,163,164] Allowing for a 10% drop out rate, we aimed to include 19 patients.
No interim statistical analyses were conducted, and no adaptations to the study design were planned or performed. Descriptive statistics were used to summarize baseline characteristics, presented as means and standard deviations (SDs) or medians and interquartile ranges (IQRs). Primary analysis was performed according to the intention-to-treat principle. A mixed-effects linear regression model was used to analyze repeated measures and estimate least-square mean differences between pyridostigmine and placebo, with treatment and treatment period (categorical) as fixed factors and patients as a random effect. The same

model was applied to secondary and exploratory outcomes. Statistical significance was determined by a p-value less than 0.05.
All statistical analyses were predefined in the study protocol. No data were missing for the primary outcome analyses and no data imputation was performed to account for missing data. All statistical analyses were conducted using RStudio (version 4.4.0).

Results

Fifty-five patients were screened for eligibility between March 22, 2023, and Feb 21, 2024, of whom 18 patients did not meet eligibility criteria, and 16 patients declined participation. Two patients failed to complete the washout period prior to randomization and were therefore excluded from participation in part one. Nineteen patients were enrolled and randomly assigned to receive either first pyridostigmine and then placebo (n=10) or vice versa (n=9); **Figure 4.1**. Two participants resumed pretrial pyridostigmine treatment prematurely on day 10 since randomization (in which the treatment was placebo) due to an intolerable increase in MG symptoms. In both patients, the primary and secondary outcome measures were determined before the pretrial medication was resumed. All 19 randomly assigned participants were included in the primary and secondary analyses.
Patient demographics and baseline characteristics are summarized in **Table 4.1**. Patients predominantly had mild to moderate generalized MG at baseline, with a small proportion of patients presenting with ocular symptoms only, according to the MGFA disease classification criteria.
Healthcare costs and non-healthcare costs in the year before inclusion are summarized in **Supplementary Table 4.1** and **Supplementary Table 4.2**, respectively. The interaction between corticosteroid use and the MGII score at baseline is shown in **Supplementary Figure 4.1**.

Table 4.2 provides the least square mean values during placebo treatment, and during pyridostigmine treatment, as well as the placebo-pyridostigmine treatment difference, for the primary and secondary outcome variables. The mean treatment difference in MGII total score between pyridostigmine and placebo (primary endpoint) was -5.3 points (95% CI -8.7, -1.9); p = 0.004). The least square mean values for the MGII total score were 32.0 (95% CI 24.9-39.2) with placebo, and 26.7 (95% CI 19.6-33.9) with pyridostigmine. Statistically significant differences were also observed across all secondary efficacy outcome measures, including MGII ocular, MGII generalized, QMG, and MG-ADL, quality of life (MG-QoL15r), and treatment satisfaction (TSQM-9). There was no evidence of a treatment-by-period interaction (**Supplementary Table 4.3**).

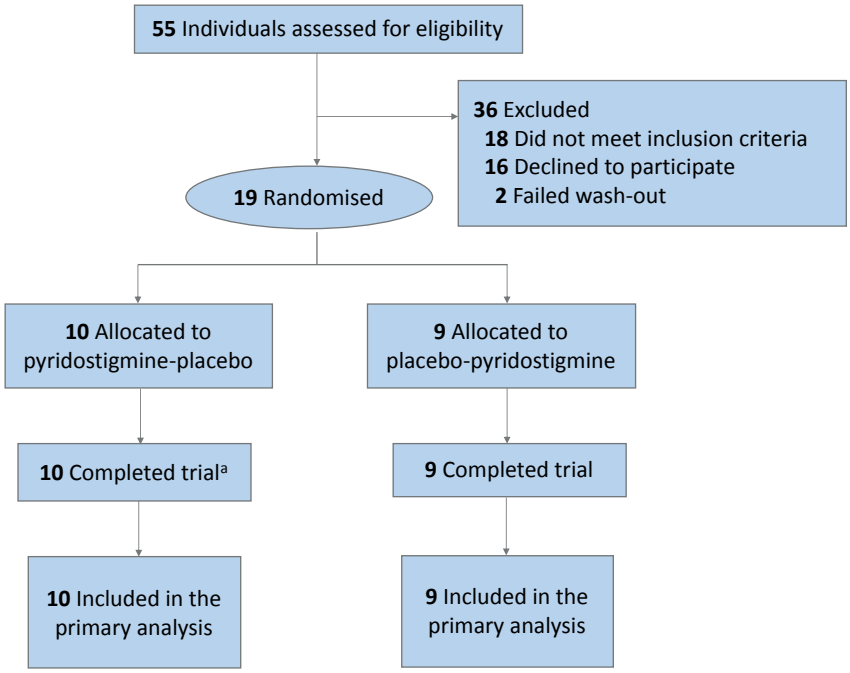


Figure 4.1 Flow diagram
[a] In two patients, primary and secondary outcome measures were obtained prematurely due to an intolerable increase in MG symptoms during the placebo treatment week.

Figure 4.2 shows the absolute values for each participant at baseline and during treatment with pyridostigmine and placebo for the MGII and EQ-5D utility score. **Supplementary Figure 4.2** presents the corresponding values for the remaining secondary outcome measures. Treatment adherence was excellent with 99% compliance with dosing (doses taken per doses scheduled) during both pyridostigmine and placebo treatment.

The number of participants who correctly guessed their treatment allocation in the preceding period was 13 (68%) during pyridostigmine treatment and 16 (84%) during placebo treatment. The investigators correctly guessed the treatment allocation in 14 participants (74%) during pyridostigmine treatment and in 17 participants (89%) during placebo treatment.
Side effects are shown in **Table 4.3**. Twelve participants (63%) reported at least one side effect during treatment with pyridostigmine, compared to 7 participants (37%) during treatment with placebo. No serious adverse events occurred in any patients during the

study. Pulse rates were lower during treatment with pyridostigmine (least square mean 70.4 [95% CI 65.3-75.5]) compared with placebo (least square mean 74.6 [95% CI 69.5-79.7], p=0.003). There was no statistically significant difference between pyridostigmine and placebo in blood pressure and respiratory rate. Results of laboratory testing and serum concentrations of pyridostigmine are shown in **Supplementary Table 4.4** and **4.5**, respectively.

Table 4.1 Baseline characteristics

	Overall, N = 19	**Pyridostigmine then placebo, N = 10**	**Placebo then pyridostigmine, N = 9**
Age, years	59 (39, 69)	60 (43, 64)	58 (36, 69)
Age at onset, years	49 (26, 60)	37 (19, 57)	53 (36, 65)
Sex, female	11 (58%)	8 (80%)	3 (33%)
BMI, kg/m2	28.2 (25.9, 28.7)	28.3 (26.5, 28.6)	26.4 (25.5, 28.7)
Disease duration, years	6 (2, 16)	13 (6, 23)	4 (2, 5)
Previous thymectomy, yes	7 (37%)	4 (40%)	3 (33%)
MGFA score at inclusion			
I	3 (16%)	2 (20%)	1 (11%)
II	14 (74%)	6 (60%)	8 (89%)
III	2 (11%)	2 (20%)	0 (0%)
Total daily dose pyridostigmine (mg)	240 (190, 300)	240 (185, 300)	250 (240, 300)
Baseline scores			
MGII total	26 (23, 36)	27 (25, 37)	24 (22, 32)
QMG	11 (9, 15)	12 (9, 15)	10 (7, 14)
MG-ADL	6 (4, 9)	7 (5, 10)	4 (3, 9)
MG-QoL15r	12 (9, 15)	13 (10, 16)	10 (9, 15)
Economic characteristics			
Utility, EQ-5D	0.77 (0.65, 0.86)	0.79 (0.62, 0.83)	0.77 (0.69, 0.91)
Utility, EQ-VAS	0.82 (0.69, 0.90)	0.80 (0.68, 0.88)	0.84 (0.77, 0.90)
Therapies in previous year			
No therapy	9 (47%)	4 (40%)	5 (56%)
Prednisolone	7 (37%)	4 (40%)	3 (33%)
Azathioprine	2 (11%)	2 (20%)	0 (0%)
Mycophenolate mofetil	4 (21%)	2 (20%)	2 (22%)
Cyclosporine	1 (5.3%)	0 (0%)	1 (11%)
Methotrexate	1 (5.3%)	1 (10%)	0 (0%)
IVIg on a regular basis	1 (5.3%)	1 (10%)	0 (0%)

Data are median (Q1-Q3) or n (%).
BMI, Body Mass Index; MGFA, Myasthenia Gravis Foundation of America; MGII, Myasthenia Gravis Impairment Index; QMG, Quantitative Myasthenia Gravis; MG-ADL, Myasthenia Gravis-Activities of Daily Living; MG-QoL15r, revised 15-item Myasthenia Gravis Quality of Life; EQ-5D, EuroQoL-5 dimensions; EQ-VAS, EuroQoL-visual analogue scale; IVIg, intravenous immunoglobulin therapy

Table 4.2 Treatment difference on primary and secondary outcome variables

	Pyridostigmine (95% CI)[a]	Placebo (95% CI)[a]	Difference (95% CI)[a]	P-value
Primary outcome				
MGII total	26.7 (19.6, 33.9)	32.0 (24.9, 39.2)	-5.3 (-8.7, -1.9)	0.004
Secondary outcomes				
MGII ocular	10.0 (6.9, 13.2)	12.4 (9.3, 15.5)	-2.4 (-4.2, -0.6)	0.012
MGII generalized	16.7 (10.7, 22.6)	19.6 (13.6, 25.5)	-2.9 (-5.4, -0.5)	0.023
QMG	10.5 (7.8, 13.2)	11.9 (9.2, 14.6)	-1.4 (-2.3, -0.5)	0.005
MG-ADL	5.9 (4.2, 7.6)	7.0 (5.4, 8.7)	-1.2 (-1.8, -0.5)	0.001
MG-QoL15r	11.4 (8.4, 14.4)	13.4 (10.3, 16.4)	-2.0 (-3.9, 0.0)	0.047
TSQM-9	35.8 (32.4, 39.1)	28.5 (25.2, 31.9)	7.2 (2.3, 12.2)	0.006

[a] Values are least square means from a mixed effects linear model that includes a fixed effect for treatment period and a random effect for participant. CI, confidence interval; MGII, Myasthenia Gravis Impairment Index; QMG, Quantitative Myasthenia Gravis; MG-ADL, Myasthenia Gravis-Activities of Daily Living; MG-QoL15r, revised 15-item Myasthenia Gravis Quality of Life; TSQM-9, 9-item Treatment Satisfaction Questionnaire for Medication

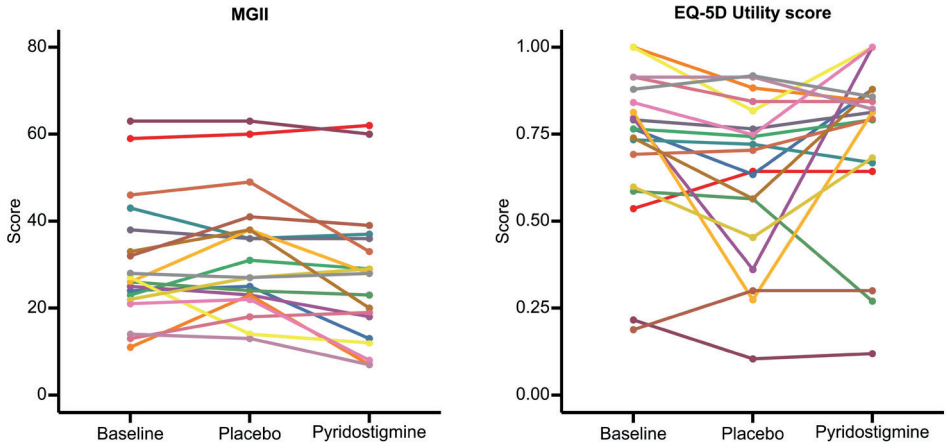


Figure 4.2 Absolute values for each participant at baseline and during treatment with pyridostigmine and placebo for the MGII and EQ-5D utility score
MGII, Myasthenia Gravis Impairment Index; EQ-5D, EuroQoL-5 dimensions.

Table 4.3 Summary of side effects in all patients

	Pyridostigmine	Placebo
Number of reported side effects	68	36
Patients having at least one side effect	12 (63%)	7 (37%)
Flatulence	7 (37%)	2 (11%)
Fatigue	6 (32%)	6 (32%)
Increased lacrimation	5 (26%)	3 (16%)
Urinary urgency	5 (26%)	2 (11%)
Increased salivation	5 (26%)	2 (11%)
Muscle weakness	4 (21%)	5 (26%)
Diarrhea	4 (21%)	1 (5%)
Muscle twitching	4 (21%)	1 (5%)
Light-headedness	3 (16%)	2 (11%)
Muscle cramps	3 (16%)	1 (5%)
Hot flushes	3 (16%)	0 (0%)
Flu-like symptoms	3 (16%)	0 (0%)
Nausea	2 (11%)	2 (11%)
Headache	2 (11%)	1 (5%)
Blushing	2 (11%)	1 (5%)
Abdominal cramps	2 (11%)	0 (0%)
Stomach ache	2 (11%)	0 (0%)
Increased bronchial secretion	1 (5%)	2 (11%)
Heartburn	1 (5%)	1 (5%)
Hyperhidrosis	1 (5%)	1 (5%)
Blurred vision	1 (5%)	1 (5%)
Irregular heartbeat	1 (5%)	1 (5%)
Slow heartbeat	1 (5%)	0 (0%)
Tremor	0 (0%)	1 (5%)

Data are number of patients (%) with one or more side effect. Excessive belching, vomiting, chest pain, skin rash, hives, and fainting were queried but not reported in either treatment period.

Table 4.4 provides the mean health economic outcomes during treatment with and without pyridostigmine, as well as the treatment difference. The cost-utility analysis showed lower annual societal costs (-€6565 [95% CI -€15945-€328]) with pyridostigmine treatment. Treatment with pyridostigmine resulted in more EQ-5D QALYs than without pyridostigmine (mean difference 0.106 [95% CI 0.019-0.210]). The cost-effectiveness plane showed that the majority of bootstrap points were located in the lower right quadrant (95.2%), indicating that treatment with pyridostigmine offers greater benefits at lower costs than treatment without pyridostigmine. The CEAC demonstrated a high probability (at least 99%) of pyridostigmine being cost-effective compared to placebo, at the €20000 per QALY threshold and higher (**Figure 4.3**). Sensitivity analyses resulted in different point estimates without altering the overall conclusion. In particular, the CEAC showed almost indistinguishable curves (**Supplementary Figure 4.3**).

Table 4.4 Estimated annual health and economic outcomes of treatment with and without pyridostigmine

	With pyridostigmine	Without pyridostigmine	Difference (95% CI)
QALYs based on EQ-5D	0.735	0.629	0.106 (0.019, 0.210)
QALYs based on EQ-VAS	0.761	0.690	0.071 (0.029, 0.125)
Long-term prednisolone use (%)	20.8	22.2	-1.4 (-13.8, 4.2)
Annual prednisolone costs (€)	7	8	-1 (-10, 2)
Annual pyridostigmine costs (€)	341	0	341 (283, 389)
Other annual healthcare costs (€)	6864	11398	-3821 (-12463, -395)
Total annual non-healthcare costs (€)	4662	7032	-1998 (-6987, 1389)
Total annual societal costs (€)	11871	18436	-6565 (-15945, 328)

Outcomes are based on non-parametric bootstrapping with 1000 replications.
QALYs, quality-adjusted life years; EQ-5D, EuroQoL-5 dimensions; EQ-VAS, EuroQoL-visual analogue scale

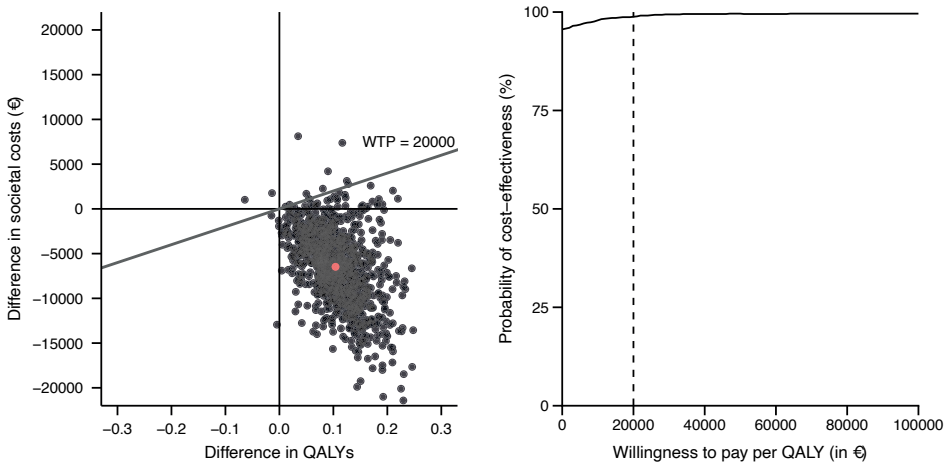


Figure 4.3 Cost−effectiveness results for the outcome of QALY, based on the societal perspective
Cost-effectiveness plane (left) and cost-effectiveness acceptability curve (right) of difference in QALYs derived from EQ-5D versus societal costs. Outcomes are based on non-parametric bootstrapping with 1000 replications.
QALYs, quality-adjusted life years; EQ-5D, EuroQoL-5 dimensions; EQ-VAS, EuroQoL-visual analogue scale; WTP, willingness to pay

Discussion

In IMPACT-MG, a randomized, double-blind, placebo-controlled crossover trial in patients with AChR myasthenia gravis chronically using pyridostigmine, we demonstrated that pyridostigmine was associated with improvements in the primary outcome measure (MGII -5.3 points) and all secondary outcome measures (MGII ocular -2.4 points, MGII generalized

-2.9 points, QMG -1.4 points, MG-ADL -1.2 points, MG-QoL15r -2 points, and TSQM-9 7.2 points) when compared to placebo. Adverse events were reported more frequently during the use of pyridostigmine in our study, with fatigue and flatulence being the most commonly reported. In the cost-effectiveness analysis, treatment with pyridostigmine was less costly and more effective than treatment without pyridostigmine. It showed a high probability of being cost-effective at a QALY threshold of €20,000, both from a societal and a healthcare perspective.

The observed mean difference for the primary outcome measure, the MGII, was below the predefined threshold for a clinically meaningful change. However, the 95% confidence interval included values that exceed this threshold, indicating that a clinically meaningful difference cannot be excluded. For the secondary efficacy outcomes, the QMG and the MG-ADL, pyridostigmine was also associated with statistically significant improvements. In contrast to the MGII, the predefined thresholds for clinically meaningful change on these outcomes fell outside the corresponding 95% confidence intervals. For the MG-ADL this may be (in part) explained by its well-known floor effect.[148] As many patients already had relatively low baseline scores, the potential for a measurable improvement was limited. This was one of the reasons why we selected the MGII as the primary outcome measure rather than the more commonly used MG-ADL. In addition, when interpreting whether the observed statistically significant differences are also clinically meaningful, it is important to consider that the thresholds for clinically meaningful improvement depend on the clinical and treatment context in which they were developed. For the MGII and QMG, these thresholds were developed in populations receiving treatments such as prednisone, IVIg, and PLEX,[156,165] which are more invasive and often associated with more severe adverse effects than pyridostigmine. It is likely that patients weigh the risks and side effects of these treatments when judging the minimal size of a beneficial effect. Consequently, a clinically meaningful improvement with a symptomatic treatment like pyridostigmine would be expected to be smaller than with these more invasive treatments. This suggests that cut-off values for a clinically meaningful improvement, developed in the context of immunomodulating therapies, may not be appropriate in trials investigating symptomatic medications. When comparing the results of our trial to other symptomatic treatments, such as the adrenergic agonist ephedrine or the ClC-1 inhibitor NMD670, the improvements demonstrated in these trials show similar effect sizes of respectively 1.0 and 1.5 points on the mean QMG score, comparable to 1.4 points in our study.[31,166] A study using terbutaline reports that 5 out of 8 patients had an improvement of more than 3 points on the QMG, while the other 3 patients had larger or unchanged QMG scores. This study did not provide the mean group values.[112]

One of the main strengths of our study is that, in addition to efficacy outcome measures, we included an analysis of the cost-effectiveness of pyridostigmine. Although pyridostigmine has been widely used for many years, assessing its cost-effectiveness remains particularly

important in an era of rising healthcare costs. While its cost-effectiveness cannot be directly compared to the effect of novel treatments, our data can serve as a benchmark for evaluating the cost-effectiveness of newer therapies. Moving forward, it is essential to include cost-effectiveness data when reporting efficacy outcomes for novel treatments, as this will provide a more comprehensive perspective on their value. Other strengths of our trial include the robust study design, consisting of a randomized, double-blinded, placebo-controlled crossover study, and the use of a wide range of both patient-reported and investigator-reported outcome measures, which effectively capture the impact of MG on patients and enable meaningful translation of results into clinical practice. Other strengths include the absence of drop-outs and the completeness of data for primary (100%) and secondary outcome measures (>99%).

Our study has some limitations. We only included patients who were currently using pyridostigmine, excluding those who had discontinued the medication or were treatment-naïve. Additionally, patients who could not tolerate the two-day washout period due to worsening of MG symptoms were excluded. Ideally, we would have selected recently diagnosed, untreated MG patients, however, this was not feasible, as identifying such patients would likely have required a multicenter, possibly multinational, trial, and it would have been ethically unacceptable to withhold treatment from untreated patients, even briefly. Although tapering pyridostigmine is common once patients are stable, some participants may not have required pyridostigmine during the trial. These factors should be considered when extrapolating our findings on efficacy to the general MG population.

The same considerations apply when evaluating tolerability. By including only patients currently using pyridostigmine, we may have selected patients with relatively few side effects, potentially leading to an overestimation of its tolerability. However, the trial data align well with a previous cross-sectional study in 410 MG patients on the effectiveness and side effects of pyridostigmine.[79]

Another limitation is the short treatment duration, which was based on the ethical decision that withholding ongoing pyridostigmine treatment from patients for a longer period was undesirable. Furthermore, a five-day treatment period was considered sufficient to adequately demonstrate the efficacy of pyridostigmine on MG symptoms compared to placebo, due to its rapid onset of action, but may have limited the likelihood of detecting infrequent adverse events. Most patients and investigators correctly identified the treatment allocation under blind conditions, potentially due to either a clear therapeutic effect or noticeable side effects. This may have introduced bias into the trial due to partial unblinding.

Several limitations related to the cost-effectiveness analysis should be noted. First, the cost-effectiveness analysis was not pre-specified as an outcome measure for this part of the study. Second, results may not be representative for other non-Dutch healthcare settings. Finally, the follow-up period was too short to directly observe the impact on healthcare use

and costs. Instead, these were extrapolated using cross-sectional baseline models, in which the estimated relationships do not need to be causal. For example, we predicted the use and impact of corticosteroid use from our baseline data, assuming that the observed cross-sectional relationships between the efficacy outcome measures and corticosteroid use were causal. In real-life, this relation is probably more complex. More accurate estimates for corticosteroid use could have been obtained from other larger data sets, however, for internal consistency, we chose to estimate all model components from our own trial data. Fortunately, the results showed that corticosteroid use had very little impact on the estimated outcomes. There is no guarantee that this also holds true for other parts of the economic model.

Conclusion

Our study demonstrated that treatment with pyridostigmine was associated with statistically significant improvements on efficacy scores, quality of life, and treatment satisfaction in patients with AChR MG chronically using pyridostigmine. In addition, pyridostigmine had a very high probability of being cost-effective at established willingness-to-pay thresholds, both from a societal and a healthcare perspective. The results from this study help define the current therapeutic landscape for patients with MG and the positioning of novel treatments with respect to finding a balance between the effectiveness, tolerability, and costs of treatment.

Supplements

Inclusion and exclusion criteria

Inclusion criteria

1. Age >18 years
2. AChR positive myasthenia gravis (ocular or generalized)
3. Current use of pyridostigmine
4. MGFA Clinical Classification I-IV
5. Receiving a stable dose of MG treatment (other than pyridostigmine). If applicable:
   a. A stable steroid regimen for 1 month
   b. Nonsteroidal immunosuppressants:
      i. Azathioprine, mycophenolate mofetil, cyclosporine or other nonsteroid immunosuppressive agents start > 3 months ago and a stable regimen for 1 month.
      ii. Rituximab start > 6 months ago, complement inhibitors and Fc receptor inhibitors start > 6 months ago and a stable regimen for 3 months.

Exclusion criteria

1. Use of intravenous immunoglobulin or plasma exchange <4 weeks or planned during the trial.
2. Thymectomy < 6 months, or thymectomy (expected) to take place during the trial
3. Use of other AChE inhibitors than pyridostigmine
4. Pregnancy, lactation or intention to become pregnant during the study
5. The patient is unable to fill out the study questionnaires or be interviewed in Dutch, or is unable to undergo the tests needed for the study, or is unable to give informed consent for participation in the study.
6. The investigator can exclude patients for this trial which are deemed not suitable for any reason.

Full list of pyridostigmine side effects queried

Abdominal cramps, stomach ache, nausea, vomiting, diarrhea, flatulence, heartburn, excessive belching, urinary urgency, headache, increased salivation, increased lacrimation, hyperhidrosis, blurred vision, increased bronchial secretion, irregular heartbeat, slow heartbeat, chest pain, light-headedness, fainting, fatigue, blushing, hot flashes, flu-like symptoms (chills, runny nose, coughing up phlegm), tremor, muscle cramps, muscle weakness, muscle twitching, skin rash and hives.

Supplementary Table 4.1 Healthcare costs in the year before inclusion, averaged for those with the costs (n) and averaged over all patients (N)

Unit	n/N	Average costs (€) per patient (n)	Average costs (€) per patient (N)
General practitioner	16/19	88	74
Practice nurse	16/19	32	27
Social worker	19/19	159	159
Physiotherapist	19/19	351	351
Occupational Therapist	19/19	45	45
Speech Therapist	19/19	4	4
Dietitian	19/19	22	22
Psychologist/Psychiatrist	19/19	170	170
Occupational Health Physician	19/19	18	18
Home care			
Household help	3/19	12708	2007
Personal care	1/19	9995	526
Nursing	0/19	0	0
Informal caregiving			
Household help	3/19	1427	225
Personal care	0/19	0	0
Practical assistance	5/19	6918	1821
Emergency department visits	19/19	49	49
Ambulance	0/19	0	0
Outpatient clinic	11/19	796	461
Day treatment	4/19	884	186
Rehabilitation centre	4/19	6191	1303
Hospital admission	1/19	3532	186
Total costs		43389	7634

Supplementary Table 4.2 Non-healthcare in the year before inclusion, averaged for those with the costs (n) and averaged over all patients (N)

Unit	n/N	Average costs (€) per patient (n)	Average costs (€) per patient (N)
Absenteeism	2/19	4779	503
Presenteeism	4/19	4327	911
Productivity losses in the context of unpaid work	7/19	11797	4346
Total costs		**20903**	**5760**

Supplementary Table 4.3 Treatment by period interaction for primary and secondary outcome measures

	Estimate (95% CI)	P-value
Primary outcome		
MGII total	-13.4 (-41.6, 14.7)	0.328
Secondary outcomes		
MGII ocular	-6.2 (-18.3, 5.9)	0.297
MGII generalized	-7.3 (-31.2, 16.7)	0.531
QMG	-0.2 (-11.3, 11)	0.975
MG-ADL	-3.7 (-10.3, 2.9)	0.256
MG-QoL15r	-5.4 (-17.1, 6.2)	0.341
TSQM-9	4.1 (-5.4, 13.7)	0.383

CI, confidence interval; MGII, Myasthenia Gravis Impairment Index; QMG, Quantitative Myasthenia Gravis; MG-ADL, Myasthenia Gravis-Activities of Daily Living; MG-QoL15r, revised 15-item Myasthenia Gravis Quality of Life; TSQM-9, 9-item Treatment Satisfaction Questionnaire for Medication

Supplementary Table 4.4 Laboratory results

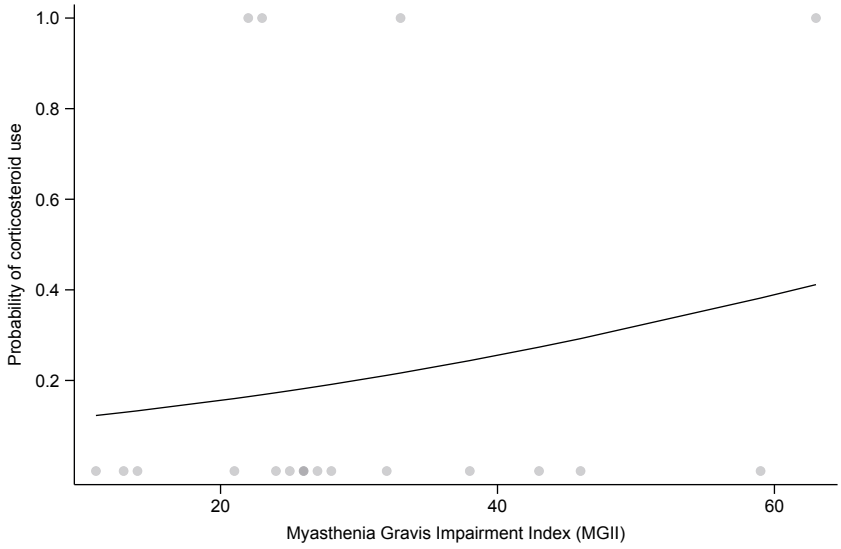
	Baseline N = 19	**Visit 3 N = 19**
Hemoglobin	8.8 (8.3, 9.6)	8.6 (8.2, 9.2)
Unknown	1	0
Hematocrite	0.4 (0.4, 0.5)	0.4 (0.4, 0.4)
Unknown	1	0
Leukocytes	6.6 (5.7, 9.6)	6.6 (5.6, 8.0)
Unknown	1	0
Thrombocytes	244.5 (211.0, 301.8)	243.0 (205.0, 297.5)
Unknown	1	0
Sodium	141.0 (140.0, 143.5)	140.0 (138.0, 142.0)
Potassium	4.6 (4.5, 4.9)	4.3 (4.2, 4.6)
Creatinine	61.0 (56.0, 78.0)	64.0 (55.0, 76.0)
eGFR	90.0 (90.0, 90.0)	90.0 (89.0, 90.0)
Aspartate aminotransferase (ASAT)	22.0 (19.5, 27.0)	21.0 (18.0, 25.0)
Alanine aminotransferase (ALAT)	24.0 (15.5, 26.5)	21.0 (16.5, 30.5)
Alkaline phosphatase	70.0 (63.5, 81.5)	73.0 (61.0, 80.0)
Gamma glutamyltransferase	13.0 (11.0, 23.5)	13.0 (11.0, 22.0)
Creatinine kinase	80.0 (60.5, 103.5)	76.0 (73.0, 122.5)
Glucose	5.0 (4.9, 5.9)	5.4 (5.0, 5.9)
AChR antibodies	9.3 (3.0, 15.7)	10.2 (3.0, 16.2)

Data are median [Q1-Q3]

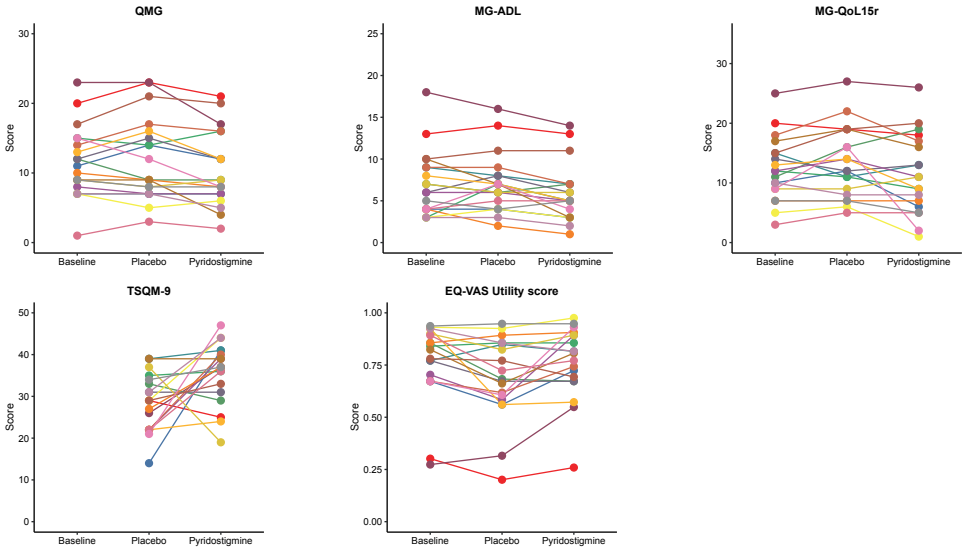
Supplementary Table 4.5 Serum pyridostigmine concentrations

	Baseline N = 19	**Placebo N = 19**	**Pyridostigmine N = 19**
Pyridostigmine concentrations	0.0 (0.0, 1.4)	0.0 (0.0, 0.0)	28.6 (17.9, 40.4)
Unknown	0	1	1

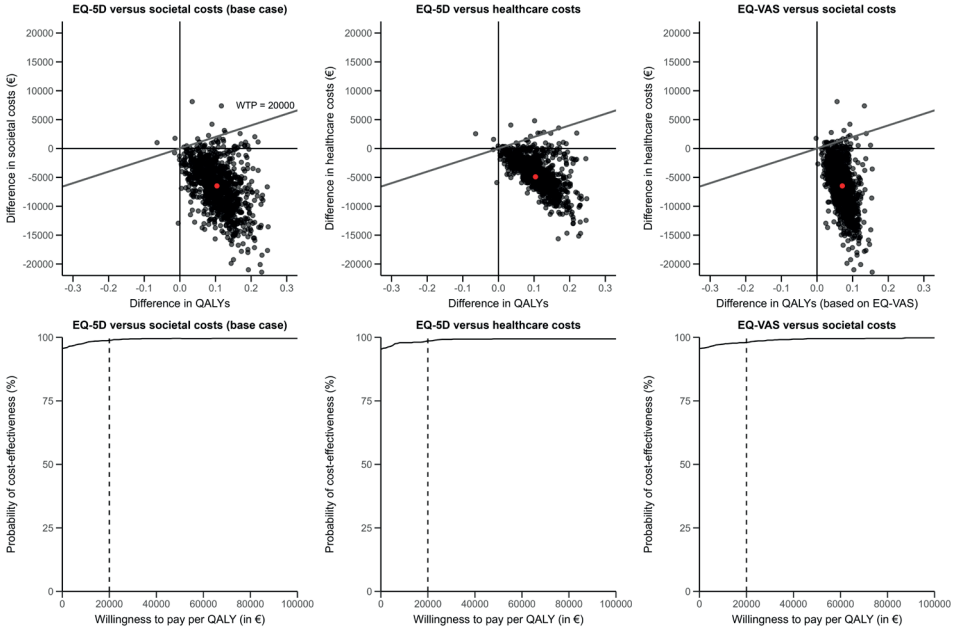
Data are median [Q1-Q3]



Supplementary Figure 4.1 Interaction between corticosteroid use and Myasthenia Gravis Impairment Index at baseline (MGII)
Derived from binary logistic regression analysis.



Supplementary Figure 4.2 Absolute values for each participant at baseline and during treatment with pyridostigmine and placebo for additional secondary outcome measures
QMG, Quantitative Myasthenia Gravis; MG-ADL, Myasthenia Gravis-Activities of Daily Living; MG-QoL15r, revised 15-item Myasthenia Gravis Quality of Life; TSQM-9, 9-item Treatment Satisfaction Questionnaire for Medication; EQ-VAS, EuroQoL-visual analogue scale



Supplementary Figure 4.3 Sensitivity analysis of cost-effectiveness analysis
Top row: cost-effectiveness plane of difference in (a) QALYs derived from EQ-5D versus societal costs (base case) b) QALYs derived from EQ-5D versus healthcare costs, and (c) QALYs derived from EQ-VAS versus societal costs. Bottom row: cost-effectiveness acceptability curves for a) QALYs derived from EQ-5D versus societal costs (base case), (b) QALYs derived from EQ-5D versus healthcare costs, and (c) QALYs derived from EQ-VAS versus societal costs.
QALYs, quality-adjusted life years; EQ-5D, EuroQoL-5 dimensions; EQ-VAS, EuroQoL-visual analogue scale

CHAPTER

5

Efficacy and safety of amifampridine

Efficacy and safety of amifampridine in myasthenia gravis:
a randomized, double-blind, placebo-controlled crossover trial
Neurology, 2026, 106(8)

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Abstract

Background and objectives

Amifampridine, a short-acting potassium-channel blocker, is the first-line treatment for Lambert-Eaton myasthenic syndrome. Its benefit as add-on to pyridostigmine in acetylcholine receptor (AChR) positive myasthenia gravis (MG) has been proposed, but no randomized controlled trials have been conducted. We aimed to evaluate the efficacy and safety of amifampridine in AChR-MG with insufficient symptom control on pyridostigmine.

Methods

A randomized, double-blind, placebo-controlled crossover trial was conducted at Leiden University Medical Center, a tertiary center for the treatment of MG in the Netherlands, as part two of the IMPACT-MG trial. Eligible patients had either completed part one (pyridostigmine vs. placebo) or failed the initial two-day pyridostigmine washout, and had an MGII score >10. Participants were randomized (1:1:1) to one of three treatment sequences. Each treatment period lasted 5 days, with a 2-day washout between treatments, during which participants received either 30 mg or 60 mg of amifampridine modified release, or placebo. The primary outcome was the difference in MGII score between the two amifampridine dosages and placebo. Pharmacokinetics, safety and tolerability were assessed throughout the trial.

Results

Between March 2023 and April 2024, twenty patients were enrolled (median age 60 years, 55% female). For the MGII score, the estimated mean differences were -1.0 (95% CI -4.1-2.0, p=0.681) for amifampridine 30 mg versus placebo and -1.7 (95% CI -4.7-1.4, p=0.379) for amifampridine 60 mg versus placebo. No statistically significant association was observed between pharmacokinetic parameters and the MGII. No serious adverse events were reported. Adverse events were more common with amifampridine 30 mg (12 patients [60%]), and 60 mg (15 patients [75%]), compared to placebo (6 patients [30%]), with paresthesia, fatigue, numbness, dizziness, and sleep disturbances most frequently reported. Three patients discontinued amifampridine early due to adverse events.

Discussion

The addition of amifampridine to pyridostigmine was not superior to treatment with pyridostigmine alone and was associated with a higher incidence of adverse events.

Introduction

Myasthenia gravis (MG) is a chronic autoimmune neuromuscular disorder characterized by fluctuating muscle weakness due to impaired signal transmission at the neuromuscular junction.

Most commonly, autoantibodies target the acetylcholine receptor (AChR), while in rarer cases, antibodies against other neuromuscular junction structures are found leading to different clinical features and in some cases, a different response to treatment.

Clinical management includes either symptomatic drugs, which directly improve muscle strength, or immunomodulating drugs, which modify immune system activity. Symptomatic treatment is universally considered to be the first step in the treatment of MG. Patients who do not have a satisfactory functional result with symptomatic treatment alone, generally start using immunosuppressive treatment consisting of corticosteroids often in combination with nonsteroidal immunosuppressive treatment.[167]

Symptomatic drugs offer several advantages over immunomodulatory therapies. A key advantage is their ability to improve muscle strength within minutes, whereas immunomodulatory agents typically require weeks to months to demonstrate effects. Additionally, immunomodulatory treatments and in particular corticosteroids, are associated with significant long-term side effects that can severely impact health-related quality of life.[168] Although significant progress has been made in developing novel immunomodulatory agents, their widespread clinical application is limited by high costs and insufficient long-term safety data. These limitations underscore the continued importance of symptomatic treatment in the management of MG.

Pyridostigmine has emerged as the preferred symptomatic drug in MG treatment [169]. However, for the majority of patients, pyridostigmine monotherapy is insufficient; currently, 69% of MG patients require immunosuppressant medication.[86] Other symptomatic treatment options are limited and there remains an unmet need for more effective symptomatic treatments for MG that can delay or reduce the need for immunosuppressive treatment. In patients with a chronic, lifelong autoimmune disease, even a relatively modest clinical benefit would have a substantial impact on long term side-effects and consequently, on quality of life.

Amifampridine, also known as 3,4-diaminopyridine, is a well-known symptomatic treatment for other diseases of the neuromuscular junction such as Lambert Eaton myasthenic syndrome (LEMS),[170] and certain subtypes of congenital myasthenic syndromes.[171] It is a short-acting potassium channel blocker, which blocks potassium efflux presynaptically. This results in a prolonged action potential of the presynaptic nerve terminal, which enhances the release of acetylcholine into the synaptic cleft by an increased calcium influx into the nerve terminal.[172,173] In the Netherlands, amifampridine base modified release (amifampridine

MR) has been used clinically since 1989 and it is the formulation most frequently used in Dutch LEMS patients. Higher peak concentrations are associated with adverse effects and it is hypothesized that amifampridine MR leads to lower peak concentrations than the immediate release amifampridine phosphate salt authorized for the treatment of LEMS. The use of amifampridine as an add-on to pyridostigmine in MG has been primarily described in patients with antibodies against muscle-specific kinase (MuSK).[32,33] In AChR MG its use has been reported only in a limited number of observational studies.[34,99,174] To our knowledge, no randomized controlled trials have systematically evaluated the therapeutic effect of add-on amifampridine treatment in patients with AChR MG already receiving pyridostigmine. In this crossover randomized trial, we therefore aimed to assess the efficacy and safety of add-on treatment with amifampridine MR for patients with AChR MG whose symptoms are insufficiently controlled with pyridostigmine alone.

Methods

Study design and participants

IMPACT-MG was a two-part, investigator-initiated, prospective, randomized, double-blind, placebo-controlled crossover trial that evaluated the efficacy and cost-effectiveness of pyridostigmine in patients with MG (part one), followed by a second randomization to assess the efficacy and safety of amifampridine in combination with pyridostigmine (part two). Part one focused on pyridostigmine optimization and is reported separately. The current manuscript specifically presents part two of the study, designed to evaluate the efficacy and safety of amifampridine MR added to stable pyridostigmine treatment. The study was performed at Leiden University Medical Center, a tertiary center for the treatment of MG in the Netherlands.

The results of part one will be published separately. The independent Medical Ethics Review Committee Leiden The Hague Delft reviewed and approved the study protocol. The protocol was revised once, four months after the first inclusion, to extend the study with an additional observational visit after three months and after six months, to evaluate the long-term effectiveness of amifampridine.

Written informed consent was obtained from each patient before study participation. Subjects gave separate consent for participating in the pharmacokinetic/pharmacodynamic (PK/PD) substudy to provide additional blood samples.

Participants

Eligible patients were aged 18 years or older and had a documented diagnosis of ocular or generalized MG with evidence of elevated anti-acetylcholine receptor antibodies. Patients

had a Myasthenia Gravis Foundation of America (MGFA) clinical classification of class I to IV at screening, were currently using pyridostigmine and had a stable dose of other MG treatments. Patients were excluded if they had a history of thymectomy in the 6 months before screening, or thymectomy (expected) to take place during the trial, use of intravenous immunoglobulin or plasma exchange <4 weeks or planned during the trial or current use of other AChE inhibitors than pyridostigmine.
To be eligible for part two, patients were required to have either completed part one or failed to complete the two-day washout period prior to randomization in part one. In addition, patients were required to have a score of >10 points on the myasthenia gravis impairment index (MGII) questionnaire at inclusion. Patients were excluded from part two if they had contra-indications for treatment with amifampridine MR: a history of epilepsy, uncontrolled asthma, inherited QT syndrome or a prolonged QT interval (as indicated by ECG), or use of any drug known to cause QTc-prolongation or any drug known to lower the epileptic threshold. A full list of the eligibility criteria is provided in the appendix.

Randomization and masking

Eligible participants were randomized in a 1:1:1 ratio and allocated to either one of three treatment sequences as per a balanced Latin square crossover design: amifampridine MR 30 mg, amifampridine MR 60 mg, and placebo (30-60-P); amifampridine MR 60 mg, placebo, and amifampridine MR 30 mg (60-P-30); or placebo, amifampridine MR 30 mg, and amifampridine MR 60 mg (P-30-60). The Latin square design ensured that each treatment appeared once in each study period, thereby balancing potential period effects. As carry-over effects were expected to be minimal given the washout period between treatment periods and the short half-live of amifampridine, and because of the small sample size which would have complicated block randomization, we chose for this specific design.
Randomization was done with the Castor Electronic Data Capturing System (Castor EDC; Amsterdam, the Netherlands) using variable block sizes (3, 6 and 9 patients). The amifampridine MR 30 mg, amifampridine MR 60 mg and placebo tablets appeared identical and were dispensed by the hospital pharmacy and distributed in identical bottles. The study medication allocation sequence was known only to the study pharmacist, while all other study personnel and participants remained blinded until database lock. The success of blinding was evaluated by assessing both the investigator's and the patient's perception of which treatment the patient had received during the past week.

Study procedures

After obtaining informed consent, screening assessments were conducted, including the MGII and an ECG. As part of the study protocol for part one, patients were instructed to discontinue their regular use of pyridostigmine. Patients who were unable to do so were

not eligible for part one, but could directly proceed to part two if they met the in- and exclusion criteria. All other patients first participated in part one for two weeks before proceeding to part two. Upon completion of part one, patients who met the inclusion and exclusion criteria were randomized for part two on the final day of assessments in part one. Participants were randomly assigned to a sequence of three treatment periods for five days separated by a 2-day washout. Patients received two times daily 15 mg (amifampridine MR 30 mg), two times daily 30 mg (amifampridine MR 60 mg) or matching placebo, in addition to their unchanged pre-study dose of pyridostigmine. The amifampridine doses were selected based on the standard dosing used in the Netherlands in patients with LEMS, with 30 mg corresponding to a commonly used low dose and 60 mg to a commonly used high dose. Throughout the trial, patients were required to maintain a stable dose and treatment schedule of their prior pyridostigmine, oral corticosteroids, or other immunosuppressive treatments. Patients had the opportunity to consult an independent neurologist specializing in neuromuscular disorders regarding symptoms and adverse effects. To maintain blinding, this neurologist was unaware of the study medication, and researchers were not informed of any patient consultations.

For evaluation of efficacy, we assessed the following validated outcome measures: the MGII,[148] the Myasthenia Gravis-Activities of Daily Living (MG-ADL) scale,[149] the Quantitative Myasthenia Gravis (QMG) score,[150] and the revised 15-item Myasthenia Gravis Quality of Life (MG-QOL15r) questionnaire.[151] Treatment satisfaction was evaluated using the 9-item Treatment Satisfaction Questionnaire for Medication (TSQM-9).[152] Health-related quality of life was measured using the 5-level EQ-5D (EQ-5D-5L).[153] The MGII was assessed at screening, baseline (without pyridostigmine treatment), and during part two on days 5, 12 and 19. MG-ADL, QMG, MG-QOL15r, and EQ-5D-5L were scheduled at baseline and on days 5, 12 and 19. TSQM-9 was assessed at day 5, 12 and 19.

Serum concentrations of amifampridine MR and pyridostigmine were measured at each visit. In addition, an optional PK/PD substudy was conducted among a subset of patients. Blood samples of pyridostigmine and amifampridine MR were collected before amifampridine MR ingestion, at 30 and 60 minutes post-ingestion, and then hourly up to 8 hours post-ingestion.

Safety evaluations included the collection of vital signs at each visit, and an electrocardiogram (ECG) to monitor corrected QT (QTc) intervals at screening, the last day of part one (or at baseline for patients only participating in part two of the study), days 5, 12 and 19. Patients were actively queried for adverse events during each visit and their severity was graded. Additionally, side effects were evaluated using a questionnaire that was previously developed to assess the side effects of pyridostigmine in a cross-sectional study.[79] For amifampridine MR, the questionnaire was adapted by compiling a list of 21 potential side effects based on the patient information leaflet of amifampridine MR and the European Public Assessment

Report (EPAR) for amifampridine immediate release phosphate. A full list of the side effects queried are in the appendix. Laboratory testing at baseline and day 19 included a blood cell count, assessment of electrolytes, renal function, liver enzymes, creatine kinase and AChR antibody titers.

After the final study visit, patients were given the option to continue using amifampridine MR in an unblinded manner at a dose of their choosing. For those patients, further assessments were conducted at three and six months after the last visit to evaluate long-term efficacy and potential side effects. An overview of the study design, including the first part of the trial, is provided in **Figure 5.1**.

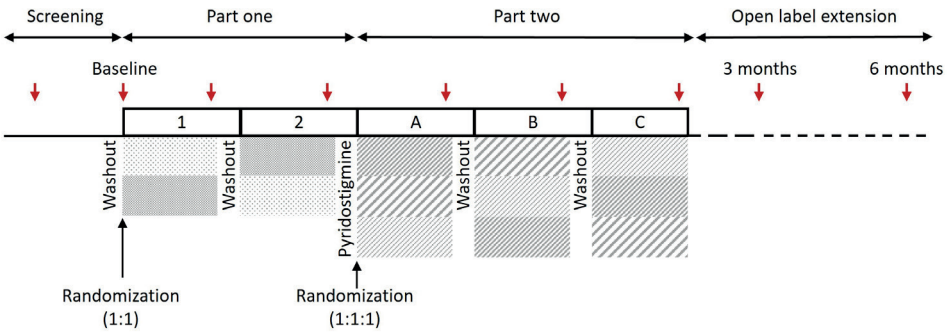


Figure 5.1 Outline of the study design
The study consisted of a screening phase, part one (pyridostigmine vs. placebo, published separately), part two, and an open-label extension. Patients unable to complete the initial washout during screening were ineligible for part one but could proceed directly to part two if inclusion and exclusion criteria were met. All other patients first completed part one (2 weeks) before entering part two. In part two, participants were randomized (1:1:1; Latin square design) to one of three treatment sequences (amifampridine 30 mg, 60 mg, and placebo), each administered double-blind for 5 consecutive days, separated by 2-day washout periods. Assessments were performed on day 5 of each treatment period. Following completion of part two, patients continuing amifampridine were followed up at 3 months (by phone) and 6 months.

Outcome measures

Both in part one and part two, the primary outcome was change in MGII total score compared to placebo. The MGII is a validated score and includes 22 patient-reported items and 6 items assessed through examination. Total scores range from 0 to 84, with higher scores reflecting greater severity of impairment. The MGII can be subdivided into ocular (8 items) and generalized (20 items) subscores. A change of ≥8 is considered a clinically meaningful improvement.[156] The MGII was chosen as the primary outcome measure because it provides a broader range of scores and has less floor effect compared to other commonly used outcome measures.[148] Prespecified secondary efficacy endpoints were changes on the MG-ADL, QMG, MG-QoL15r, and TSQM-9 compared to placebo. A clinically meaningful

improvement was defined as a 2-point change in the MG-ADL score and a 3-point change in the QMG score. Trial medication compliance was documented and compared between trial medication and placebo treatment.
In the PK/PD substudy, PK parameters were maximum concentration (C_{max}); area under the concentration-time curve (AUC), τ = dosing interval (AUCτ), time of maximum concentration (t_{max}), clearance (CL) and serum half-life ($t_{1/2}$). MGII was used as a PD endpoint to assess a possible dose response relationship. Safety outcomes were incidences of adverse events and side effects, vital signs, and QTc intervals.

Statistical analysis

Based on previous data with a similar population of MG patients,[156,163,164] a sample size of 17 participants was expected to have 90% power to detect a difference of 8 points on the MGII with a two-sided p value of 0.05. We assumed a standard deviation of repeated measurements of MGII between patients of 13 and a conservative intraparticipant correlation of 0.7.[156,163,164] Allowing for a 10% drop out rate, we aimed to include 19 patients in both parts of the study.
Descriptive statistics were used to summarize baseline characteristics, presented as means and standard deviations (SDs) or medians and first and third quartiles (median [Q1-Q3]). Primary analysis was performed according to the intention-to-treat principle. In addition, we performed as treated analysis for all efficacy outcomes. A mixed-effects linear regression model was used to analyze repeated measures and estimate mean differences between amifampridine MR 30 mg (30), amifampridine MR 60 mg (60), and placebo (P), with treatment and treatment period (categorical) as fixed factors and patients as a random effect. Significance was defined as a p-value lower than 0.025, to account for multiple comparisons. We analyzed safety endpoints in all participants who were exposed to at least one dose of the trial medication and summarized these data using descriptive statistics.
For the PK/PD analysis actual blood sampling times were used for PK analysis. Amifampridine concentrations were measured in plasma with a UPLC/MS-MS assay, validated according to the ICH/EMA guidelines,[175] in the ISO15189 certified clinical pharmaceutical laboratory. A population PK-model for amifampridine MR was developed in NONMEM version 7.5. For the development of the model we included the observed data for all patients including the patients not participating in the PK/PD substudy. One- and two-compartment disposition kinetics with linear and nonlinear elimination were tested to describe the structural model of amifampridine MR. Model development and inclusion of covariates followed a stepwise approach based on physiological plausibility and improvement in model diagnostics. Tested covariates were age, sex, weight, height, BMI, creatinine and NAT genotype. Estimated individual PK parameters (AUCτ, C_{max}, C_{min}, $t_{1/2}$, t_{max}, CL) were extracted from the final PK model. Associations between PK parameters and the primary outcome measure were

evaluated using Pearson correlation coefficients and linear regression models.
All statistical analyses were predefined in the study protocol. No data were missing for the primary outcome analyses and no data imputation was done to account for missing data. All statistical analyses were conducted using RStudio (version 4.4.0).

Results

Between March 22, 2023 and Feb 21, 2024 21 patients were screened for eligibility, of whom nineteen patients completed part one of the trial, and two patients failed to complete the washout period prior to randomization in part one. Of these, one did not meet the eligibility criteria for part two, and was therefore excluded from further participation. Twenty patients were enrolled and randomly assigned to one of three consecutive treatment sequences: 30-60-P (7 patients); 60-P-30 (7 patients); or P-30-60 (6 patients) (**Figure 5.2**).
Three participants discontinued treatment prematurely due to adverse events. Among them, two participants withdrew during treatment with amifampridine 60 mg on the third day of treatment, and one participant withdrew during both the amifampridine 30 mg and amifampridine 60 mg treatment periods on the second day of treatment. These patients were included in the intention-to-treat analysis but were excluded from the per-protocol analysis.

Patient demographics and clinical characteristics across treatment sequences is shown in **Table 5.1**.
Eleven patients (55%) were female and nine (45%) were male. The median age of the cohort was 60 years (IQR 41-70), and the median age at onset was 51 years (IQR 26-63). Median MGII score at inclusion was 29 (IQR 21-40). Data on daily doses of pyridostigmine and AChR antibody status for each participant is provided in **Supplementary Table 5.1** and **5.2**, respectively.

Table 5.2 provides the estimated mean values for the primary and secondary outcome variables during treatment with amifampridine 30 mg and 60 mg, as well as for the placebo treatment. The treatment differences between amifampridine 30 mg and placebo, and between amifampridine 60 mg and placebo, are also provided. The mean treatment difference for the primary outcome, the MGII score, was -1.0 point (95% CI -4.1, 2.0); p = 0.379) for amifampridine 30 mg and -1.7 (95% CI -4.7,1.4; p = 0.379) for amifampridine 60 mg when compared to placebo. Similar to the primary outcome measure, no statistically significant differences were observed across all secondary efficacy outcome measures, including MGII ocular, MGII generalized, QMG and MG-ADL, quality of life (MG-QoL15r), and

treatment satisfaction (TSQM-9). There was no evidence of a treatment-by-period interaction. (**Supplementary Table 5.3**).

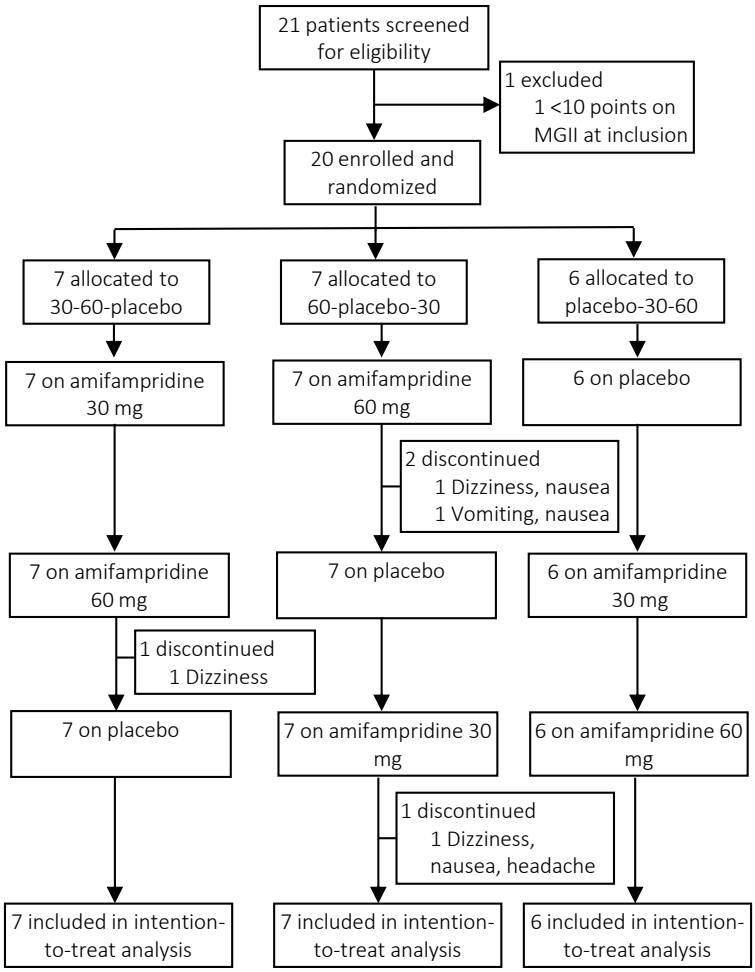


Figure 5.2 Flow diagram

[a] In two patients, primary and secondary outcome measures were obtained prematurely due to an intolerable increase in MG symptoms during the placebo treatment week.

Table 5.1 Baseline characteristics

	P-30-60[1] N = 6	**60-P-30[2] N = 7**	**30-60-P[3] N = 7**
Age, years	60 (44, 73)	60 (37, 75)	59 (47, 63)
Age at onset, years	52 (34, 63)	58 (23, 64)	45 (31, 55)
Sex, female	4 (67%)	3 (43%)	4 (57%)
BMI, kg/m2	27.4 (26.4, 28.3)	25.3 (23.7, 28.7)	28.4 (26.0, 29.4)
Disease duration, years	5 (3, 16)	8 (2, 11)	5 (4, 12)
Previous thymectomy, yes	2 (33%)	1 (14%)	4 (57%)
MGFA score at inclusion			
I	1 (17%)	2 (29%)	0 (0%)
II	4 (67%)	4 (57%)	7 (100%)
III	1 (17%)	1 (14%)	0 (0%)
MGII total score at inclusion	35 (23, 45)	30 (26, 50)	21 (21, 30)
NAT2 enzyme acetylator phenotype			
Fast	1 (17%)	4 (57%)	4 (57%)
Slow	5 (83%)	3 (43%)	3 (43%)
QTc, milliseconds	403 (400, 419)	403 (393, 404)	395 (389, 405)
Therapies in previous year			
No therapy	3 (50%)	4 (57%)	3 (43%)
Prednisolone	3 (50%)	2 (29%)	2 (29%)
Azathioprine	1 (17%)	1 (14%)	0 (0%)
Mycophenolate mofetil	2 (33%)	1 (14%)	1 (14%)
Cyclosporine	0 (0%)	1 (14%)	0 (0%)
Methotrexate	0 (0%)	0 (0%)	1 (14%)
IVIg on a regular basis	1 (17%)	0 (0%)	0 (0%)

Data are median (Q1-Q3) or n (%). Of the 20 participants, 19 had also participated in part one of the trial.
Abbreviations: MGFA, Myasthenia Gravis Foundation of America; MGII, Myasthenia Gravis Impairment Index; NAT2, N-acetyltransferase 2; QTc, corrected QT interval; IVIg, intravenous immunoglobulin therapy.
[1] Placebo-amifampridine 30 mg-amifampridine 60 mg
[2] Amifampridine 60 mg-placebo-amifampridine 30 mg
[3] Amifampridine 30 mg-amifampridine 60 mg-placebo

Table 5.2 Estimated mean scores and treatment differences on primary and secondary outcome variables

	Estimated mean scores[a] (95% CI)			Treatment difference (95% CI)					
Outcome	**Placebo**	**Amifampridine 30 mg**	**Amifampridine 60 mg**	**Amifampridine 30 mg vs. placebo**	**Unadjusted p-value**	**Adjusted p-value**	**Amifampridine 60 mg vs. placebo**	**Unadjusted p-value**	**Adjusted p-value**
Primary outcome									
MGII total	26.6 (19.2, 33.9)	25.5 (18.2, 32.8)	24.9 (17.6, 32.2)	-1.0 (-4.1, 2.0)	**0.681**	**1.000**	-1.7 (-4.7, 1.4)	**0.379**	**1.000**
Secondary outcomes									
MGII ocular	9.3 (6.3, 12.4)	9.0 (6.0, 12.0)	8.5 (5.5, 11.5)	-0.3 (-1.6, 1.0)	0.814		-0.9 (-2.2, 0.5)	0.263	
MGII generalized	17.2 (11.2, 23.2)	16.5 (10.5, 22.5)	16.4 (10.4, 22.4)	-0.7 (-3.3, 1.9)	0.784		-0.8 (-3.5, 1.8)	0.725	
QMG	10.7 (8.0, 13.4)	10.6 (7.9, 13.2)	10.2 (7.5, 12.9)	-0.1 (-1.2, 0.9)	0.943		-0.5 (-1.6, 0.6)	0.482	
MG-ADL	5.9 (4.1, 7.7)	5.3 (3.5, 7.1)	5.8 (4.0, 7.6)	-0.6 (-1.5, 0.3)	0.253		-0.1 (-1.0, 0.8)	0.975	
MG-QoL15r	11.1 (7.9, 14.3)	10.1 (6.9, 13.3)	12.2 (9.0, 15.4)	-1.0 (-3.0, 0.9)	0.402		1.1 (-0.8, 3.0)	0.351	
TSQM-9	35.8 (33.0, 38.5)	37.0 (34.2, 39.8)	36.1 (33.3, 38.9)	1.2 (-3.3, 5.8)	0.792		0.4 (-4.2, 4.9)	0.979	

[a] Values are least square means from a mixed effects linear model that includes a fixed effect for treatment period and a random effect for participant.
Significance for pairwise comparisons vs. placebo was assessed using a Bonferroni correction for two tests ($p < 0.025$ considered significant). CI, confidence interval; MGII, Myasthenia Gravis Impairment Index; QMG, Quantitative Myasthenia Gravis; MG-ADL, Myasthenia Gravis-Activities of Daily Living; MG-QoL15r, revised 15-item Myasthenia Gravis Quality of Life; TSQM-9, 9-item Treatment Satisfaction Questionnaire for Medication.

The number of participants who correctly guessed their treatment allocation in the preceding period was 12 (60%) during amifampridine 30 mg treatment, 9 (45%) during treatment with amifampridine 60 mg, and 12 (60%) during placebo treatment. The investigators correctly guessed the treatment allocation in 10 participants (50%) during amifampridine 30 mg treatment, 9 participants (45%) during amifampridine 60 mg treatment, and in 11 participants (55%) during placebo treatment.
Following completion of the trial, 16 out of 20 participants elected to continue treatment with amifampridine, of whom 13 participants were still using amifampridine at 6 months, with a median daily dose of 37.5 mg (range 15.0-60.0 mg). **Supplementary Table 5.4** presents the patient and disease characteristics of the subgroup that continued treatment compared to those who discontinued. Median age and age at disease onset were 59 vs. 73 years (p=0.178) and 36 vs. 62 years (p = 0.122), respectively. Immunosuppressive therapy was used by 62% of those who continued amifampridine vs. 29% who discontinued (p=0.350).
During amifampridine 30 mg treatment, 12 (60%) participants correctly identified their treatment allocation, and 9 (45%) participants did so during amifampridine 60 mg treatment. During the placebo period, 12 (60%) participants guessed their allocation correctly. Similarly, investigators correctly predicted the treatment allocation for 10 (50%) participants during amifampridine 30 mg treatment, 9 (45%) participants during amifampridine 60 mg treatment, and 11 (55%) participants during the placebo period.
Side effects were reported by 6 (30%), 12 (60%), and 15 (71%) participants treated with placebo, amifampridine 30 mg, and amifampridine 60 mg, respectively (**Table 5.3**). Most frequently reported side effects with amifampridine were paresthesia, fatigue, numbness, dizziness, and sleep disturbances. Three patients discontinued treatment during the study: two during treatment with amifampridine 60 mg, and one during treatment with both amifampridine 30 mg and 60 mg. These patients specifically reported numbness, paresthesia, anxiety, dizziness and nausea as the reason for treatment discontinuation. All participants' adverse events improved after discontinuation of the study drug. No severe adverse events were reported. There was no statistically significant difference in vital signs (blood pressure, pulse rate, and respiratory rate) and QTc interval between placebo and treatment with amifampridine 30 mg or 60 mg. Results of laboratory testing are shown in **Supplementary Table 5.5**.

Amifampridine MR pharmacokinetics were best described by a two-compartment model with linear elimination and first-order absorption. The covariate NAT genotype was significantly correlated with amifampridine clearance. Model derived PK parameters of amifampridine MR were a mean C_{max} of 18.3 ± 6.4 ng/mL for the 30 mg dose, and 38.3 ± 16.3 ng/mL for the 60 mg dose. T_{max} was 98.7 ± 43.3 min for the 30 mg dose, and 91.6 ± 29.7

min for the 60 mg dose. C_{trough} was 2.1 ± 2.3 ng/mL for the 30 mg dose, and 5.8 ± 5.0 ng/mL for the 60 mg dose. The mean steady-state AUCτ was 117.7 ± 47.8 ng·h/mL for the 30 mg dose, and 248.5 ± 126.5 ng·h/mL for the 60 mg dose. The elimination half-life (t½) was comparable between doses, with 4.4 ± 1.5 hours for 30 mg and 4.4 ± 1.4 hours for 60 mg,

Table 5.3 Adverse events by treatment condition

	Placebo	**Amifampridine 30 mg**	**Amifampridine 60 mg**
Participants with ≥1 treatment emergent adverse event	6 (30%)	12 (60%)	15 (75%)
Treatment-emergent adverse events leading to discontinuation of the study drug	0	1 (11%)	3 (50%)
Numbness	0	1 (5%)	3 (15%)
Paresthesia	0	1 (5%)	2 (10%)
Anxiety	0	1 (5%)	2 (10%)
Dizziness	0	1 (5%)	2 (10%)
Nausea	0	0	2 (10%)
Treatment-emergent adverse events occurring in any participant in any treatment group			
Paresthesia	0 (0%)	4 (20%)	10 (50%)
Fatigue	3 (15%)	4 (20%)	8 (40%)
Numbness	0 (0%)	3 (15%)	8 (40%)
Dizziness	3 (15%)	4 (20%)	7 (35%)
Sleep disturbances	1 (5%)	5 (25%)	6 (30%)
Diarrhea	1 (5%)	3 (15%)	6 (30%)
Muscle spasms or muscle twitches	1 (5%)	1 (5.0%)	6 (30%)
Headache	1 (5 %)	5 (25%)	5 (25%)
Gastric discomfort	1 (5%)	3 (15%)	5 (25%)
Nausea	1 (5%)	2 (10%)	5 (25%)
Raynaud's syndrome	1 (5%)	2 (10%)	5 (25%)
Drowsiness	1 (5%)	2 (10%)	4 (20%)
Stomach ache	0 (0%)	2 (10%)	4 (20%)
Blurred vision	1 (5%)	2 (10%)	3 (15%)
Excessive or viscous mucus in the airways	1 (5%)	2 (10%)	3 (15%)
Rapid or irregular heartbeat	0 (0%)	0 (0%)	3 (15%)
Anxiety	0 (0%)	1 (5%)	2 (10%)
Cough	1 (5%)	1 (5%)	1 (5%)
Asthma	0 (0%)	1 (5%)	1 (5%)
Chorea	0 (0%)	0 (0%)	1 (5%)
Other[a]	2 (10%)	4 (20%)	1 (5%)

Data are number of patients (%) with one or more adverse event. Seizures were queried but not reported in either treatment period. [a] Other treatment-emergent adverse events:
- During placebo treatment: urinary urgency and left foot.
- During amifampridine 30 mg treatment: urinary urgency, hyperhidrosis, muscle weakness, and bowel symptoms.
- During amifampridine 60 mg treatment: urinary urgency.

respectively. No associations were found between the mean steady-state AUCτ, and C_{max}, and the primary outcome measure (**Figure 5.3**). The estimated odds ratio for dose-limiting side effects per ng/mL increase in C_{max} was 1.05 (95% CI: 0.99-1.12, p = 0.06), and the estimated odds ratio per ng·h/mL increase in steady-state AUCτ was 1.01 (95% CI: 1.00-1.02, p = 0.06).

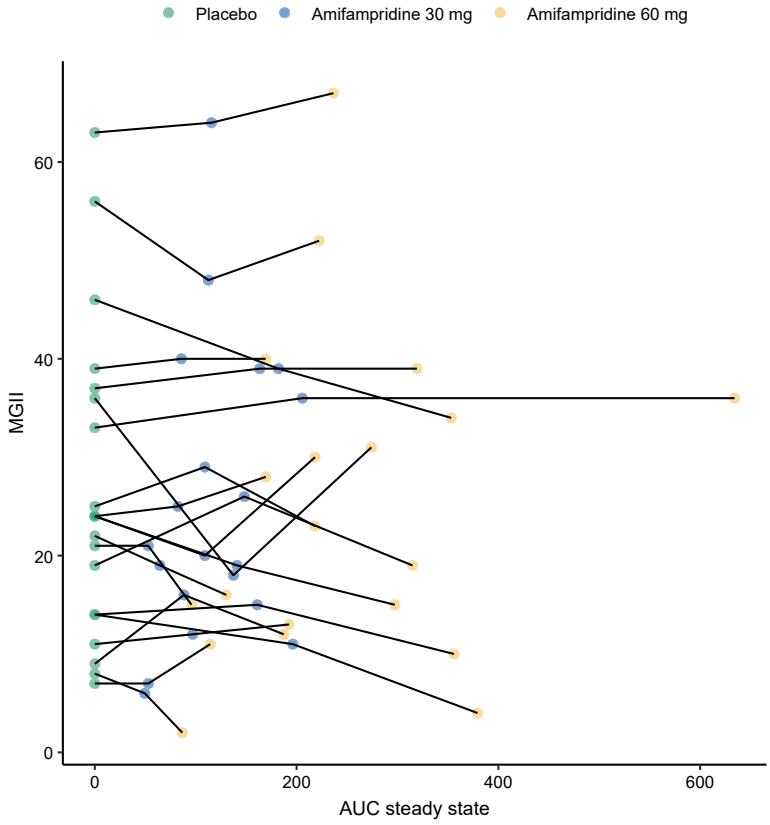


Figure 5.3 Association between area-under-the curve steady state and the primary outcome by treatment condition

Each point represents an individual patient, with lines connecting measurements from the same patient across different treatments. Colors indicate treatment conditions: placebo, amifampridine 30 mg, and amifampridine 60 mg. Data are based on a population pharmacokinetic model for amifampridine modified release, developed using a two-compartment linear approach. The dataset included 20 patients, with observed AUC data available for 8; in the remaining 12 patients, exposure was predicted using the model. This figure illustrates that AUC steady state increases with higher doses of amifampridine, with MGII scores varying independently of AUC steady state across patients.
MGII, Myasthenia Gravis Impairment Index; AUC, area under the concentration-time curve.

Discussion

IMPACT-MG, a randomized, double-blind, placebo-controlled crossover trial, evaluated the efficacy and safety of amifampridine MR in patients with AChR MG who remained symptomatic while on pyridostigmine therapy. The primary outcome, the mean change in MGII total score, showed no statically significant difference between amifampridine 30 mg, amifampridine 60 mg and placebo. Furthermore, no differences were observed across all secondary outcome measures, including the MGII ocular and MGII generalized subscores, as well as QMG, MG-ADL, MG-QoL15r, and TSQM-9, between amifampridine 30 mg, amifampridine 60 mg, and placebo. In addition, adverse events were reported more frequently with amifampridine than with placebo, with paresthesia, fatigue, numbness, dizziness, and sleep disturbances being the most commonly reported. In three patients, treatment-emergent adverse events were severe enough to necessitate discontinuation of amifampridine. These findings are supported by the additional PK/PD analyses, which did not show a statistically significant association between pharmacokinetic parameters and the primary outcome measure.

The strengths of our study include carefully controlled study procedures, e.g., all patients were evaluated throughout the study by the same investigator, and the inclusion of a comprehensive set of both patient-reported and investigator-assessed outcome measures allowing for a thorough evaluation of the impact of the treatment. Additionally, the study was marked by strong data integrity, with no drop-outs and complete data for primary outcomes (100%) and nearly complete data for secondary outcomes (>99%).

The study also had some limitations. First, the study design employed two fixed doses of amifampridine; 30 mg and 60 mg. However, some patients experienced a favorable response at 30 mg, but developed significant side effects at 60 mg, whereas others showed no response at 30 mg but did benefit from the 60 mg dose. Since it was deemed methodologically unsound to select the best individual dose post hoc and compare it to placebo, it is possible that an initial dose-titration phase to identify the optimal dose could have resulted in a demonstrable treatment effect. This could explain why such a substantial number of participants chose to continue using amifampridine once they were allowed to self-titrate their dosage. What argues against this, however, is the lack of an association between the PK parameters and the MGII. Second, the sample size in this study was relatively small. We did not account for multiple testing in the sample size calculation for this part of the trial, which in retrospect would have been appropriate. Even after applying this correction, however, 20 patients would still have been required (without adjustment for drop-out), corresponding to the number actually included. This sample size was estimated to provide sufficient power to detect a difference of 8 points on the MGII, corresponding to the established cutoff for a clinically meaningful difference at the group level. However, two

factors not accounted for in advance may have reduced its power. In part one of the IMPACT-MG trial, which assessed the efficacy of pyridostigmine in a largely similar patient population, the effect size was smaller than anticipated, with a highly significant mean improvement of 5.3 points. Therefore, the estimated minimal additional effect of amifampridine (an improvement of 8 points), was likely greater than could reasonably be expected. In addition, by the time participants were randomized into part two, they had already resumed pyridostigmine treatment, resulting in milder symptoms that made achieving an 8-point improvement more challenging compared to part one. Nonetheless, even if a significant difference were to be demonstrated with a larger sample size, the observed effect size of amifampridine in this study was small (1.7), and the clinical relevance of such an effect remains debatable.

In conclusion, the addition of amifampridine to pyridostigmine did not demonstrate a significant benefit compared to placebo on the prespecified primary and secondary outcome measures. In addition, more adverse events were observed during treatment with amifampridine. This trial provides insufficient support to justify adding amifampridine to standard treatment with pyridostigmine.

Supplements

Full list of amifampridine side effects queried

Paresthesia, numbness, gastric discomfort, nausea, diarrhea, stomach ache, cough, excessive or viscous mucus in the airways, blurred vision, rapid or irregular heartbeat, fatigue, headache, anxiety, dizziness, sleep disturbances, drowsiness, chorea, muscle spasms or muscle twitches, seizures, asthma and Raynaud's syndrome.

Supplementary Table 5.1 Pyridostigmine daily doses for each participant

Patient	Daily dose of pyridostigmine	Participation in:	
		Part one	Part two
1	30 mg four times daily	yes	yes
2	60 mg five times daily	yes	yes
3	60 mg five times daily	yes	yes
4	60 mg four times daily	yes	yes
5	60 mg five times daily	yes	yes
6	60 mg five times daily	yes	yes
7	60 mg six times daily	yes	yes
8	60 mg five times daily	yes	yes
9	60 mg five times daily	yes	yes
10	50 mg five times daily	yes	yes
11	60 mg four times daily	yes	yes
12	60 mg three times daily	yes	yes
13	1dd 90 mg and 4dd 80 mg	yes	yes
14	60 mg four times daily	yes	yes
15	60 mg four times daily	yes	yes
16	50 mg four times daily	yes	yes
17	60 mg three times daily	yes	yes
18	60 mg three times daily	yes	yes
19	60 mg four times daily	yes	yes
20	60 mg five times daily	no	yes

Supplementary Table 5.2 Acetylcholine receptor antibody status for each participant

Patient	AChR antibody titer (nmol/L) at baseline
1	11.8
2	3.3
3	5.5
4	15.8
5	17.2
6	2.7
7	15.6
8	19.6
9	2.5
10	17.4
11	13.8
12	8.1
13	1.4
14	10.1
15	1.2
16	1.2
17	21.1
18	7.0
19	9.3
20	18.4

AChR antibodies were measured by using a standard dilution of the sample in a radioimmunoassay (RSR Limited, Cardiff, UK). This assay provides a numerical value that is linearly correlated with actual serum concentrations up to a value of 20 nmol/L.

Supplementary Table 5.3 Treatment by period interaction for primary and secondary outcome measures

		Amifampridine 30 mg		Amifampridine 60 mg	
	Visit	**Estimate (95% CI)**	**P-value**	**Estimate (95% CI)**	**P-value**
Primary outcome					
MGII total	Second	9.8 (-21.5, 41.1)	0.517	-15.2 (-45.3, 14.9)	0.303
MGII total	Third	21.0 (-9.2, 51.1)	0.161	9.8 (-21.5, 41.1)	0.519
Secondary outcomes					
MGII ocular	Second	8.1 (-4.0, 20.2)	0.174	-5.3 (-16.9, 6.4)	0.353
MGII ocular	Third	13.6 (1.9, 25.2)	0.025	6.9 (-5.2, 19.0)	0.244
MGII generalized	Second	1.7 (-25.4, 28.8)	0.897	-9.9 (-36, 16.2)	0.436
MGII generalized	Third	7.4 (-18.7, 33.5)	0.560	2.9 (-24.3, 30.0)	0.827
QMG	Second	0.7 (-11.6, 13.0)	0.907	-2.2 (-14.0, 9.6)	0.701
QMG	Third	1.8 (-10.0, 13.6)	0.751	1.1 (-11.2, 13.4)	0.851
MG-ADL	Second	1.8 (-5.4, 9.0)	0.609	-4.8 (-11.7, 2.2)	0.166
MG-ADL	Third	7.4 (0.5, 14.3)	0.038	2.7 (-4.5, 9.9)	0.441
MG-QoL15r	Second	7.3 (-6.2, 20.8)	0.272	-3.7 (-16.7, 9.3)	0.560
MG-QoL15r	Third	9.9 (-3.2, 22.9)	0.129	5.8 (-7.8, 19.3)	0.384
TSQM-9	Second	-0.5 (-11.0, 9.9)	0.916	6.2 (-4.0, 16.4)	0.226
TSQM-9	Third	1.8 (-8.4, 11.9)	0.728	4.7 (-5.7, 15.2)	0.364

CI, confidence interval; MGII, Myasthenia Gravis Impairment Index; QMG, Quantitative Myasthenia Gravis; MG-ADL, Myasthenia Gravis-Activities of Daily Living; MG-QoL15r, revised 15-item Myasthenia Gravis Quality of Life; TSQM-9, 9-item Treatment Satisfaction Questionnaire for Medication

Supplementary Table 5.4 Patient and disease characteristics of patients who continued vs. discontinued amifampridine treatment

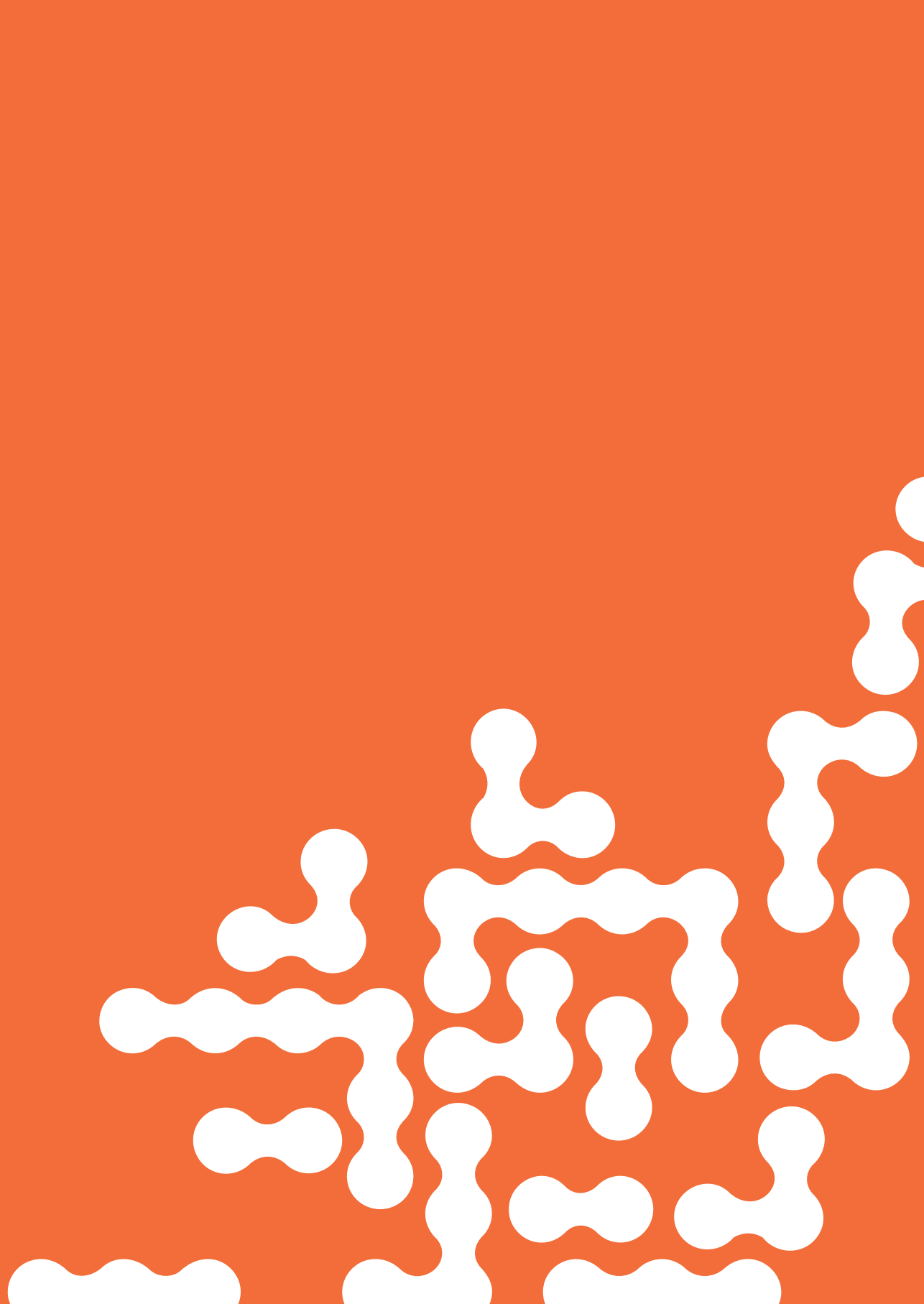
	Discontinued amifampridine after trial completion N = 7	**Continued amifampridine after trial completion** N = 13
Age, years	73 (55, 77)	59 (36, 64)
Age at disease onset, years	62 (51, 72)	36 (25, 57)
Sex, female	3 (43%)	8 (62%)
BMI, kg/m2	26.2 (24.8, 28.7)	28.2 (26.4, 28.7)
Disease duration, years	5 (2, 8)	6 (2, 19)
Previous thymectomy, yes	2 (29%)	5 (38%)
MGFA score at inclusion		
I	2 (29%)	1 (7.7%)
II	5 (71%)	10 (77%)
III	0 (0%)	2 (15%)
MGII total score at inclusion	29 (19, 34)	30 (21, 45)
NAT2 enzyme acetylator phenotype		
Fast	2 (29%)	7 (54%)
Slow	5 (71%)	6 (46%)
QTc, milliseconds	403 (398, 415)	401 (391, 404)
Therapies in previous year		
No therapy	5 (71%)	5 (38%)
Prednisolone	1 (14%)	6 (46%)
Azathioprine	0 (0%)	2 (15%)
Mycophenolate mofetil	1 (14%)	3 (23%)
Cyclosporine	1 (14%)	0 (0%)
Methotrexate	0 (0%)	1 (7.7%)
IVIg on a regular basis	0 (0%)	1 (7.7%)

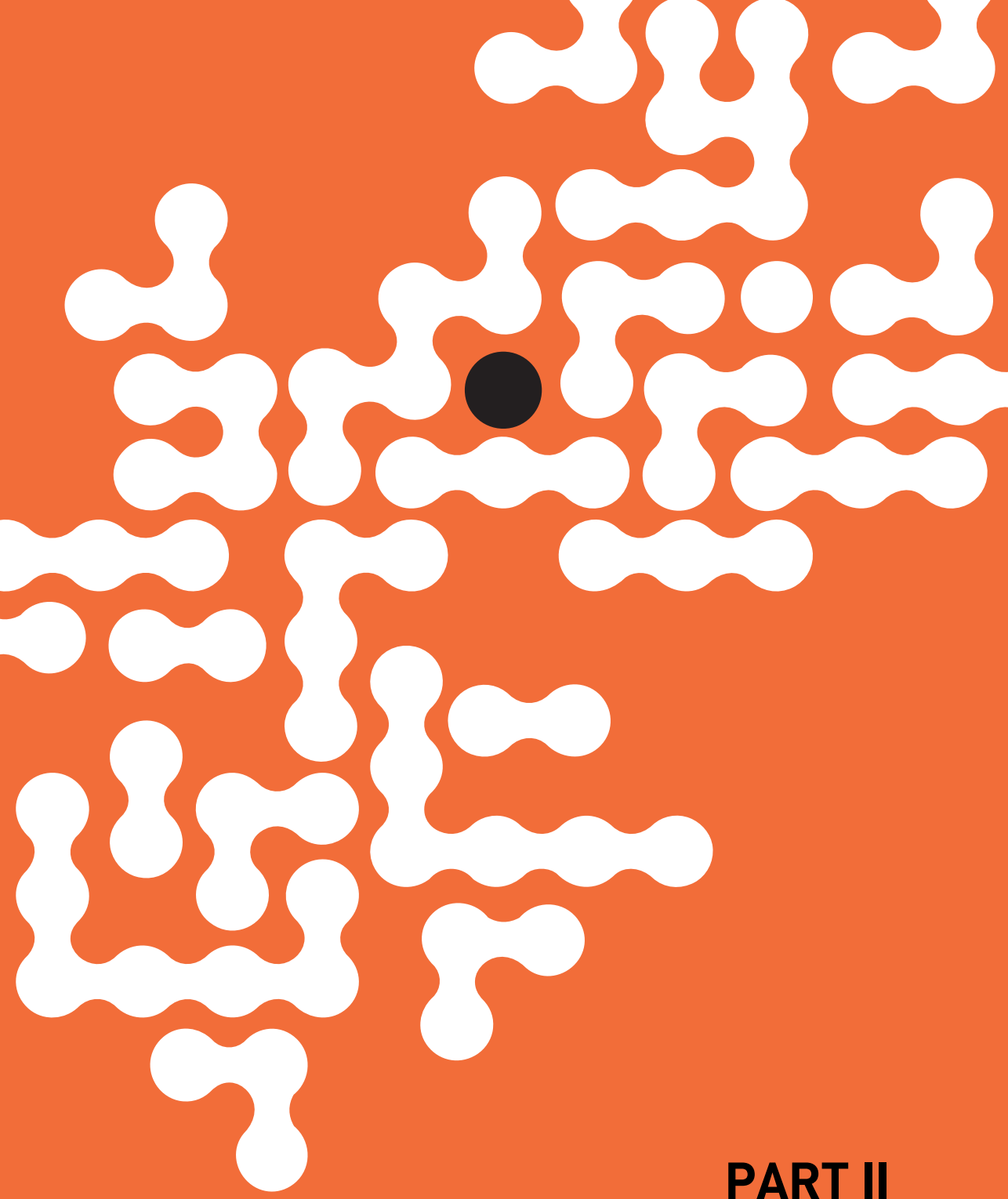
Data are median (Q1-Q3) or n(%). Abbreviations: MGFA, Myasthenia Gravis Foundation of America; MGII, Myasthenia Gravis Impairment Index; NAT2, N-acetyltransferase 2; QTc, corrected QT interval; IVIg, intravenous immunoglobulin therapy.

Supplementary Table 5.5 Laboratory results

	Baseline N = 20	**Last visit N = 19**
Hemoglobin	8.7 (8.3, 9.6)	8.4 (7.6, 8.9)
Unknown	1	0
Hematocrite	0.4 (0.4, 0.5)	0.4 (0.4, 0.4)
Unknown	1	0
Leukocytes	6.8 (5.7, 9.4)	5.9 (5.1, 8.1)
Unknown	1	0
Thrombocytes	238.0 (210.0, 291.5)	234.5 (199.5, 284.3)
Unknown	1	0
Sodium	141.0 (139.8, 143.3)	141.0 (138.0, 143.0)
Unknown	0	1
Potassium	4.6 (4.4, 4.8)	4.2 (4.1, 4.4)
Unknown	0	1
Creatinine	62.5 (56.5, 78.0)	63.0 (54.5, 76.0)
Unknown	0	1
eGFR	90.0 (88.3, 90.0)	90.0 (89.5, 90.0)
Unknown	0	1
Aspartate aminotransferase (ASAT)	21.5 (19.8, 26.5)	20.5 (18.0, 23.5)
Unknown	0	1
Alanine aminotransferase (ALAT)	22.5 (15.8, 26.3)	21.0 (16.0, 23.0)
Unknown	0	1
Alkaline phosphatase	70.0 (59.8, 80.3)	67.0 (62.5, 79.5)
Unknown	0	1
Gamma glutamyltransferase	12.5 (11.0, 22.8)	13.0 (10.5, 23.0)
Unknown	0	1
Creatinine kinase	79.5 (55.3, 102.8)	93.0 (68.5, 118.5)
Unknown	0	1
Glucose	5.0 (4.9, 5.7)	5.2 (4.8, 6.5)
Unknown	0	1
AChR antibodies	9.7 (3.2, 16.2)	8.8 (3.2, 12.4)

Data are median (Q1-Q3).





PART II

Immunomodulatory therapies

CHAPTER

6

Treatment patterns in myasthenia gravis

Longitudinal treatment patterns in myasthenia gravis:
an analysis from the prospective Dutch-Belgian patient registry
Journal of neuromuscular diseases, 2026, 13(3), 445-452

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Abstract

Background

Understanding treatment patterns in myasthenia gravis (MG) is crucial for clinical practice. However, longitudinal data are limited.

Objective

This study aimed to provide an overview of treatment patterns in different subgroups throughout the disease course.

Methods

All adult patients with acetylcholine receptor (AChR), muscle-specific kinase (MuSK), or seronegative MG enrolled in the Dutch-Belgian myasthenia registry were included. AChR-MG patients were classified into early-onset (EOMG) (≤50 years) and late-onset (LOMG) (>50 years) MG. Mixed-effects regression was used to compare differences between the different subgroups.

Results

A total of 506 patients were included (147 AChR EOMG, 265 AChR LOMG, 26 MuSK, and 94 seronegative patients). Data were available within a year of diagnosis for 84 patients. Most patients initially started treatment with pyridostigmine (93%; 78/84), followed by corticosteroids (52%; 44/84) and azathioprine or mycophenolate mofetil (30%; 25/84). After ten years, the use of pyridostigmine (65%; 54/83) and corticosteroid use (36%; 30/83) declined, whereas the use of azathioprine and mycophenolate mofetil increased (55%; 46/83). Seronegative patients received fewer treatments than AChR MG patients, independent of functional status and disease duration (OR 0.38, 95% CI 0.17-0.84, p=0.018), and were less likely to use corticosteroids (OR 0.15, 95% CI [0.05-0.41] , p<0.001). Patients with LOMG reported more frequently immunosuppressive use compared to patients with EOMG (OR 4.69, 95% CI 1.16-19.02, p=0.031).

Conclusions

This analysis offers valuable insights into treatment patterns in different subgroups of patients with MG. A substantial proportion of patients continues to rely on corticosteroids, suggesting an ongoing need for alternative treatment options.

Introduction

Myasthenia gravis (MG) is a rare, chronic autoimmune disorder characterized by muscle fatigability resulting from autoantibodies aimed at components of the neuromuscular junction.[6] Various studies have identified distinct subgroups of MG, primarily based on antibody status and clinical features.[3] Patients may present with antibodies directed against the acetylcholine receptor (AChR), muscle-specific kinase (MuSK), or may be seronegative, demonstrating no detectable antibodies. In addition to serological classification, disease characteristics have been shown to differ across age groups.[3] Early-onset MG (EOMG), defined as disease onset before the age of 50, predominantly affects young women and is commonly associated with AChR antibodies, thymic hyperplasia, and the HLA-A1-B8-DR3 haplotype. In contrast, late-onset MG (LOMG), which manifests after the age of 50, occurs more frequently in men and is associated with thymic atrophy and the HLA-DRB1*15:01 allele.[2]

The treatment goal for MG is achieving Minimal Manifestation Status or better, with no functional limitations and only mild medication side effects.[25] This often involves a sequential approach, requiring trial and error to identify the most effective regimen for each patient. First-line pharmacological treatment for MG consists of acetylcholinesterase inhibitors, which enhance neuromuscular transmission by increasing the amount of acetylcholine in the synaptic cleft. Although various acetylcholinesterase inhibitors exist, pyridostigmine is the preferred agent for symptomatic treatment in clinical practice.[25] If symptom control proves insufficient with pyridostigmine alone, corticosteroids are introduced, often in combination with non-steroidal immunosuppressive agents. Corticosteroids play a central role in MG treatment due to their faster onset of action compared to other immunosuppressive therapies, affordability, and wide availability.[176] Nevertheless, long term corticosteroid use is known to be associated with significant adverse effects.[177] Non-steroidal immunosuppressant therapies (NSISTs), such as azathioprine, mycophenolate mofetil, ciclosporin, and rituximab, can be added to the treatment regimen to reduce the cumulative corticosteroid dose. However, due to lack of comparative studies, there is considerable global variation in the preferred use of these therapies.[39] For patients experiencing acute exacerbations, intravenous immunoglobulin therapy (IVIg) or plasma exchange (PE) is used. Additionally, IVIg may be considered as a long-term treatment option in patients in whom prior therapeutic interventions fail to achieve adequate disease control, although a recent trial did not demonstrate the efficacy of this strategy.[178]

In recent years, significant advances have been made in the treatment of MG with the introduction of complement inhibitors and FcRn blockers. At present, in most countries, these new treatments are reserved for patients with severe, refractory disease, partly due to their high costs. However, the definition of refractory MG is not clearly established. In

general, refractory disease refers to the failure of multiple prior therapies, and it is common practice that several other treatments must first be attempted before initiating these new agents. Therefore, in order to estimate who may benefit from emerging treatments, it is essential to understand how current therapies, including pyridostigmine, corticosteroids, NSISTs, IVIg, and PE, are used throughout the disease course. These insights are key to refining treatment guidelines and optimizing therapeutic strategies for patients with MG. This study therefore aims to provide a comprehensive overview of longitudinal treatment patterns of currently available treatment options in different subgroups of MG patients.

Patients and Methods

Data source

We used data from the Dutch-Belgian registry for neuromuscular junction disorders, a prospective clinical registry in the Netherlands and Belgium, with the primary aim to study the natural disease history, treatment response, and potential risk factors. Full details of the design of the registry, along with the primary results, have been reported previously.[86] In short, the registry is a collaborative initiative of the Dutch patient support organization for neuromuscular diseases and Leiden University Medical Center, a tertiary center for the treatment of neuromuscular junction disorders. The registry is an ongoing longitudinal database which collects medical information obtained from both patients and their treating physicians. The study was reviewed and approved by the independent Medical Ethics Review Committee Leiden The Hague Delft. All patients provided written informed consent before participation in the registry.

Outcomes

In this analysis, we included data from all patients with a confirmed diagnosis of MG, collected from August 2016 to March 2025, including those with disease onset at ≥18 years of age.

Diagnosis was based on clinical signs or symptoms suggestive of auto-immune MG, along with a positive serologic test for AChR antibodies or MuSK antibodies, or a diagnostic electrophysiological investigation consistent with a neuromuscular transmission disorder. Registry participants with congenital myasthenic syndrome or Lambert-Eaton myasthenic syndrome were excluded.

Data collected at baseline included patient demographics, disease characteristics such as antibody status, date of diagnosis, thymectomy (yes/no), and functional status, as well as treatment characteristics, including the use of the following treatments either at the time of data collection or within the past three months: pyridostigmine, prednisolone,

azathioprine, mycophenolate mofetil, rituximab, ciclosporin, methotrexate, cyclophosphamide, IVIg, or PE. In addition, patients were asked whether they had ever been treated with IVIg or PE at any point during the course of their disease, and whether they had ever had to discontinue treatments for MG due to side effects. During each scheduled annual follow-up questionnaire, patients were again asked about their functional status, treatments they were receiving either at the time of data collection or within the past three months, as well as whether they had ever had to discontinue treatments for MG due to side effects.

Seronegative patients were defined as patients who had no detectable antibodies before or at the time of enrollment in the registry. Early-onset MG (EOMG) was defined as an age of onset of 50 years or younger, and late-onset MG (LOMG) as an age of onset greater than 50 years. Data are presented by disease duration, with each duration representing a prevalent patient group. While there is overlap between groups, not all patients appear in every duration group.

Statistical analysis

Mean and standard deviation (SD), or median and interquartile range [IQR] were used to report continuous variables where appropriate. Comparisons were based on either student's T-test (normal distribution) or Mann-Whitney U test (non-normal distribution). Binary and categorical variables were reported according to frequencies and percentages. Categorical data are presented as proportions (%) and comparisons were assessed by the Chi-Square test. P-values were considered significant when <0.05. Missing data were reported and excluded from the analyses.

To investigate the association between treatment characteristics and disease characteristics, we applied several mixed-effects regression models. For ordinal outcomes, such as the number of treatments used over the course of the disease and the functional status of patients, mixed-effects ordinal regression was used. In the first model, we examined how the number of treatments varied in relation to disease onset or antibody subtype, disease duration, and functional status, including patients as a random effect. In the second model, functional status was analyzed as the outcome variable, with disease onset or antibody subtype and disease duration as fixed effects, and again, a random intercept for patients. For binary outcomes, including the use of specific treatment modalities such as pyridostigmine, corticosteroids, NSISTs, and IVIg/PE, and side effects, we employed mixed-effects logistic regression models. Each model assessed the association between treatment use or side effects and disease onset or antibody subtype, adjusting for disease duration and functional status, while accounting for repeated measures by including patients as a random effect. All data analyses were conducted using RStudio (version 4·4.0)

Results

Overall, 506 patients with MG enrolled in the Dutch-Belgian MG registry were included in this analysis (mean age 61 [SD 14]), of whom 247 (49%) were female. The cohort consisted of 412 patient with AChR MG, of whom 147 patients with EOMG and 265 patients with LOMG, 26 patients with MuSK MG, and 94 patients with seronegative MG. The median number of annual observations was 4 (range 1, 8). Patient demographics and disease characteristics, stratified by the different subgroups are summarized in **Table 6.1**.

Data were available for 84 patients within the first year after diagnosis. During this period, nearly all patients (99%; 83/84) initiated treatment. Most started with pyridostigmine (93%; 78/84), over half began corticosteroids (52%; 44/84), and nearly one-third started azathioprine or mycophenolate mofetil (30%; 25/84). Corticosteroids or other NSISTs were not used in 38 patients. While the difference was not statistically significant, there was a tendency for these patients to be more frequently female (55%; 21/38) than those who did receive such treatments (35%; 16/46), p=0.060. No difference was found in age at diagnosis, thymectomy, symptoms at baseline (ocular vs. generalized), or functional status.
At the five-year mark, treatment patterns shifted somewhat: 63% (107/170) of patients remained on pyridostigmine, 44% (74/170) continued corticosteroids, and the use of azathioprine or mycophenolate mofetil increased to 46% (79/170). After ten years, a majority (65%; 54/83) were still using pyridostigmine, corticosteroid use declined to 36% (30/83), while more than half (55%; 46/83) received treatment with azathioprine or mycophenolate mofetil. As the disease duration increased, the number of treatments decreased (OR 0.85, 95% CI [0.80-0.90], p<0.001).
In **Figure 6.1**, the number of treatments used by patients within the first ten years of the disease is shown, stratified by the different subgroups. Seronegative patients received fewer treatments, independent of functional status and disease duration, compared to AChR MG patients (OR 0.38, 95% CI 0.17-0.84, p=0.018). No association was found between disease onset (EOMG vs. LOMG) and the number of treatments after adjusting for disease duration and functional status (OR 0.69, 95% CI [0.29-1.67], p=0.414), nor was there an association between thymectomy (yes vs. no) in AChR-MG and the number of treatments (OR 1.41, 95% CI [0.75-2.65], p=0.281).

Table 6.1 Baseline characteristics

	Missing, n (%)	Overall, n = 506	AChR EOMG, n = 147	AChR LOMG, n = 265	MuSK, n = 26	Seronegative, n = 94
Age at diagnosis	0 (0%)	53 (16)	36 (10)	64 (8)	49 (14)	51 (13)
Age at enrollment in registry	0 (0%)	61 (14)	49 (14)	68 (8)	57 (13)	57 (12)
Gender, female	0 (0%)	247 (49%)	105 (71%)	85 (32%)	18 (69%)	57 (61%)
Disease duration, median (IQR)	0 (0%)	4 (1, 11)	11 (4, 21)	3 (1, 6)	5 (1, 12)	3 (0, 13)
BMI	17 (3.4%)	27.4 (5.4)	26.2 (5.8)	28.0 (5.0)	27.4 (4.9)	27.5 (5.5)
Thymectomy, yes	211 (42%)	97 (33%)	64 (67%)	29 (18%)	3 (20%)	4 (9.8%)
Symptoms at baseline, generalized	16 (3.0%)	482 (93%)	141 (97%)	239 (93%)	26 (100%)	76 (85%)
Functional status	4 (0.8%)					
Fully active, no limitations		153 (30%)	46 (32%)	78 (30%)	5 (19%)	29 (31%)
Fully dependent, entirely bed or chair-bound		4 (0.8%)	0 (0%)	3 (1.1%)	0 (0%)	1 (1.1%)
Limited self-care, mostly bed or chair-bound		17 (3.4%)	5 (3.4%)	7 (2.7%)	1 (3.8%)	5 (5.3%)
Restricted heavy activity, light work capable		230 (46%)	63 (43%)	131 (50%)	15 (58%)	36 (38%)
Self-care possible, unable to work, mostly out of bed		98 (20%)	32 (22%)	43 (16%)	5 (19%)	23 (24%)

Numbers are mean (SD) or n (%) unless otherwise specified. Abbreviations: AChR, acetylcholine receptor; EOMG, early-onset myasthenia gravis; LOMG, late-onset myasthenia gravis; MuSK, muscle-specific kinase; BMI, body mass index.

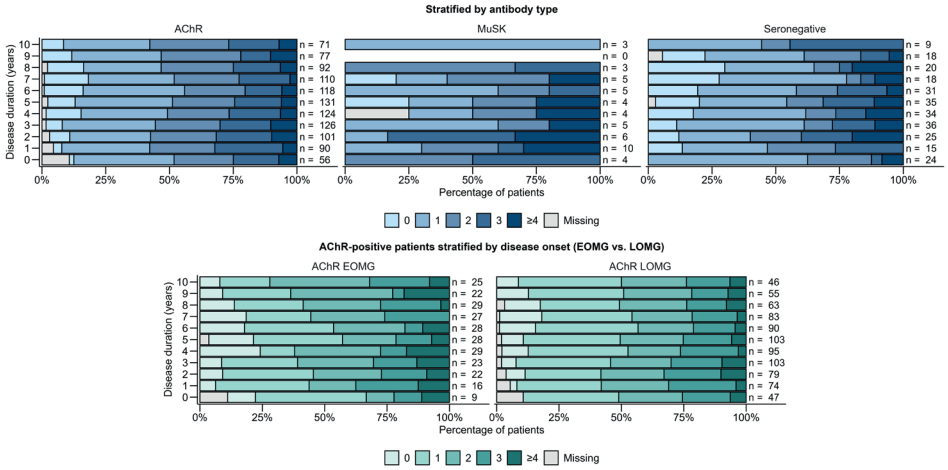

Figure 6.1 Number of concurrent treatments by disease duration in different subgroups of MG patients
Each bar represents a prevalent patient group at a certain disease duration, and patients in one disease duration group may not necessarily be present in other duration groups. Treatments include pyridostigmine, corticosteroids, non-steroidal immunosuppressant therapies, intravenous immunoglobulin therapy, and plasma exchange. Abbreviations: AChR, acetylcholine receptor; MuSK, muscle-specific kinase; EOMG, early-onset myasthenia gravis; LOMG, late-onset myasthenia gravis.

Figure 6.2 shows the evolution of different treatment modalities (pyridostigmine, corticosteroids, NSIST, and IVIg/PE) throughout the disease course, stratified for the different subgroups. Pyridostigmine was the most commonly used treatment in all subgroups except MuSK MG, with its use declining as the disease duration increased (OR 0.53, 95% CI [4.62-0.61], p<0.001). Patients with LOMG reported less frequent use of pyridostigmine compared to patients with EOMG in the first ten years of the disease, adjusted for disease duration and functional status (OR 0.05 95% CI 0.01-3.04, p=0.001). Additionally, patients who underwent a thymectomy used pyridostigmine more frequently than those who did not (OR 8.54, 95% CI [1.75-41.55], p=0.008). Corticosteroid use declined with a longer disease duration, independent of functional status (OR 0.79, 95% CI [0.73-0.85], p<0.001). Seronegative patients used corticosteroids less frequently compared to AChR MG patients (OR 0.15, 95% CI [0.05-0.41] , p<0.001). A trend was observed, although not statistically significant, suggesting that patients with EOMG used corticosteroids less frequently compared to those with LOMG (OR 2.53, 95% CI [0.91-7.09], p=0.077), after accounting for differences in disease duration and functional status. Corticosteroid use did not differ between patients who underwent a thymectomy and those who did not (OR 0.67, 95% CI [0.26-1.72], p=0.400). The use of NSISTs increased with a longer disease duration across all

patients (OR 1.18, 95% CI [1.09-1.28], p<0.001). MuSK MG patients appeared to use NSISTs more frequently than AChR MG patients (OR 14.70, 95% CI [1.64-131.97], p=0.016), while there was a trend, although not statistically significant, suggesting that seronegative patients used NSISTs less frequently than those with AChR MG (OR 0.32, 95% CI [0.10-1.06], p=0.063). The use of NSISTs did not differ between patients who underwent a thymectomy and those who did not (OR 1.42, 95% CI [0.60-3.36], p=0.428).
Patients with LOMG reported more frequent use of immunosuppressive therapies in general (corticosteroids, either alone or in combination with a NSIST) compared to patients with EOMG (OR 4.69, 95% CI 1.16-19.02, p=0.031). The use of immunosuppressive therapies was comparable between female and male patients (OR 2.35, 95% CI [0.68-8.17], p=0.179).
There was no difference in frequency of use of IVIg/PE between the different subgroups. Out of all patients, 33.6% (179 patients) reported to have ever used IVIg during the disease course, and 16.4% (87 patients) reported to have ever been treated with PE. After adjusting for functional status, a longer disease duration was associated with a reduced likelihood of receiving plasma exchange (OR 0.78, 95% CI [0.63-0.95], p=0.014), while it was not associated with the likelihood of receiving intravenous immunoglobulin (OR 0.97, 95% CI [0.94-1.01], p=0.167). No significant differences in the likelihood of receiving plasma exchange or IVIg were observed between antibody subtypes. However, there was a trend that patients with LOMG were less likely to receive IVIg (OR 0.32, 95% CI [0.11-0.96], p=0.043) and plasma exchange (OR 0.17, 95% CI [0.03-1.11], p=0.064).

Treatment discontinuation due to side effects was most frequently reported in in the first year after diagnosis: 19/84 patients (23%) discontinued treatment due to side effects. After five years, this number decreased to 20/170 patients (12%), and after ten years, 9/83 patients (11%). The likelihood of experiencing side effects decreased throughout the first ten years of the disease course (OR 0.87, 95% CI [0.81-0.94], p<0.001). There was no difference between antibody subtype, or EOMG and LOMG.
In **Figure 6.3** the evolution of the functional status throughout the first ten years of the disease course is shown, stratified for the different subgroups. There appears to be a trend indicating that seronegative patients have a worse functional status compared to AChR MG patients, after adjusting for disease duration (OR 2.40, 95% CI [0.96-6.00], p=0.061). No difference was found between functional status between EOMG and LOMG.

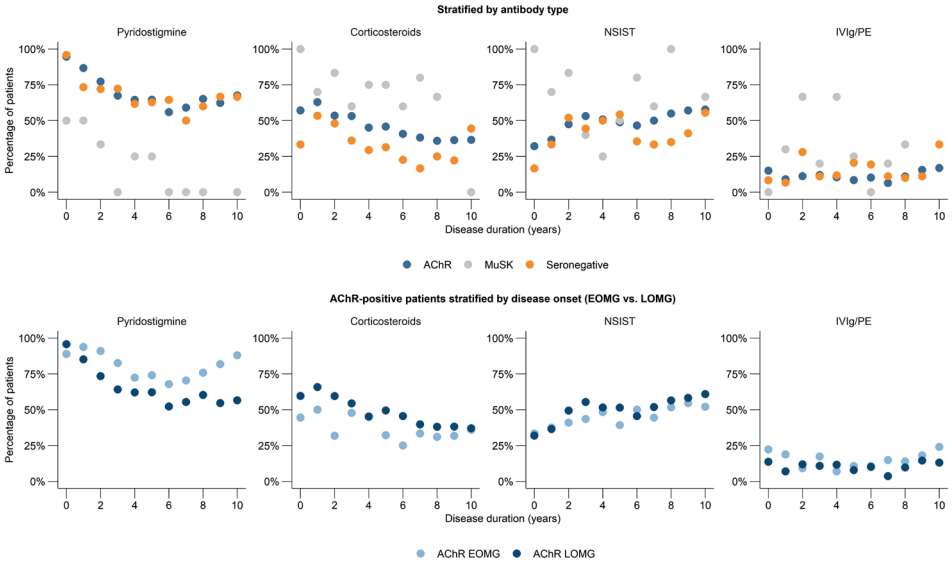

Figure 6.2 Evolution of treatment modalities throughout the disease course
Abbreviations: AChR, acetylcholine receptor; MuSK, muscle-specific kinase; EOMG, early-onset myasthenia gravis; LOMG, late-onset myasthenia gravis; NSIST, non-steroidal immunosuppressant therapies (azathioprine, mycophenolate mofetil, ciclosporin, methotrexate, rituximab, cyclophosphamide); IVIg, intravenous immunoglobulin therapy; PE, plasma exchange

Discussion

The current study provides an overview of longitudinal characteristics and treatment patterns of currently available pharmacological treatment options in a large cohort of MG patients. This cohort describes a time period in which complement inhibitors and FcRn blockers were not yet widely prescribed for patients with MG in the Netherlands, enabling the identification of unmet medical needs and potential therapeutic opportunities for emerging treatments. The observed treatment patterns in this study align well with our experiences in clinical practice; most patients require pharmacological treatment for MG; the majority of patients are initially treated with pyridostigmine, but this proportion subsequently declines to levels similar to those previously reported;[79] the use of corticosteroids declines after the first two years, while the use of NSIST increases during the disease course.

Our analysis shows that ten years after disease onset, 30-40% of patients continue to rely on corticosteroids, although the national guideline emphasizes the need for efforts to minimize their use. These findings are supported by previous studies that, while describing a shorter disease duration of 3 and 5 years, report similar percentages of patients using

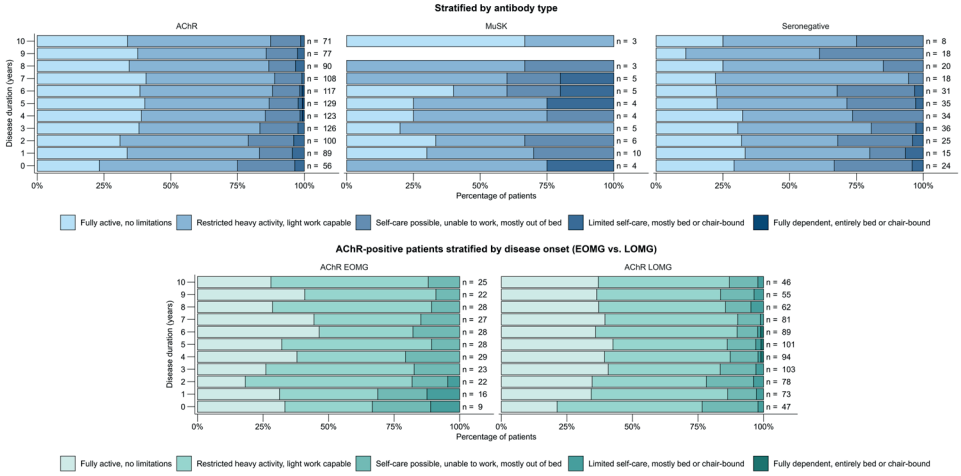

Figure 6.3 Functional status by disease duration in different subgroups of MG patients
Abbreviations: AChR, acetylcholine receptor; MuSK, muscle-specific kinase; EOMG, early-onset myasthenia gravis; LOMG, late-onset myasthenia gravis

corticosteroids (42%,[179] and 45%,[180] respectively). Although, based on our data, we cannot differentiate between patients who use corticosteroids chronically and those requiring (short-term) corticosteroids to manage disease fluctuations, these findings suggest an ongoing need for alternative treatment options.

In this study, we described treatment patterns among seronegative MG patients. Since antibody status is only assessed at the time of registry inclusion, it cannot be ruled out that some of these patients may have tested positive for antibodies later in the disease course. Nonetheless, we demonstrate that this group received fewer treatments overall and was less frequently treated with immunosuppressive therapies. Interestingly, they did not appear to receive IVIg or plasma exchange less often.

In addition, it is notable that patients with LOMG more frequently received immunosuppressive treatment compared to those with EOMG, despite EOMG being associated with a more refractory disease course.[181,182] One possible explanation is that patients with EOMG more often require treatments such as IVIg and plasma exchange, thereby reducing the need for long-term immunosuppressive therapies.

During the first ten years after disease onset, 10-15% of patients reported treatment with IVIg annually, and a smaller proportion (<3%) of patients reported treatment with PE. The proportion of IVIg use reported in this study is higher than previously described; earlier studies have reported proportions ranging between 2% and 10%.[180,183,184] Several factors may explain this difference. More severe patients may have been overrepresented in our

cohort due to the voluntary nature of the registry and the fact that patients treated at the national expert center, who are often more complex cases, are more likely to be encouraged to join the registry than those treated in other hospitals. Moreover, in the Netherlands, IVIg is easily accessible, with the option for home administration, and it is fully reimbursed under the basic health insurance package, which may contribute to its frequent use.

This study has several limitations. First, treatment data in the registry are patient-reported, making them susceptible to subjectivity and recall bias. For example, patients may have misunderstood the question regarding treatment use at the time of data collection or within the past three months, and may have reported treatments they had ever used instead. Furthermore, although cyclophosphamide is queried in the registry, to our knowledge, it is not prescribed in the Netherlands for this indication. It is possible that patients may have confused it with ciclosporin, or that they were treated with cyclophosphamide for other indications. Second, information on medication dosages is not collected, limiting conclusions regarding treatment intensity. Third, treatment data are recorded annually and reflect therapies received either at the time of reporting or in the preceding three months. As a result, short-term treatments such as corticosteroids, IVIg, or PE administered in the preceding nine months may not have been captured. Fourth, the number of patients with MuSK MG was limited in this cohort. Therefore, the results for this subgroup should be interpreted with caution. Fifth, validated outcome measures (such as the MG-ADL) are unfortunately not collected in the registry. Therefore, we chose to use the question regarding functional outcome as a proxy for disease severity. As this is not a validated measure, it limits the interpretability of our findings. Sixth, data on thymectomy were missing in a substantial number of patients (44%), which may have affected the reliability of related analyses. Finally, the vast majority of patients have been treated in the Netherlands; differences in healthcare systems and reimbursement policies in other countries may influence treatment practices and limit generalizability. Despite these limitations, we believe that this real-world analysis provides important insights into treatment patterns throughout the disease course in patients with MG.

In conclusion, in this study we demonstrate that a substantial proportion of patients continues to rely on corticosteroids or IVIg/PE throughout the course of the disease. These findings suggest an ongoing need for alternative therapeutic options. The introduction of novel therapies may help to address these challenges, but their long-term impact on treatment patterns will need to be evaluated in future research.

CHAPTER

7

Efgartigimod in refractory myasthenia gravis

Efgartigimod in refractory autoimmune myasthenia gravis
Muscle & nerve, 2024, 70(3), 325-332

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Abstract

Introduction/Aims

Efgartigimod, a neonatal Fc-receptor inhibitor, has recently been approved as treatment for myasthenia gravis (MG). In this retrospective cohort study, we aimed to systematically assess short- and long-term effectiveness of efgartigimod in patients with refractory MG.

Methods

Sixteen patients with refractory autoimmune acetylcholine receptor MG were treated with efgartigimod. Data were collected from January 2021 to March 2023 on Myasthenia Gravis Activities of Daily Living (MG-ADL), Quantitative Myasthenia Gravis score (QMG), Myasthenia Gravis Composite score (MGC) and the 15-item revised version of the Myasthenia Gravis Quality of Life questionnaire (MG-QoL15r).

Results

A favorable outcome was seen in 56% of patients at the last measurement. Out of 16 patients, 50% were an MG-ADL responder after the first treatment cycle. After four weeks, a clinically meaningful improvement compared to baseline was seen on the MG-ADL, QMG, and MGC. There was a statistically significant improvement on the MGQoL15r from baseline to week 4. The improvement was maintained until the last measurement for the MGC and the MGQoL15r. At the last visit, all patients had discontinued 4-weekly dosages, shifting to administration frequencies of one, two, or three weeks. Drug doses could be decreased for prednisolone (n=7), azathioprine (n=2) and intravenous immunoglobulins (n=9). Frequency of plasma exchange was decreased in nine patients.

Discussion

In patients with refractory MG, efgartigimod was effective for at least half of all patients. Patients required more frequent dosing compared to the ADAPT phase 3 trial. In 80% of the patients concurrent medication could be reduced or ceased.

Introduction

In recent years, the neonatal Fc receptor has become an important target for new therapies in several antibody-mediated autoimmune diseases, including myasthenia gravis (MG).[185,186] In 2021, the efficacy and tolerability of intravenous efgartigimod, a neonatal Fc receptor antagonist, was demonstrated in patients with generalized MG.[62] Efgartigimod was effective in improving muscle strength and overall disease status in patients with generalized MG. Treatment was generally well-tolerated, with the most common adverse events being mild to moderate in nature. Interim results of the ADAPT+ have shown the long-term efficacy and safety of efgartigimod in generalized MG.[187] However, its effectiveness in patients with refractory MG has not been established. Although there are various definitions of refractory MG,[25,57,129,188,189] it is estimated that in approximately 10 percent of patients with generalized MG, conventional therapies are not sufficient.[190] Patients with refractory MG are generally excluded from clinical trials due to the requirement of intensive treatment with multiple drugs, chronic IVIg or plasma exchange and frequent adjustments in the dosing of their medical treatment.

Following approval by the European Medicines Agency, an expanded access program (EAP) was initiated to provide treatment with efgartigimod to patients prior to marketing authorization. In this study, we present the results of long-term treatment with efgartigimod in a cohort of patients with refractory MG.

Methods

Patients

In this retrospective cohort study, all patients with MG in whom treatment with efgartigimod was initiated between January 2021 and March 2023 as part of the EAP, were included. Patients were treated at the department of neurology at Leiden University Medical Center, a tertiary center for the treatment of MG in the Netherlands. As efgartigimod at the time of treatment was not a registered drug in the Netherlands, efgartigimod was provided by argenx (Ghent, Belgium) on a named patient basis. A named patient program is a regulatory mechanism enabling individual patient access to investigational drugs for serious medical conditions outside of clinical trials, with strict oversight from the supervisory authority responsible for monitoring the quality and safety of healthcare and youth care services in the Netherlands, the Health and Youth Care Inspectorate. All requests for access to efgartigimod were unsolicited. Apart from providing efgartigimod for use, argenx was not involved in any clinical decision, nor did the manufacturer contribute to the process of data collection and data analysis. The eligibility criteria for approval for the EAP were: clinical

criteria of MGFA class II, III, IVa, or IVb; patient's screening Myasthenia Gravis Activities of Daily Living (MG-ADL)-score of at least 5, with at least 50% of the total score attributed to non-ocular symptoms; treatment with pyridostigmine for at least 4 weeks; treatment with prednisolone for at least 6 weeks; treatment for at least 3 months with at least one of the following: azathioprine, mycophenolate mofetil, cyclosporine, or methotrexate. If medication had to be discontinued due to side effects, minimum treatment periods did not apply as a restriction.

Moreover, we required all patients to have refractory MG, as defined by 1) unsatisfactory functioning due to either persistent MG symptoms or side effects of current treatments, without clinical evidence of other significant serious diseases 2) treatment with two or more immunosuppressive therapies for 12 months without symptom control OR at least one immunosuppressive therapy with intravenous immunoglobulin or plasma exchange given at least four times per year6. The decision whether or not to treat a patient with efgartigimod was made as part of clinical care through collaborative consultation between the treating physician and the patient.

Treatment

Efgartigimod was administered intravenously at a dose of 10 mg/kg with one infusion per week during four weeks. All efgartigimod administrations were given in the hospital. After four weeks the dosing frequency was adjusted based on clinical symptoms, IgG level and side effects at the discretion of the treating physician. All participating patients were evaluated weekly up to 4 weeks. Thereafter outcome measures were recorded at each hospital visit. The decision to initiate rescue treatment was at the treating physician's discretion.

Outcome measures

All measurements conducted were performed within the scope of routine clinical care. In order to fully optimize and understand the clinical effect of this novel drug on patients within the EAP, we established a standardized evaluation protocol, consisting of QMG, MG-ADL, MGC and the 15-item revised version of the Myasthenia Gravis Quality of Life questionnaire (MG-QoL15r) before each administration of efgartigimod. Measurements were conducted by a trained nurse practitioner. A clinically meaningful improvement was defined as an improvement of ≥2 points (MG-ADL) or ≥3 points (QMG + MGC) from baseline11. A responder was defined as a patient who had an improvement of ≥2 points (MG-ADL) or ≥3 points (QMG + MGC) at 4 weeks, sustained for at least 4 consecutive weeks. In cases where patient data was not available at 8 weeks, the closest available value was used. Demographic and clinical data (age, sex, medical history, clinical phenotype of MG, laboratory tests including IgG levels and acetylcholine receptor (AChR) antibody titers), comorbidities and concomitant medications or procedures were retrospectively collected. Chronic

intravenous immunoglobulin therapy (IVIg) or chronic plasma exchange was defined as treatment given periodically (at predetermined fixed intervals) or any patient who had received ≥4 treatment cycles in the previous year. To establish baseline IgG values, we obtained the most recent IgG level before the introduction of efgartigimod that was unaffected by plasma exchange or IVIg. IgG levels determined within 28 days after administration of IVIg12 and within 6 weeks after plasma exchange13 were excluded in all analyses. The semi-quantitative determination of AChR antibodies in serum was performed in the context of clinical practice by using a standard dilution of the sample in a radioimmunoassay (RSR Limited, Cardiff, UK). This assay provides a numerical value that is linearly correlated with actual serum concentrations up to a value of 20 nmol/L. A favorable outcome was defined as: 1) a clinically meaningful improvement on at least two outcomes measures (MG-ADL, QMG or MGC) or 2) discontinuation of prednisolone or a prednisolone dose reduction of >10 mg per day or 3) discontinuation of chronic plasma exchange or IVIg, and 4) no use of rescue medication.
All data were gathered in the process of routine clinical care and a formal medical ethical approval was therefore not required for this particular study according to Dutch law. All patients provided written informed consent for the collection and analysis of these data.

Statistical analyses

Sample size calculations were not performed given the descriptive nature of this study. Demographic and clinical data at baseline were evaluated using descriptive statistics. A Kaplan-Meier plot was generated to illustrate efgartigimod persistence over time. For the analysis of clinical effectiveness, the mean change from baseline for each outcome measure (MG-ADL, QMG, MGC, MG-QoL15r) was evaluated. Analyses of change from baseline for each visit were performed using the paired t-test. Missing values were not imputed.
All statistical analyses were performed with IBM SPSS Statistics (version 25.0). All graphs were made with GraphPad Prism software (version 9.3.1 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com").

Results

Baseline characteristics

Efgartigimod was evaluated in sixteen patients. Median follow-up time was 29.5 (IQR 10-50.5) weeks. Demographics and clinical characteristics at baseline are summarized in **Table 7.1**. All patients met the criteria of refractory MG. At baseline, before the initiation of efgartigimod, four patients were treated with chronic IVIg, and five patients with chronic plasma exchange.

Treatment characteristics

A total of 309 efgartigimod infusions were provided until data cutoff. Treatment characteristics are summarized in **Table 7.2**.

At the last visit, none of the patients were still undergoing treatment in cycles of 4-weekly dosages. All patients switched to a one, two or three weekly frequency of administrations (**Figure 7.1**).

Table 7.1 Baseline characteristics

	All patients (n=16)
Age (years) , mean (SD)	47.3 (16.8)
Sex	
Male	5 (31%)
Female	11 (69%)
Myasthenia gravis duration (years) , median (IQR)	12 (4-17)
Previous thymectomy	12 (75%)
Previous thymoma	6 (38%)
Immunomodulatory treatments at baseline	
Corticosteroids	11 (75%)
Any NSIST	11 (75%)
Intravenous immunoglobulin therapy	
≤4 weeks before efgartigimod initiation	4 (25%)
Multiple treatments in the preceding year	9 (56%)
Plasma exchange	
≤6 weeks before efgartigimod initiation	7 (44%)
Multiple treatments in the preceding year	9 (56%)
No treatment[$]	1 (6%)
History of immunomodulatory treatments	
Corticosteroids	16 (100%)
NSIST	
1	3 (19%)
2	7 (44%)
≥3	6 (38%)
Intravenous immunoglobulin therapy	15 (94%)
Plasma exchange	14 (88%)
Outcome measures, mean (SD)	
MG-ADL	7.9 (4.5)
QMG	14.1 (7.0)
MGC	13.1 (6.9)
MG-QoL15r	17.8 (7.5)

Data are n (%) unless otherwise specified. Abbreviations: NSIST, non-steroidal immunosuppressant therapy.[$] Patient declined to take any medication.

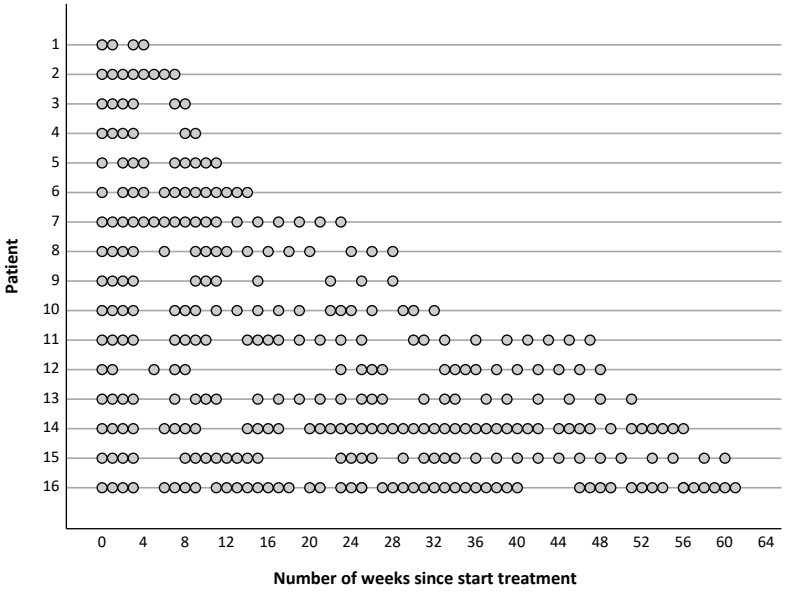


Figure 7.1 Efgartigimod administrations since treatment start for each individual patient

Clinical effectiveness

Data of 16 patients, were evaluated at 4 weeks after start of the treatment. At 4 weeks, one patient had received 2 infusions of efgartigimod, all other patients received 4 infusions. Eight (50%) were an MG-ADL responder (**Table 7.3**). Nine patients fulfilled our criteria for a favorable outcome (56.3%) (**Table 7.2**).

Four patients continued the use of efgartigimod, although they did not fulfill the criteria for a favorable outcome. For three patients, the decision to continue efgartigimod was based on the observed subjective clinical improvement by the patient and the clinician.

A clinically meaningful improvement compared to baseline was seen on the MG-ADL (mean -4.37 points, 95% CI [-2.39 to -6.36], p<0.001), QMG (mean -4.93 points, 95% CI [-2.42 to -7.45], p<0.001), and MGC (mean -7.60 points, 95% CI [-4.48 to -10.72], p<0.001). The MGQoL15r showed a statistically significant improvement between baseline and week 4 (mean -5.75 points, 95% CI [-2.16 to -9.35], p=0.005) (**Figure 7.2**).

The last measurement was performed at a median of 28 weeks (IQR 9-52) for the MG-ADL, 16 weeks (IQR 5-28) for the QMG, 20 weeks (IQR 8-48) for the MGC and 24 weeks (IQR 14-33) for the MG-QoL15r. The improvement was sustained until the last measurement for the MGC (mean -3.31 points, 95% CI [-0.39 to -6.24], p=0.029 and the MGQoL15r (mean -4.71

points, 95% CI [-1.62 to -7.81], p=0.006), but not for the MG-ADL (mean -2.31 points, 95% CI [0.15 to -4.78], p=0.064) and QMG (mean -2.25 points, 95% CI [0.39 to -4.89], p=0.089). All four patients who discontinued treatment with efgartigimod, stopped within the first hundred days of treatment (**Figure 7.3**). Out of these four patients, three patients discontinued because of lack of efficacy. One patient declined further treatment with efgartigimod for reasons not further specified. Rescue medication was used in five patients (**Table 7.2**) and consisted of plasma exchange (n=5), intravenous immunoglobulins (n=1), prednisolone (n=3), (re)start of non-steroidal immunosuppressant drugs such as azathioprine (n=1), mycophenolate mofetil (n=1), rituximab (n=2) and eculizumab (n=2). Median time until the first rescue medication was 56 days (IQR 24-126 days).

One patient did not use any medication at the initiation of efgartigimod. Out of the other 15 patients, 12 (80%) were able to reduce any medication. In seven patients, the dose of prednisolone could be lowered (**Figure 7.4A**). The median decrease in prednisolone dose was 5 mg (IQR 0 to 20 mg). Out of six patients, three patients were able to lower the dose of azathioprine (**Figure 7.4B**). In all thirteen patients treated with IVIg or plasma exchange, either as needed or chronically, in the period before the initiation of efgartigimod, the number of IVIg doses (**Figure 7.4C**) and plasma exchanges (**Figure 7.4D**) was lower after the initiation of efgartigimod.

Table 7.2 Efgartigimod treatment characteristics

	All patients (n=16)
Favorable outcome	9 (56%)
Discontinued treatment	4 (25%)
Treatment duration (days), median (IQR)	208 (78-352)
Number of doses, median (IQR)	17 (9-25)
Lowest IgG level achieved, mean (SD)	3.4 (±1.3)
Max. IgG change vs. baseline[#] (%), mean (SD)	64.8 (±13.8)
Rescue medication	5 (31%)

Data are n (%) unless otherwise specified. [#]Missing values, n=7

Table 7.3 Summary of outcome measures

	MG-ADL	**QMG**	**MGC**
Clinically meaningful improvement			
4 weeks	10/16 (63%)	9/15 (60%)	11/15 (73%)
Last measurement	7/16 (44%)	8/15 (53%)	8/15 (53%)
Responder	8/16 (50%)	6/11 (55%)	5/10 (50%)

Abbreviations: MG-ADL, Myasthenia Gravis Activities of Daily Living; MGC, Myasthenia Gravis Composite score; QMG, Quantitative Myasthenia Gravis score

IgG levels and AChR antibody titers

In 9 patients, changes in IgG levels from baseline were analyzed (**Table 7.2**). **Figure 7.5** shows serum IgG levels and AChR antibody titers for each patient during treatment with efgartigimod. Despite frequent dosing, the lowest IgG level achieved stayed above 2.0 g/L in 15 out of 16 patients. In only one patient, an IgG level of 1.7 g/L was seen, which returned to levels above 2.0 g/L after the discontinuation of efgartigimod. Patients who empirically ended up with a treatment schedule with weekly administrations demonstrated a consistently higher AChR antibody titer throughout the entire treatment period compared to patients who were in need of treatment every two or three weeks.

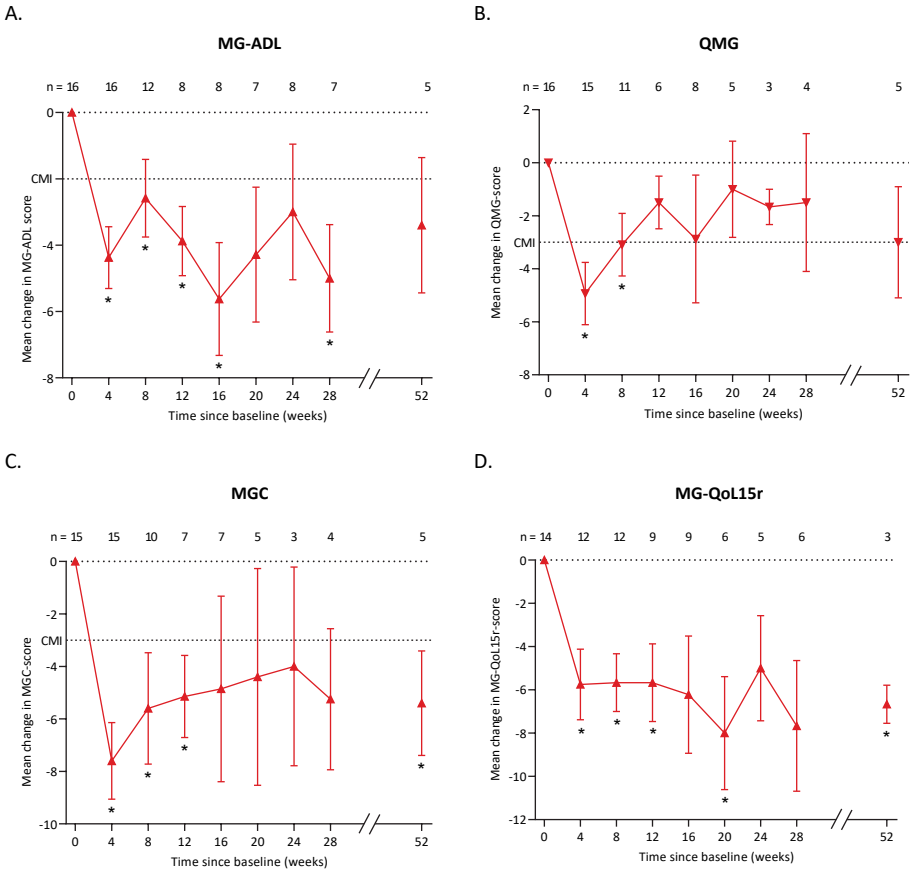


Figure 7.2 Mean change in Myasthenia Gravis Activities of Daily Living (A), Quantitative Myasthenia Gravis score (B), Myasthenia Gravis Composite score (C), and 15-item revised version of the Myasthenia Gravis Quality of Life questionnaire (D) during 28 weeks and at 52 weeks

*During the first four weeks efgartigimod was administered weekly, after these four weeks the dosing frequency was adjusted at the discretion of the treating physician. *Indicates a statistically significant change from baseline (P<0.05). Error bars show standard error. CMI; clinically meaningful improvement.*[191]

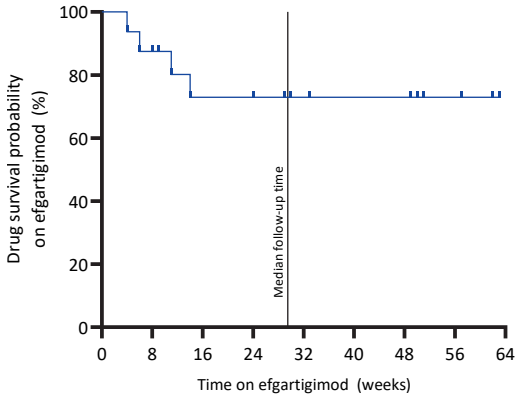


Figure 7.3 Kaplan-Meier curve illustrating efgartigimod persistence in 16 patients with refractory myasthenia gravis
Median follow-up time 29.5 weeks (interquartile range 10-50.5).

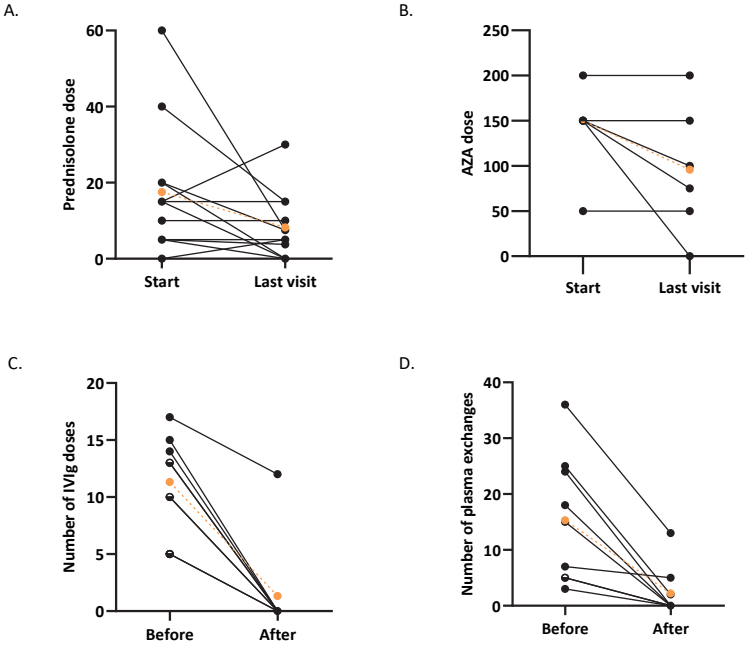
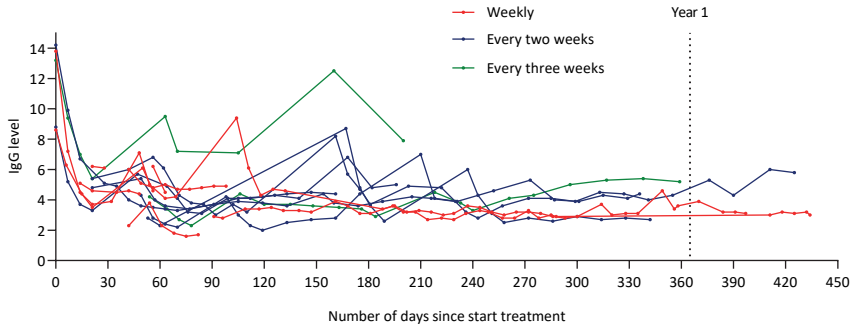


Figure 7.4 Change in prednisolone dose (panel A) and azathioprine dose (panel B) from start of efgartigimod to the last visit for each patient and change in number of IVIg doses (panel C) and plasma exchanges (panel D) in the period before and after start of efgartigimod for each patient
The orange line plot represents the mean change. Abbreviations: AZA, azathioprine; IVIg, intravenous immunoglobulin.

A.



B.

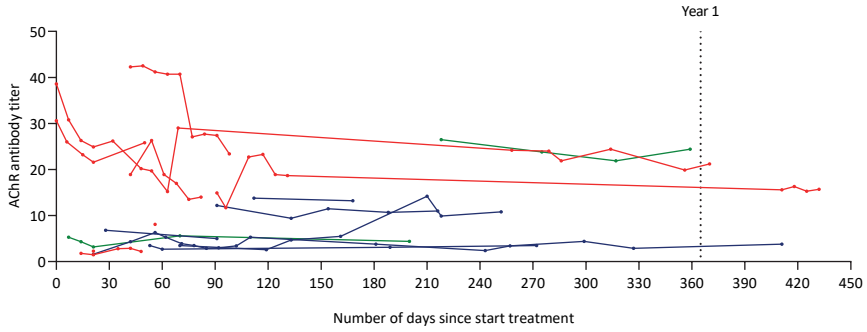


Figure 7.5 A. Serum immunoglobulin (IgG) levels and B. Acetylcholine receptor antibody titer after treatment with efgartigimod for each patient. Red, blue and green colors represent patients with different regimens of efgartigimod dosing at the last visit
IgG levels and AChR antibody titers determined within 4 weeks after treatment with IVIg and within 6 weeks after plasma exchange are excluded.

Discussion

In this retrospective study efgartigimod was effective for at least half of all patients; in 56% of patients a favorable outcome was seen at the last measurement, even though our cohort comprised only patients with refractory MG. The proportion of patients who were MG-ADL responders after 4 weeks of treatment was lower than in the ADAPT phase 3 trial (50% versus 68%).[62] Even in a subgroup analysis of patients with refractory myasthenia gravis in ADAPT, the MG-ADL responder rate was higher (67.5%) than what was observed in our cohort.[192] Other clinical outcome measures (a clinically meaningful improvement on MG-ADL, QMG, MGC and MG-QoL15r) were comparable to the results of the ADAPT-trial after 4 weeks.

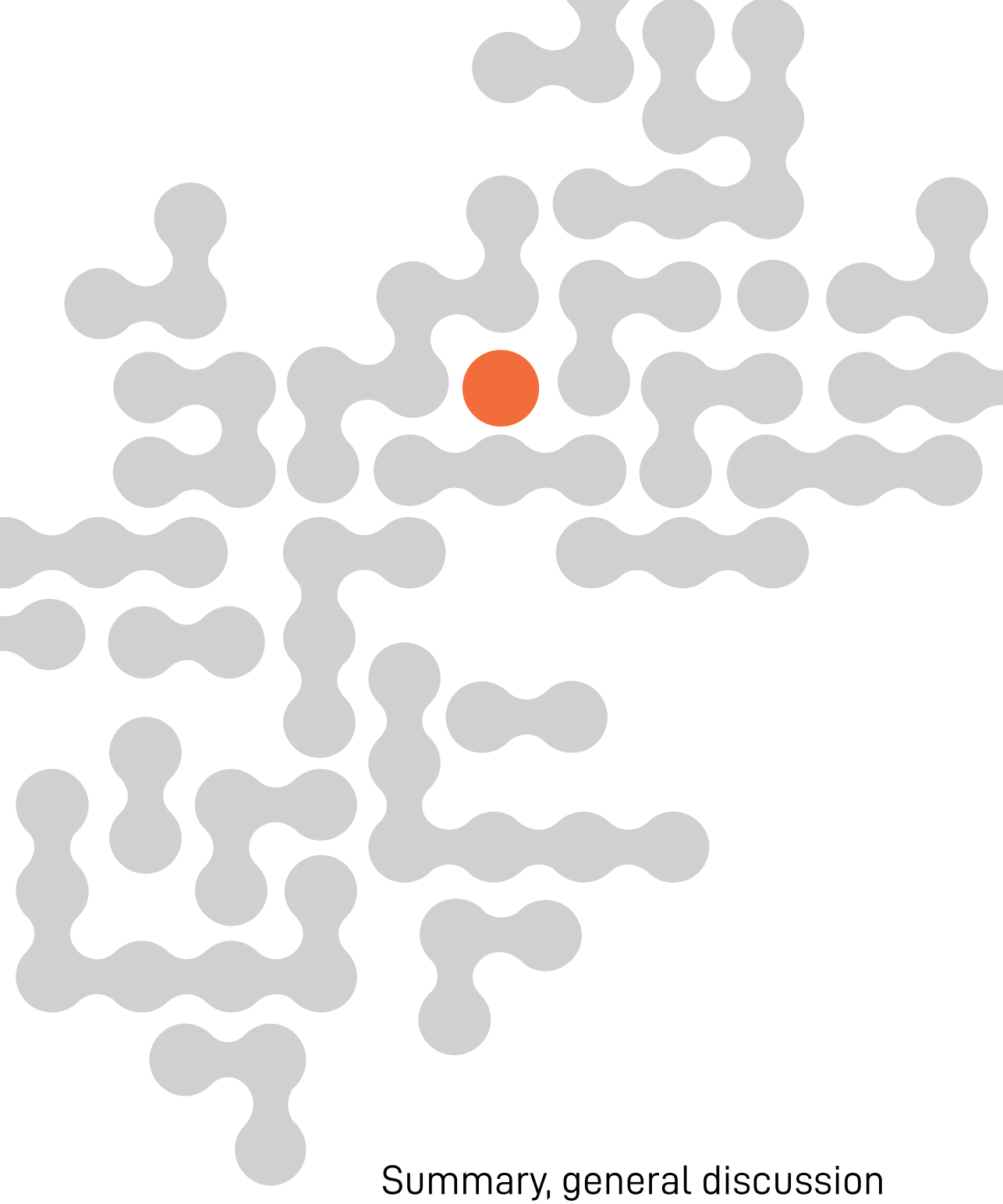
The data in this cohort suggest that, in the long term, the effect of efgartigimod does not appear to persist; patients seem to exhibit a worsening of symptoms after 8-12 weeks of treatment (QMG, MGC and MG-ADL). This observation could suggest reduced effectiveness of efgartigimod, or it may be attributed to withdrawal of concomitant medication. In contrast, long-term results of the MG-QoL15r appear to indicate a persistent effect, and a significant number of patients (80%) were able to reduce other MG treatments such as prednisolone, azathioprine, plasma exchange and IVIg. However, a crucial caveat should be noted. The interpretation of these long-term effects is complicated by the diminishing number of patients contributing to the outcome over time, rendering these data less representative. Patients in our cohort required frequent dosing of efgartigimod. At the last visit, all patients switched to a one, two or three weekly frequency of administrations. Interestingly, with a regimen of more frequent administrations, IgG levels remained stable, and no further reductions below safety thresholds were seen.

This study has some limitations. First, we did not systematically evaluate side effects of efgartigimod in our dataset; however, no major adverse events occurred, and no patients discontinued efgartigimod due to side effects, indicating that efgartigimod was well tolerated. Secondly, several measurements had to be omitted from subsequent analyses because a substantial portion of patients (69%) underwent treatment with either plasma exchange or IVIg in the month before the initiation of efgartigimod. Consequently, the analyses of changes from baseline for both IgG levels and outcome measures are based on a limited number of patients and should be interpreted with caution. Thirdly, the determination of the AChR antibody titers was performed infrequently (**Figure 7.5)** and values were not always within the linear range of the assay. Therefore, it was not possible to correlate change in AChR antibody titers to the IgG levels or to clinical outcome measures. Lastly, due to the retrospective nature of this study, assessment of treatment outcomes was not mandatory and data from a considerable proportion of patients was lacking on various outcomes during the follow-up period, resulting in a potential risk of bias in the estimation of these outcome measures.

In conclusion, efgartigimod was shown to be effective in a relevant subset of patients with refractory MG. However, patients required more frequent dosing and fewer patients showed a significant improved MG-ADL response compared to the ADAPT trial. Our experience provides insights to better delineate the long-term effectiveness in a cohort of patients with refractory MG.

CHAPTER

8



Summary, general discussion and future perspectives

Summary

The aim of this thesis was to provide an overview of the therapeutic landscape for patients with myasthenia gravis (MG), with Part I focusing on existing and emerging symptomatic treatment strategies, and Part II on immunomodulatory therapies and their current use in clinical practice.

Part I: Symptomatic treatment

Chapter 2 presents an extensive literature review on symptomatic therapies for MG. These therapies target the neuromuscular junction directly, although for certain drugs like sympathomimetics, their precise mode of action remains unclear.
Three main groups of currently available therapies are described; acetylcholinesterase inhibitors, potassium channel blockers, and sympathomimetic agents. Acetylcholinesterase inhibitors like pyridostigmine and ambenonium chloride, enhance cholinergic transmission by inhibiting the breakdown of acetylcholine in the synaptic cleft. According to national and international guidelines, pyridostigmine is widely accepted as the cornerstone of symptomatic treatment for MG. This is based on observational studies, the earliest of which date back to the mid-1930s, as well as decades of clinical experience. However, no randomized clinical trial has quantified its effect size, and systematic evaluation of its side effect burden is lacking. As a result, it remains challenging to position pyridostigmine relative to other symptomatic therapies. The role of short-acting potassium channel blockers (e.g., amifampridine), which enhance acetylcholine release by prolonging the presynaptic action potential, as well as sympathomimetic agents (e.g., ephedrine, salbutamol, terbutaline), remains unclear due to a lack of robust comparative studies.
In addition to currently available treatments, three experimental compounds are discussed: CLC-1 chloride channel inhibitors, fast-skeletal muscle troponin activators, and antisense oligodeoxynucleotides. The latter two are no longer under active development, limiting their future clinical applicability. CLC-1 chloride channel inhibitors appear to be promising with potentially fewer side effects than the current standard. Hopefully, phase III trials will provide greater insight into their efficacy and help clarify their role in the symptomatic treatment of MG patients.

In **chapter 3** we characterized the effectiveness and side effects of pyridostigmine in a cross-sectional study. Data were collected from 410 out of 642 invited patients participating in the Dutch-Belgian myasthenia patient registry. In a dynamic, for this study developed questionnaire, effectiveness, side effects and net benefit were evaluated. Almost all patients

reported to have used pyridostigmine at some point during the course of their disease, with approximately two thirds (62%) continuing its use. The reported net benefit and effectiveness were moderate with frequently reported side effects. Out of all patients currently using pyridostigmine, 91% reported at least one side effect compared to 54% in the control group. The most frequently reported side effects included gastro-intestinal symptoms (flatulence, diarrhea, and abdominal cramps), urinary urgency, muscle cramps, blurred vision, hyperhidrosis, increased salivation, light-headedness and flu-like symptoms. Side effects were cited as the primary reason for discontinuation by 26% of respondents. Factors associated with discontinuation of pyridostigmine were male sex and the presence of MuSK antibodies.

In **chapter 4** and **chapter 5** we describe the results of IMPACT-MG, a randomized double-blinded, placebo controlled crossover trial that evaluated the efficacy and cost-effectiveness of pyridostigmine (part one), followed by a second randomization to assess the efficacy and safety of amifampridine in combination with pyridostigmine (part two) in patients with AChR MG.

The findings of part one of IMPACT-MG are described in **chapter 4**. Nineteen participants were assigned to two five-day treatment periods separated by a two-day washout. Participants received pyridostigmine followed by placebo, or vice versa. All participants had been using pyridostigmine chronically before enrollment, and their trial dose was identical to their usual pre-study dose.

The primary endpoint was the within-patient difference in MGII score, a validated score measuring MG disease severity, during pyridostigmine use compared to placebo. Secondary outcome measures included the MG-ADL and QMG, evaluating efficacy; the MG-QoL15r, evaluating quality of life; and the TSQM-9, evaluating treatment satisfaction. Adverse events were systematically recorded, and an economic evaluation was performed from both societal and healthcare perspectives.

Treatment with pyridostigmine improved the MGII score by -5.3 points compared with placebo. All secondary endpoints also favored pyridostigmine (QMG -1.4 points, MG-ADL -1.2 points, MG-QoL15r -2 points, and TSQM-9 7.2 points). Adverse events occurred more frequently during pyridostigmine use than during placebo, with fatigue and flatulence being the most commonly reported events. In the cost-utility analysis, pyridostigmine treatment showed lower annual healthcare and societal costs, and annual improved QALYs, compared to treatment without pyridostigmine. In addition, the probability of cost-effectiveness at a willingness-to-pay threshold of €20,000 per QALY was high (>99%), from both societal and healthcare perspectives.

Chapter 5 presents the findings from the second part of IMPACT-MG, which evaluated the addition of amifampridine to treatment with pyridostigmine. Amifampridine is a presynaptic potassium channel blocker that prolongs the action potential and thereby enhances acetylcholine release in the synaptic cleft. It is primarily used in patients with Lambert-Eaton myasthenic syndrome and congenital myasthenic syndrome, while its use in AChR MG has been hypothesized but has not previously been studied in randomized controlled trials.
To be eligible for participation in part two, patients had either completed part one of the study or failed to complete the two-day washout period prior to randomization in part. A total of twenty patients were randomized to one of three treatment sequences, each consisting of three treatment periods of five days, separated by a two-day washout period. During these periods, patients received either 30 mg amifampridine, 60 mg amifampridine, or placebo. The primary and secondary efficacy and safety outcomes were identical to those used in part one. An additional pharmacokinetic/pharmacodynamic (PK/PD) substudy was conducted to assess PK parameters and evaluate potential dose-response relationships.
For the primary outcome, measured by the MGII score, the mean treatment difference compared with placebo was -1 point for amifampridine 30 mg and -1.7 points for amifampridine 60 mg. Similar to the primary outcome, no statistically significant differences were observed across any of the secondary efficacy endpoints. In contrast, adverse events occurred more frequently during treatment with amifampridine. The most commonly reported events included paresthesia, fatigue, numbness, dizziness, and sleep disturbances. In three cases, treatment-emergent adverse events were severe enough to necessitate discontinuation of amifampridine. Within the PK/PD substudy, no associations were found between mean steady-state AUCτ, and C_{max} and the primary outcome measure.

Part II: Immunomodulatory therapies

In **chapter 6**, we provide an overview of current treatment patterns in the Netherlands across different subgroups of MG patients throughout the disease course. We analyzed longitudinal data from adult patients enrolled in the Dutch-Belgian Myasthenia Registry, stratifying them by age at disease onset (≤50 years as early-onset MG [EOMG], and >50 years as late-onset MG [LOMG]) and antibody status (AChR, MuSK, and seronegative). Within the first year after diagnosis, the majority patients initiated pyridostigmine (93%), and after five years, approximately two-thirds continued its use. Furthermore, this study demonstrates that a substantial proportion of patients remains dependent on corticosteroids ten years after disease onset (30-40%). In addition, about one third of patients reported the use of intravenous immunoglobulin therapy at some point during the course of the disease, and 16% of patient reported treatment with plasma exchange.

In **chapter 7** we describe our clinical experience with sixteen patients with refractory MG treated with efgartigimod, an FcRn blocker, in a retrospective cohort study. A favorable outcome was defined as either a clinically meaningful improvement on at least two outcome measures (MG-ADL, QMG, or MGC) or discontinuation of prednisolone, a prednisolone dose reduction of >10 mg per day, or discontinuation of chronic plasma exchange or IVIg, without the use of rescue medication. A favorable outcome was observed in 56% of patients at the last assessment. Moreover, in 80% of patients, MG-related treatments (prednisolone, azathioprine, plasma exchange, and IVIg) were reduced compared with treatment prior to efgartigimod. All patients had discontinued the treatment regimen as used in the pivotal ADAPT-trial (one infusion per week for four weeks, followed by a variable interval before the next treatment cycle) to administration intervals of 1, 2, or 3 weeks.

General discussion

Treatment of myasthenia gravis (MG) consists of two main therapeutic approaches: symptomatic therapy with pyridostigmine on the one hand, and immunomodulatory treatments on the other, including agents such as prednisolone, non-steroidal immunosuppressive agents, intravenous immunoglobulin (IVIg), and plasma exchange. As these treatments target different aspects of MG pathophysiology, they are often used in combination to achieve disease control.
As described in chapter 6, nearly all patients (93%) initiate treatment with pyridostigmine, and approximately two-thirds continue its use later in the disease course, either as monotherapy or in combination with immunomodulatory therapy. It is therefore noteworthy that, prior to the studies presented in this thesis, despite the widespread and long-standing use of pyridostigmine, systematic literature on its efficacy and adverse effects was scarce. This is of particular relevance, as information on the use of pyridostigmine not only helps to inform patients about the expected effects and side effects, but also provides a basis for comparing new symptomatic treatments with the current standard.

When considering the effect size of pyridostigmine, many (myasthenia) experts may recall cases with remarkable responses to a single dose of an acetylcholinesterase inhibitor. However, chapter 3 shows that only a small minority of patients reported experiencing a very good effect on ocular, bulbar or generalized symptoms, indicating that such remarkable responses are at least uncommon.
In chapter 4, we quantified the effect size of pyridostigmine on outcomes reflecting myasthenia severity (MGII, MG-ADL and QMG), quality of life (MG-QoL15r), and treatment

satisfaction (TSQM-9) in a randomized double-blinded, placebo-controlled crossover trial. All measures demonstrated statistically significant, yet rather small, improvements compared with placebo. When comparing these results to the predefined thresholds for clinically meaningful improvement, the cutoff value for the primary outcome measure (MGII) was found to lie within the 95% confidence interval. This implies that a clinically meaningful effect cannot be excluded. For the secondary outcome measures, the MG-ADL and the QMG, the predefined thresholds for clinically meaningful change fell outside the corresponding 95% confidence intervals. This raises the question of whether this simply indicates that pyridostigmine does not produce a clinically meaningful improvement (on our secondary outcome measures) within this specific population, or whether the established cutoff values may not be truly appropriate within this therapeutic context with a symptomatic treatment such as pyridostigmine. To address this question, it is useful to provide some background on how a Minimal Clinically Important Difference (MCID) is determined. Two main approaches can be distinguished: anchor-based and distribution-based methods. In the anchor-based approach, the change in the outcome measure is linked to an external reference, such as a global rating of change or a clinical outcome measure. This method is therefore highly dependent on the choice of reference. In the distribution-based approach, the MCID is derived from the statistical distribution of the measurements, for example by using 0.5 standard deviation as an approximation of a clinically meaningful improvement. While this method is more objective, it does not capture the subjective perception of clinical benefit. For both approaches, the values obtained are strongly influenced by the study population and the treatment under investigation. For the MGII, for example, the MCID was established in a population receiving prednisone, IVIg, and PLEX.[156] The nature of these treatments is fundamentally different from that of pyridostigmine, as they are more invasive and often associated with more (severe) adverse effects. Consequently, a clinically meaningful improvement with these treatments would be expected to be larger than with a symptomatic treatment like pyridostigmine. This should be taken into account when interpreting the results; rather than considering the MCID as a gold standard, it should be viewed in the context of the specific study population and the treatment under investigation.

When comparing our study population with other MG-trials, patients enrolled in IMPACT-MG had milder symptoms, as expected for a symptomatic treatment rather than an immunomodulatory therapy. However, this implies that the potential for improvement in IMPACT-MG was smaller than in populations with a higher disease burden, which likely contributed to the observed small effect size. This anticipated limitation was one of the reasons why the MGII was chosen as the primary endpoint rather than the more commonly used MG-ADL or QMG, as the MGII is known to have minimal floor effects compared with other outcome measures.[163]

This may explain why a clinically meaningful improvement could not be excluded for the MGII (as the cut-off value for a clinically meaningful difference fell within the 95% confidence interval of the mean difference between pyridostigmine and placebo) and, in contrast, could not be demonstrated for the MG-ADL and the QMG (for which the cut-off value fell outside the 95% confidence interval).

Only limited research has been conducted on alternative symptomatic therapies, but in line with our study findings, the (small) trials that are available demonstrate modest effect sizes. In a double-blind, placebo-controlled, crossover pilot study, the short-term efficacy of terbutaline, a β2-adrenergic agonist, was assessed in eight patients with generalized MG.[112] The primary efficacy endpoint, a ≥3-point reduction in QMG score, was achieved in 63% of patients, with estimated mean improvements of approximately 3 points compared to placebo treatment. Similarly, in a randomized, double-blind, n-of-1 trial, four patients were treated with ephedrine, an adrenergic agonist, or placebo as add-on therapy to pyridostigmine.[31] All included patients had mild residual symptoms that did not warrant immunosuppressive therapy but were inadequately controlled by pyridostigmine monotherapy. Ephedrine produced a modest mean improvement of 1.0 points on the QMG score compared to placebo. More recently, a randomized, double-blind, placebo-controlled crossover trial evaluated the ClC-1 inhibitor NMD670 in 12 patients.[166] Two single doses of NMD670 showed statistically significant improvements in QMG scores compared to placebo, with least square mean changes of -1.5 and -1.0 points for the lower and higher doses, respectively. While these three trials demonstrate consistent statistical significance, the improvements in efficacy outcome scores with all symptomatic therapies remain modest (ranging from 1.0 to 3.0 points on the QMG score), comparable to the changes observed in our study (1.4 points).

Another important consideration is that, in our study, the observed effect reflects the difference relative to placebo rather than the change from baseline. In most clinical trials, and also in the determination of the MCID, outcomes are typically assessed as a change over time, that is, relative to baseline. Given the presence of a placebo effect, the change from baseline is likely to be larger than the change relative to placebo. This may have influenced the observed effect size and contributed to the smaller effects seen in IMPACT-MG compared with those reported in previous clinical trials. **Table 8.1** provides an overview of the most recent MG-trials investigating immunomodulatory treatments, and it presents the effect sizes relative to placebo for both the MG-ADL and the QMG. Although it is interesting to compare these differences side by side, it is important to recognize that the study populations in the newer trials and the pyridostigmine trial were not the same. Patients in the latter generally had milder symptoms, whereas those in the more recent trials were more often refractory to previous treatments. Direct comparisons of treatment

effects should therefore be made with caution. Nevertheless, it is noteworthy that when the effect size is presented relative to placebo rather than to baseline measurements, treatments such as ravulizumab and nipocalimab, for example, do not exceed the threshold for a clinically meaningful difference (≥3 for the QMG and ≥2 for the MG-ADL). This raises the question of whether a clinically relevant improvement should be assessed in relation to baseline measurements alone, or whether the placebo effect should also be taken into account.

Table 8.1 Effect sizes in phase 3 MG research relative to placebo measurements

	QMG			MG-ADL		
	Treatment	**Placebo**	**Difference**	**Treatment**	**Placebo**	**Difference**
Eculizumab[57]	-4.6	-1.7	2.9	-4.1	-2.3	1.8
Ravulizumab[59]	-2.8	-0.8	2.0	-3.1	-1.4	1.7
Zilucoplan[60]	-6.2	-3.3	2.9	-4.4	-2.3	2.1
Efgartigimod#[62]	-6.2	-1.0	5.2	-4.6	-1.8	2.8
Rozanolixizumab[64]						
7 mg/kg	-5.4	-1.9	3.5	-3.4	-0.8	2.6
10 mg/kg	-6.7	-1.9	4.8	-3.4	-0.8	2.6
Nipocalimab[65]	-4.9	-2.1	2.8	-4.7	-3.3	1.5
Pyridostigmine			1.2			1.4

Approximate values were derived from published figures, as the original numerical data were not available in the text.
QMG, Quantitative Myasthenia Gravis; MG-ADL, Myasthenia Gravis-Activities of Daily Living

One of the main strengths of IMPACT-MG is that it included a cost-effectiveness analysis. At baseline, we collected data on healthcare and productivity costs over the preceding year, along with corresponding MGII scores and utility values. Using these baseline measures, we were able to estimate how changes in MGII and utility during the two treatment periods in part one of the trial, translated into total societal costs of pyridostigmine after one year. In addition to improving quality-adjusted life years after one year, pyridostigmine was also associated with a reduction in societal costs of €6565 per patient per year. While its cost-effectiveness cannot be directly compared with that of novel treatments, our data can serve as a benchmark for evaluating the cost-effectiveness of newer therapies, which is particularly important in an era of rising healthcare costs.

Limited data on the cost-effectiveness of novel therapies are available, and the existing studies mostly estimate utilities using proxy measures, such as QMG scores, rather than directly observed values. This complicates the development of rational and cost-effective treatment strategies, and it is important to be transparent and to include cost-effectiveness data, such as utilities, when reporting efficacy outcomes.

Before extrapolating our findings to the general MG population, it is important to recognize that IMPACT-MG included a specific subset of MG patients. As noted earlier, we enrolled patients with relatively mild disease, but in addition, all participants were required to be able to temporarily discontinue pyridostigmine. Consequently, patients who experienced substantial symptomatic benefit from pyridostigmine were less likely to participate in the study or discontinued during the washout phase prior to randomization (which occurred in two patients). In an ideal scenario during the study design phase, we would have selected MG patients who had been recently diagnosed and had never received any treatment. However, we deliberately decided against this approach due to feasibility concerns, as recruiting a sufficient number of patients would likely have required a multicenter, and potentially even multinational, trial. Nevertheless, this limitation regarding patient inclusion should be considered when interpreting the observed effect size.

In chapters 3 and 4 we characterized the side effects of pyridostigmine. We showed in chapter 3 that a notable 91% of patients currently using pyridostigmine reported experiencing at least one side effect. It should be noted, however, that in this study side effects were actively queried, and patients were presented with a symptom checklist based on the Summary of Product Characteristics for pyridostigmine. It is well established that this method of actively querying side effects leads to higher reporting rates compared to spontaneous reporting.[134] Nevertheless, the 91% prevalence is substantially higher than the 54% reported in the control group, which consisted of patients not using pyridostigmine at the time of completing the questionnaire.

In chapter 3, we also showed that patients with MuSK antibodies were more likely to discontinue pyridostigmine, consistent with previous studies indicating that acetylcholinesterase inhibitors are less effective in these patients and that they experience more side effects.[126-128] In addition, female patients reported side effects more frequently than males, in line with literature showing that women are 50 to 75% more likely to experience an adverse drug reaction than men,[132] likely due to sex-based differences in pharmacokinetics and pharmacodynamics.[133]

In addition to identifying the most commonly reported side effects, which in this cross-sectional study

were gastrointestinal symptoms (flatulence, diarrhea, and abdominal cramps), urinary urgency, muscle cramps, blurred vision, hyperhidrosis, increased salivation, light-headedness, and flu-like symptoms, it is also important to consider the severity of these side effects. Patients who discontinued pyridostigmine experienced considerable discomfort from gastrointestinal symptoms, and these side effects were often reason for discontinuation or dose reduction, as has been widely recognized. Notably, hyperhidrosis was reported as particularly bothersome in both current users and those who had discontinued treatment, highlighting the importance of addressing this side effect in clinical practice.

In patients discontinuing pyridostigmine, approximately one-quarter of patients cited side effects as reason for discontinuation. This reflects a substantial subgroup of patients who do not tolerate the only registered and guideline-recommended symptomatic treatment in national and international protocols. Consequently, there appears to be a need for alternative symptomatic treatment options.

In chapter 2, we evaluated existing and emerging symptomatic therapies. The only symptomatic therapy currently under active investigation is the ClC-1 channel blocker NMD670. Other treatments, such as tirasemtiv, a fast-skeletal muscle troponin activator, and monarsen, an antisense oligodeoxynucleotide, have shown modest improvements in phase II trials; however, no plans have been announced to further develop these therapies. In chapter 5, we studied the effect of amifampridine, a well-established therapy for myasthenic disorders such as Lambert-Eaton myasthenic syndrome (LEMS) and congenital myasthenic syndromes. The use of amifampridine in patients with MG has been described, and it is occasionally prescribed off-label as an alternative to, or in addition to, pyridostigmine. Through a randomized, double-blind, placebo-controlled crossover trial, conducted immediately following the study described in chapter 4, we evaluated whether the addition of amifampridine to pyridostigmine provided added benefit.
We found no significant benefit of amifampridine compared to placebo on both primary and secondary outcome measures, while it was accompanied by an unfavorable safety profile, with a higher frequency of adverse events which led to treatment discontinuation in some patients. In addition, no association was found between pharmacokinetic parameters and the primary outcome measure.
Although several factors in the design of this part of the IMPACT-MG study may have influenced the observed outcomes, it seems unlikely that these factors played a decisive role in demonstrating the efficacy of amifampridine in addition to pyridostigmine. Even with a larger sample size, the small effect size of amifampridine (1.7 points on the MGII) raises questions about the clinical relevance of such a difference, regardless of statistical significance. Nevertheless, a few design elements are worth considering in relation to these outcomes.

- Patients were assigned a fixed dose of amifampridine (either 15 mg or 30 mg twice daily). During the trial, it became apparent that some patients preferred the lower dose, while others experienced greater benefit from the higher dose. A dose-titration phase prior to randomization was initially considered during the study design phase. However, it was ultimately decided against in order to minimize the risk that patients would become familiar with the medication and its potential side effects, which could have compromised blinding. Furthermore, PK/PD data demonstrated no correlation between drug concentrations and the primary outcome, suggesting that even with a dose-titration phase,

there would likely still have been no meaningful difference between placebo and amifampridine.

- Our power calculation was based on detecting an improvement of 8 points. However, the observed effect in the pyridostigmine study was "only" 5.3 points. Given clinical experience with amifampridine in MG, it seems unlikely that its effect size would exceed that of pyridostigmine. This would imply that we would have needed to include significantly more patients in the trial. For example, if we had aimed to demonstrate a difference of 3 points compared to placebo, a similar study design would have required over 140 patients, and to detect a difference of 1.7 points (the observed effect size in the trial), more than 435 patients would have been needed. This suggests that a substantially larger sample size might have led to a statistically significant difference; however, one could question whether such a small effect size would be clinically relevant, even for a symptomatic treatment.
- The baseline symptom severity at treatment initiation was higher in the first part of the study evaluating pyridostigmine compared to the second part evaluating amifampridine, with a baseline MGII score of 32.0 in the first part and 26.6 in the second part. This difference in baseline severity likely contributed to the small effect size found for amifampridine, as there were approximately six more points available for improvement during treatment with pyridostigmine than during treatment with amifampridine. It is possible that including patients with more severe MG symptoms could have resulted in a greater effect of amifampridine. However, one could question whether this would be the appropriate patient population for this treatment in a clinical setting.

It is noteworthy that two treatments, both of which increase the amount of acetylcholine in the synaptic cleft, show different effects in MG and LEMS. This raises questions about our understanding of the pathomechanism at the synaptic level. To understand why one treatment is more effective in MG (pyridostigmine) and the other in LEMS (amifampridine), it is important to recognize that presynaptic acetylcholine efflux from synaptic vesicles occurs directly opposite a cluster of acetylcholine receptors.[193] Although many graphic representations in textbooks suggest that acetylcholine diffuses freely across the synaptic cleft, the released acetylcholine in the synaptic cleft diffuses exclusively within a cluster of acetylcholine receptors, a "functional unit." Consequently, if a functional unit contains a sufficient number of acetylcholine receptors (as in LEMS), increasing local acetylcholine availability may have only limited benefit. In this situation, it is more effective to activate additional functional units.

In MG, an additional factor comes into play: due to the reduced number of acetylcholine receptors, acetylcholine efflux into the synaptic cleft is upregulated through cleaved LRP4 which binds to an as-yet unidentified presynaptic receptor.[16] The action of amifampridine

effectively mimics this mechanism, suggesting that amifampridine will only be effective in patients in whom this compensatory upregulation is insufficient.

In the second part of this thesis, we provide an overview of treatment patterns in the Netherlands over a period of nearly ten years (chapter 6). During this timeframe, the use of novel therapies, including complement inhibitors and FcRn blockers, remained limited in the Netherlands. A large proportion of patients relies on symptomatic treatment: the majority of patients initiate pyridostigmine within the first year after diagnosis (93%), and approximately two-thirds continues to use pyridostigmine in the long term. Furthermore, we show that a substantial proportion (30-40%) of patients remain dependent on corticosteroids ten years after disease onset. The latter is particularly undesirable, given the well-documented short- and long-term morbidity associated with corticosteroid use.[177,194] The key question is how these treatment patterns will shift with the introduction of complement inhibitors and FcRn blockers. In chapter 7, we describe our clinical experience with efgartigimod, an FcRn blocker, in sixteen patients with refractory MG. In this difficult-to-treat population, a considerable proportion of patients (80%) were able to reduce MG-related treatments, including prednisolone, azathioprine, plasma exchange, and IVIg.
It is highly likely that these novel therapies will substantially change current treatment patterns, leading to a reduction in corticosteroid use and, likely, the need for symptomatic treatments as well. At present, however, the use of novel treatments remains limited due to high costs. Over time, costs are expected to decrease, which may allow these therapies to be introduced earlier in the disease course. Nevertheless, even with these novel therapies, many patients do not achieve sustained disease control, and they are not effective in all patients. For example, approximately 30% of patients do not respond to complement inhibition.[19] This implies that, in the era of emerging immunomodulatory treatments, symptomatic treatment will continue to play an important role in the management of MG, and it is important that efforts to optimize symptomatic treatments are not overshadowed by advances in immune-targeted therapies.

Future perspectives

In the past decades, only 6% of all clinical trials have focused on symptomatic treatments.[39] At present, pyridostigmine remains the only symptomatic therapy recommended in national and international guidelines for MG. There is, however, as shown in this thesis need for alternative symptomatic treatments to improve efficacy, reduce side effects, and enhance ease of use.

Through the studies included in this thesis, we have gained insights into several considerations that need to be taken into account in future research on symptomatic treatments.

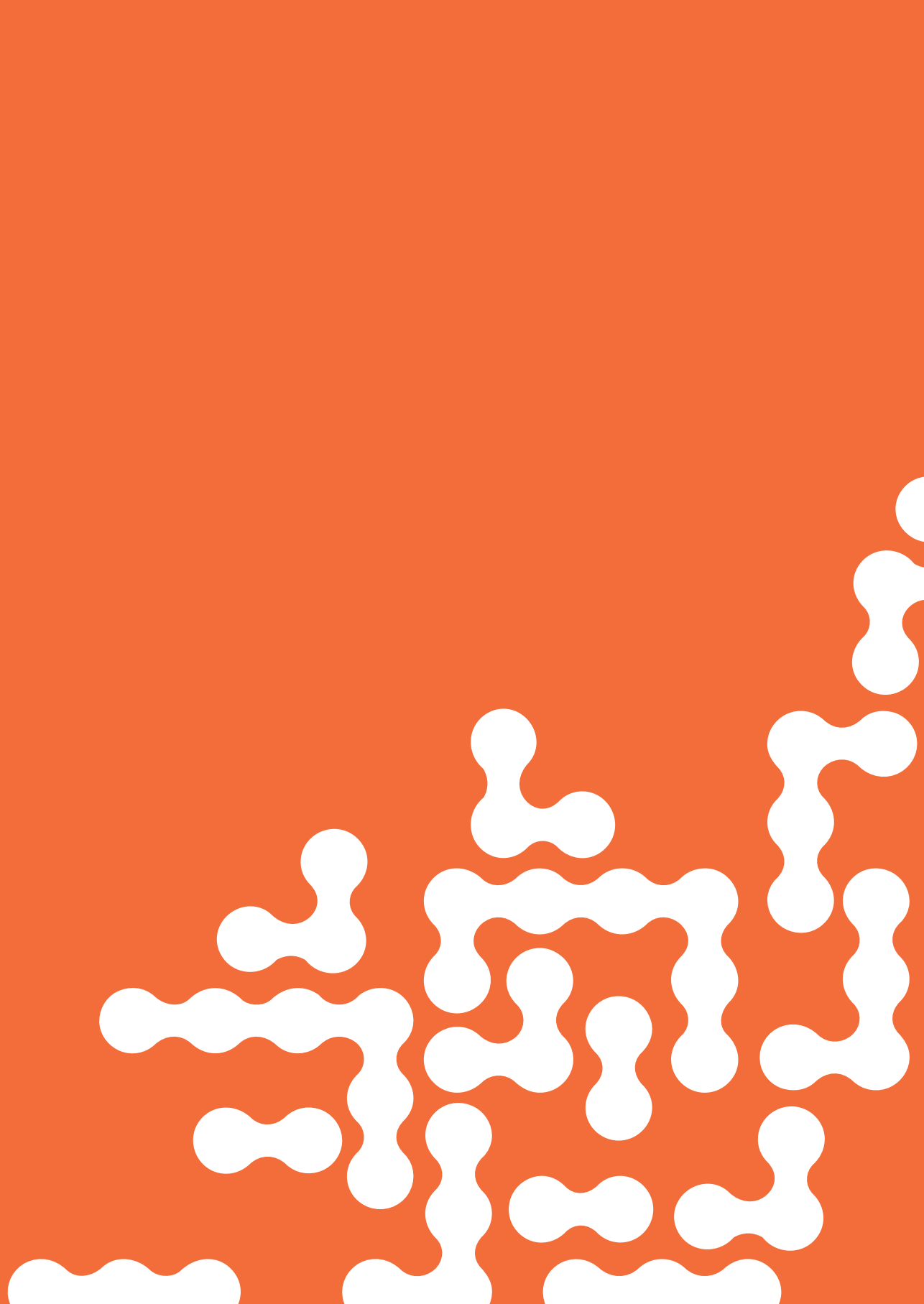
First, careful consideration is required when interpreting whether an observed change is clinically meaningful, rather than automatically applying the established cut-off values, which are likely not appropriate for symptomatic therapies. Ideally, these parameters should be redefined for the most relevant outcome measures (the MGII, MG-ADL, and QMG) in the context of symptomatic treatments in future research. Pyridostigmine could serve as a reference standard for this purpose, as it remains the only available symptomatic therapy.

Second, when selecting outcome measures, particular attention should be paid to the 'floor effect' of commonly used outcomes. The floor effect refers to an outcome measure's limited sensitivity to detect changes in patients presenting with mild symptoms. This is especially relevant for the MG-ADL, often chosen as a primary outcome measure, in which the floor effect is a well-known limitation.[191] For the MGII, the floor effect appears to play a more limited role, making this outcome measure preferable for assessing symptomatic treatments.[148]

Third, it is important to consider whether a treatment is intended to be used as an alternative to, or in addition to, pyridostigmine. In the latter case, several factors complicate the study design. For example, it must be considered how to account for the effect of pyridostigmine in the interpretation of the results. Given that pyridostigmine is fast-acting with a relatively short duration of action, the interval between its administration and the performance of the test affects the outcome. This is best approached by selecting an outcome measure that captures a longer period of time, since a single time-point assessment (such as the QMG or the examination items in the MGII) can be influenced by the timing of pyridostigmine administration. Furthermore, the study population should be selected with symptoms severe enough to adequately capture the expected treatment effects. Since these patients will often be those who would otherwise be eligible for immunosuppressive treatment, careful consideration must be given to ensure that patient safety is not compromised.

Finally, it is crucial that cost-effectiveness analyses are routinely included in clinical trials and that parameters relevant for determining the cost-effectiveness of new treatments are publicly accessible.

In a rapidly evolving field such as MG, these analyses are essential to ensure that long-term healthcare costs remain manageable.



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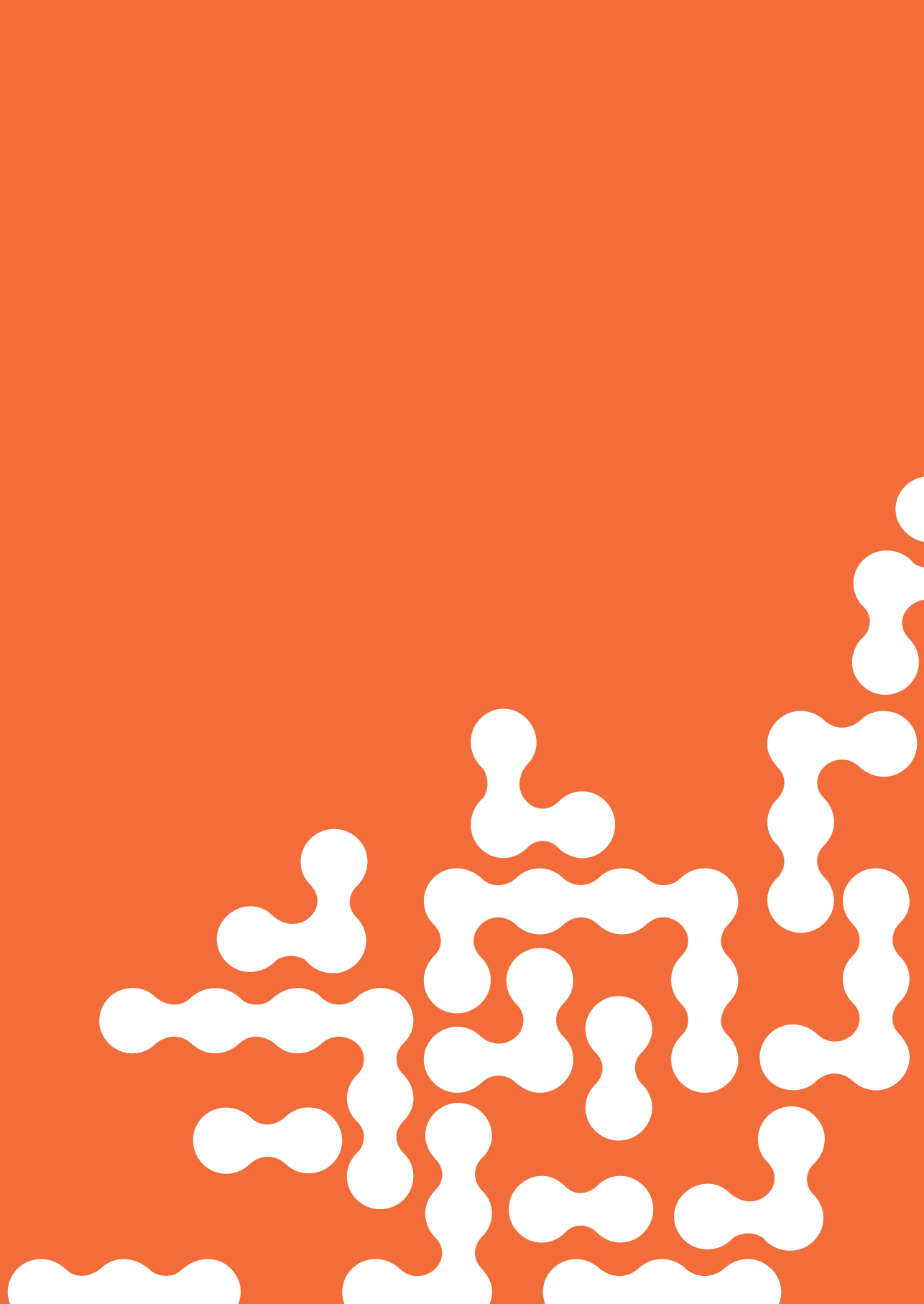

Appendices

Nederlandse samenvatting
List of publications
Dankwoord
Curriculum vitae

Nederlandse samenvatting

Het doel van dit proefschrift was om een overzicht te geven van het behandellandschap voor patiënten met myasthenia gravis (MG), waarbij deel I zich richtte op bestaande en nieuwe symptomatische behandelingen en deel II op immuunmodulerende therapieën en de huidige toepassing daarvan in de klinische praktijk.

Deel I: Symptomatische behandeling

Hoofdstuk 2 beschrijft een uitgebreide literatuurstudie naar symptomatische behandelingen voor MG. Deze therapieën grijpen direct aan op de neuromusculaire overgang, hoewel voor bepaalde geneesmiddelen, zoals sympathicomimetica, het precieze werkingsmechanisme nog onduidelijk is.

Er worden drie hoofdgroepen van momenteel beschikbare behandelingen beschreven: acetylcholinesteraseremmers, kaliumkanaalblokkers en sympathicomimetische middelen. Acetylcholinesteraseremmers zoals pyridostigmine en ambenoniumchloride versterken de cholinerge transmissie door de afbraak van acetylcholine in de neuromusculaire overgang te remmen. Volgens nationale en internationale richtlijnen wordt pyridostigmine algemeen aanvaard als de hoeksteen van de symptomatische behandeling van MG. Dit is gebaseerd op observationele studies, waarvan de vroegste dateren uit het midden van de jaren 1930, evenals op tientallen jaren klinische ervaring. Er is echter geen gerandomiseerde klinische studie die de effectgrootte heeft gekwantificeerd, en een systematische evaluatie van het bijwerkingenprofiel ontbreekt. Hierdoor blijft het moeilijk om pyridostigmine te positioneren ten opzichte van andere symptomatische behandelingen. De rol van kortwerkende kaliumkanaalblokkers (bijv. amifampridine), die de afgifte van acetylcholine verhogen door de presynaptische actiepotentiaal te verlengen, evenals sympathicomimetische middelen (bijv. efedrine, salbutamol, terbutaline), blijft onduidelijk vanwege het ontbreken van robuuste vergelijkende studies.

Naast de momenteel beschikbare behandelingen worden drie experimentele behandelingen besproken: CLC-1-chloridekanaalremmers, fast-skeletal muscle troponine-activatoren en antisense-oligodeoxynucleotiden. De laatste twee worden niet langer actief ontwikkeld, wat hun toekomstige klinische toepasbaarheid beperkt. CLC-1-chloridekanaalremmers lijken veelbelovend, met mogelijk minder bijwerkingen dan de huidige standaardtherapie. Hopelijk zullen fase-III-studies meer inzicht geven in hun werkzaamheid en helpen hun rol in de symptomatische behandeling van MG-patiënten verder te verduidelijken.

In **hoofdstuk 3** hebben we de effectiviteit en bijwerkingen van pyridostigmine in kaart gebracht in een cross-sectioneel onderzoek. De gegevens werden verzameld van 410 van de 642 uitgenodigde patiënten die deelnamen aan het Nederlands-Belgische myasthenie-

register. Met behulp van een dynamische, speciaal voor deze studie ontwikkelde vragenlijst werden effectiviteit, bijwerkingen en het netto klinisch voordeel geëvalueerd. Bijna alle patiënten rapporteerden dat zij pyridostigmine op enig moment tijdens het ziektebeloop hadden gebruikt, waarbij ongeveer twee derde (62%) het middel bleef gebruiken. Het gerapporteerde netto voordeel en de effectiviteit waren matig, terwijl bijwerkingen frequent werden gemeld. Van alle patiënten die op dat moment pyridostigmine gebruikten, rapporteerde 91% ten minste één bijwerking, vergeleken met 54% in de controlegroep. De meest frequent gerapporteerde bijwerkingen waren gastro-intestinale klachten (winderigheid, diarree en buikkrampen), vaker plassen, spierkrampen, wazig zien, overmatig zweten, toegenomen speekselvloed, licht gevoel in het hoofd en griepachtige klachten. Bijwerkingen werden door 26% van de respondenten genoemd als de belangrijkste reden om met pyridostigmine te stoppen. Factoren die geassocieerd waren met het staken van pyridostigmine waren mannelijk geslacht en de aanwezigheid van MuSK-antilichamen.

In **hoofdstuk 4** en **hoofdstuk 5** beschrijven we de resultaten van IMPACT-MG, een gerandomiseerde, dubbelblinde, placebogecontroleerde cross-overstudie waarin de effectiviteit en kosteneffectiviteit van pyridostigmine werden onderzocht (deel één), gevolgd door een tweede randomisatie om de effectiviteit en veiligheid van amifampridine in combinatie met pyridostigmine te evalueren (deel twee) bij patiënten met AChR MG.
De bevindingen van deel één van IMPACT-MG worden beschreven in **hoofdstuk 4**. Negentien deelnemers werden behandeld in twee behandelperioden van vijf dagen, gescheiden door een wash-outperiode van twee dagen. De deelnemers kregen pyridostigmine gevolgd door placebo, of dezelfde behandelingen in omgekeerde volgorde. Alle deelnemers gebruikten vóór inclusie reeds chronisch pyridostigmine, waarbij de tijdens de studie gebruikte dosering identiek was aan de gebruikelijke dosering voorafgaand aan de studie.
Het primaire eindpunt was het verschil in de MGII-score, een gevalideerde maat voor de ernst van MG, tijdens het gebruik van pyridostigmine in vergelijking met placebo. Secundaire uitkomstmaten waren de MG-ADL en QMG ter evaluatie van de effectiviteit, de MG-QoL15r ter beoordeling van kwaliteit van leven en de TSQM-9 ter evaluatie van behandeltevredenheid. Bijwerkingen werden systematisch geregistreerd en daarnaast werd een economische evaluatie uitgevoerd vanuit zowel maatschappelijk als zorgperspectief.
Behandeling met pyridostigmine resulteerde in een verbetering van de MGII-score met -5.3 punten ten opzichte van placebo. Ook op alle secundaire eindpunten werd een gunstig effect van pyridostigmine gezien (QMG -1.4 punten, MG-ADL -1.2 punten, MG-QoL15r -2 punten, and TSQM-9 7.2 punten. Bijwerkingen traden vaker op tijdens behandeling met pyridostigmine dan tijdens placebo, waarbij vermoeidheid en winderigheid het meest frequent werden gerapporteerd. In de kosten-utiliteitsanalyse ging behandeling met pyridostigmine gepaard met lagere jaarlijkse zorgkosten en maatschappelijke kosten,

evenals met een jaarlijkse toename van QALY's, vergeleken met behandeling zonder pyridostigmine. Daarnaast was de kans op kosteneffectiviteit bij een willingness-to-pay threshold van €20.000 per QALY hoog, zowel vanuit maatschappelijk als vanuit zorgperspectief.

In **hoofdstuk 5** presenteren we de bevindingen van het tweede deel van IMPACT-MG, waarin de toevoeging van amifampridine aan behandeling met pyridostigmine werd geëvalueerd. Amifampridine is een presynaptische kaliumkanaalblokker die de actiepotentiaal verlengt en daarmee de afgifte van acetylcholine in de synaptische spleet versterkt. Het middel wordt voornamelijk gebruikt bij patiënten met het Lambert-Eaton myastheen syndroom en congenitaal myastheen syndroom. Het gebruik bij AChR-MG is verondersteld, maar tot op heden niet onderzocht in gerandomiseerde gecontroleerde studies.
Om in aanmerking te komen voor deelname aan deel twee moesten patiënten ofwel deel één van de studie hebben voltooid, ofwel er niet in zijn geslaagd de tweedaagse wash-outperiode voorafgaand aan randomisatie in deel één te voltooien. In totaal werden twintig patiënten gerandomiseerd naar één van drie behandelsequenties, die elk bestonden uit drie behandelperioden van vijf dagen, gescheiden door een wash-outperiode van twee dagen. Tijdens deze behandelperioden werden patiënten met ofwel 30 mg amifampridine, 60 mg amifampridine, of placebo behandeld. Als primaire en secundaire uitkomsten voor effectiviteit en veiligheid werden dezelfde uitkomsmaten gebruikt als in deel één. Daarnaast werd een farmacokinetische/farmacodynamische (PK/PD) substudie uitgevoerd om PK-parameters te analyseren en mogelijke dosis-responsrelaties te evalueren.
Op de primaire uitkomstmaat (MGII-score) bedroeg het gemiddelde verschil ten opzichte van placebo -1 punt voor amifampridine 30 mg en -1.7 punten voor amifampridine 60 mg. De secundaire effectiviteitsuitkomstmaten toonden, net als de primaire uitkomstmaat, geen statistisch significante verschillen. Daarentegen traden bijwerkingen vaker op tijdens behandeling met amifampridine. De meest gerapporteerde bijwerkingen waren paresthesieën, vermoeidheid, verdoofd gevoel, duizeligheid en slaapstoornissen. In drie gevallen waren de tijdens de behandeling opgetreden bijwerkingen ernstig genoeg om amifampridine te staken. In de PK/PD-substudie werden geen associaties gevonden tussen de gemiddelde steady-state AUCτ en Cmax en de primaire uitkomstmaat.

Part II: Immuunmodulerende behandelingen
In **hoofdstuk 6** beschrijven we een overzicht van de huidige behandelpatronen in Nederland binnen verschillende subgroepen van MG-patiënten gedurende het ziektebeloop. Hiervoor analyseerden wij longitudinale gegevens van volwassen patiënten die waren opgenomen in het Nederlands-Belgische Myasthenia Register. Patiënten werden gestratificeerd op basis van leeftijd bij ziekteaanvang (≤50 jaar als early-onset MG [EOMG] en >50 jaar als late-onset

MG [LOMG]) en op basis van antistofstatus (AChR, MuSK en seronegatief). In het eerste jaar na diagnose startte de meerderheid van de patiënten met pyridostigmine (93%), en na vijf jaar gebruikte ongeveer twee derde dit middel nog steeds. Daarnaast laat deze studie zien dat een aanzienlijk deel van de patiënten tien jaar na het begin van de ziekte afhankelijk is van corticosteroïden (30–40%). Verder rapporteerde ongeveer een derde van de patiënten het gebruik van intraveneuze immunoglobulinetherapie op enig moment tijdens het ziektebeloop, en werd 16% van de patiënten behandeld met plasmaferese.

In **hoofdstuk 7** beschrijven we onze klinische ervaring met zestien patiënten met refractaire MG die werden behandeld met efgartigimod, een FcRn-blokker, in een retrospectieve cohortstudie. Een gunstige uitkomst werd gedefinieerd als óf een klinisch relevante verbetering op ten minste twee uitkomstmaten (MG-ADL, QMG of MGC), óf het staken van prednisolon, een dosisreductie van prednisolon van >10 mg per dag, of het stoppen van chronische plasmaferese of IVIg, zonder gebruik van noodmedicatie. Bij 56% van de patiënten werd bij de laatste evaluatie een gunstige uitkomst vastgesteld. Bovendien werd bij 80% van de patiënten de MG-gerelateerde behandeling (prednisolon, azathioprine, plasmaferese en IVIg) verminderd ten opzichte van de situatie vóór start van efgartigimod. Alle patiënten weken af van het behandelschema zoals toegepast in de ADAPT-studie (één infusie per week gedurende vier weken, gevolgd door een variabel interval tot de volgende behandelcyclus) en kregen toedieningen met intervallen van één, twee of drie weken.

List of publications

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Remijn-Nelissen L, Verschuuren JJGM, Tannemaat MR. The effectiveness and side effects of pyridostigmine in the treatment of myasthenia gravis: a cross-sectional study. Neuromuscular Disorders. 2022 Oct 1;32(10):790-9.

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Other publications

Dekker AM*, Remijn-Nelissen L*, Vincenten SCC*, van der Heijden OWH, Schreuder-Stolzenbach DTFG, de Die-Smulders CEM, Wadman RI, Verschuuren JJGM, Voermans NC. Zwangerschap en neuromusculaire ziekten. Handvatten voor preconceptionele counseling, zwangerschap, anesthesie en partus in erfelijke en verworven aandoeningen. Nervus. 2022 Mar; 1

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* These authors contributed equally.

Dankwoord

Allereerst dank aan iedereen die heeft bijgedragen aan dit proefschrift. Dit werk is niet alleen het resultaat van mijn eigen inspanningen, maar vooral ook van de hulp, kennis en aanmoediging van velen om mij heen.

Veel dank aan de patiënten die hebben deelgenomen aan de onderzoeken; jullie inzet en vertrouwen zijn allesbehalve vanzelfsprekend.

Mijn oprechte dank gaat uit naar mijn (co)promotoren:
Jan, ik heb ongelooflijk veel van je geleerd en je hielp me telkens met frisse inzichten verder. Minstens zo waardevol is wat je me naast de wetenschap hebt meegegeven; je bent een voorbeeld voor me met jouw eindeloze geduld en je vermogen om altijd het goede in de mens te zien.
Martijn, jij was een constante bron van enthousiasme en ideeën. Met jouw energie wist je me steeds opnieuw te inspireren. Ik kon voor alles bij je terecht, van kleine twijfels tot moeilijke momenten, en je steun heb ik enorm gewaardeerd.
Teun, jij bracht een frisse wind vanuit de farmacologie met nieuwe inzichten waar we in onze 'neurologie-bubbel' niet altijd bij stilstonden. Je was altijd laagdrempelig bereikbaar en bood telkens weer een nieuw perspectief.

Veel dank aan mijn paranimfen. Wisse, je sprong altijd bij waar nodig en hebt veel werk verzet voor IMPACT-MG. Ik waardeer je rustige karakter en het feit dat ik altijd op je kon rekenen. En wie had aan het begin van onze PhD's gedacht dat we nu samen langs de lijn staan op het voetbalveld bij onze zoons? Nadine, al sinds het begin van de opleiding ben je mijn maatje. Het is dan ook extra speciaal dat we samen aan dit traject in de spierballengroep zijn begonnen. In de afgelopen jaren hebben we veel gelachen, maar vonden we ook steun bij elkaar als het even tegenzat. Gelukkig blijven we collega's, al is het op een afstand(je).

Mijn MG-buddies: Annabel, dank dat je mijn vraagbaak was. Ik hoop dat we in de toekomst nog vele congressen samen zullen bezoeken. Yvonne, dank dat ik blindelings op je kon vertrouwen, je bent altijd oprecht en hebt het hart écht op de juiste plaats. Kevin, ons bezoek aan de MGFA in Miami was een absoluut hoogtepunt, je bent een heerlijke down-to-earth reisgenoot (behalve dan in het vliegtuig).

Dank aan iedereen op de spierballenkamer en de dependance. Esther, op onze zes vierkante meter hebben we elkaar goed leren kennen; het werkte perfect dat we elkaar steun boden maar ook goed in stilte konden werken. Dank ook aan Michel, Evelien en Janne voor jullie gezelligheid.

Dank aan het hele neuromusculaire team. Jeanette, je bent hardwerkend, recht door zee en altijd bereid om te helpen. Laten we onze workflow zo doorzetten! Chiara, jij vervulde al vele rollen: als opleider, stagebegeleider, mede-onderzoeker én als maatje in Londen. Jouw vertrouwen gaf me de ruimte om te groeien. Umesh, veel dank voor jouw kritische blik en je enorme kennis en kunde. Marjolein, Annemarie en Yvonne; jullie deur stond altijd open en ik ben dankbaar dat ik zo vaak een beroep mocht doen op jullie expertise.

Dank aan alle verpleegkundigen van de dagbehandeling; ik voelde me altijd welkom en ben dankbaar voor jullie hulp en flexibiliteit.

Wilbert, veel dank voor je begeleiding van de kosteneffectiviteitsanalyses; je wist me uit te dagen en de juiste weg te wijzen. Dirk Jan Moes, hartelijk dank voor je onmisbare hulp bij de PK/PD-analyses. Linda van de Hulst, dankzij jouw daadkrachtige hulp vanuit de apotheek liep alle logistiek vlekkeloos.

Dank aan alle preklinische myasthenie-onderzoekers. Ik heb veel geleerd van onze meetings en waardeer jullie heldere uitleg. In het bijzonder dank aan Maartje; ik kijk enorm op naar wat jij nu al hebt bereikt. Dana en Laurent, bedankt dat jullie altijd klaarstonden om dingen uit te leggen. William and Manon, thanks for your guidance during my internship; you gave me the confidence to take my first baby steps in the lab.

Lotte, Sophie en Tamara; ook al wonen we niet om de hoek, ik waardeer het enorm dat ik altijd bij jullie terechtkan en blind op jullie kan bouwen.

Veel dank aan mijn (schoon)familie; dankzij jullie interesse heb ik me altijd gesteund gevoeld. Lieve Andrea, ik geniet enorm van onze gesprekken aan de keukentafel en de ruimte die je me geeft om mezelf te zijn. Lieve Suzan en Guus, jullie zijn mijn veilige haven. Jullie kennen al mijn gebreken en ik waardeer dat jullie zo achter me staan.

Lieve pap en mam, zonder jullie steun en eindeloze vertrouwen was ik nooit op dit punt gekomen. Zoals jullie zelf ook altijd zeggen: “Jullie kennen me beter dan ik mezelf ken”, en daardoor weten jullie altijd de juiste snaar te raken.

Thijs, woorden schieten tekort voor wat je voor me betekent. Je vult me perfect aan en geeft me altijd vertrouwen. Dank dat je blijft luisteren naar mijn verhalen over het werk en altijd zo betrokken bent. Lieve Abel en Cas, ik ben zo trots dat ik jullie mama mag zijn. Jullie hebben me geleerd wat echt belangrijk is in het leven.

Curriculum vitae

Linda Remijn-Nelissen was born in Heesch, the Netherlands on 6 October 1988. She grew up in Heesch together with her sister Suzan. She graduated from secondary school (Mondriaan College, Oss) in 2007 and started studying Biomedical Sciences at the Radboud University Nijmegen, the Netherlands. After obtaining her propaedeutic diploma, Linda started medical school at the Radboud University Nijmegen, the Netherlands.

In 2014, after obtaining her medical degree, she started working as a neurology resident not in training at the Rijnstate hospital in Arnhem (supervisor dr. van Oostrom). In October 2016 she started her Neurology Residence program at the Leiden University Medical Center, under supervision of prof. dr. J.J. van Hilten and dr. C.S. Straathof. During her residency, she joined the board of the medical residents' association (VAA) at Leiden University Medical Center, and the board of the national association for Neurology residents (VAAN).
From 2021, she started to work on her PhD project that resulted in this thesis, under supervision of prof. dr. J.J.G.M. Verschuuren, dr. M.R. Tannemaat, and prof. dr. T. van Gelder. During her PhD she revised the Dutch myasthenia gravis guideline, was responsible for driving the implementation of home administration of complement inhibitors and FcRn blockers, and, in the role of co-applicant, contributed to successfully securing research funding for a proposal on efgartigimod in Lambert-Eaton myasthenic syndrome.
In 2024 she was awarded the "TNN Top Article Residency Award" for her article about new immunotherapies in myasthenia gravis. She has presented several results at national and international conferences, including the 14th and 15th Myasthenia Gravis Foundation of America International Conference on myasthenia and related disorders.

From September 2026 onwards, Linda will be working as a neurologist at Leiden University Medical Center. She is married to Thijs Remijn and together they have two sons, Abel and Cas.