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Autoimmune myasthenia gravis : the impact of heterogeneous patterns of muscle weakness on outcome measures and diagnosis

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Autoimmune myasthenia gravis

**The impact of heterogeneous patterns of muscle weakness
on outcome measures and diagnosis**

Robert Henri Pasquale de Meel

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Autoimmune myasthenia gravis

The impact of heterogeneous patterns of muscle weakness on outcome measures and diagnosis

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Voor Hans de Meel

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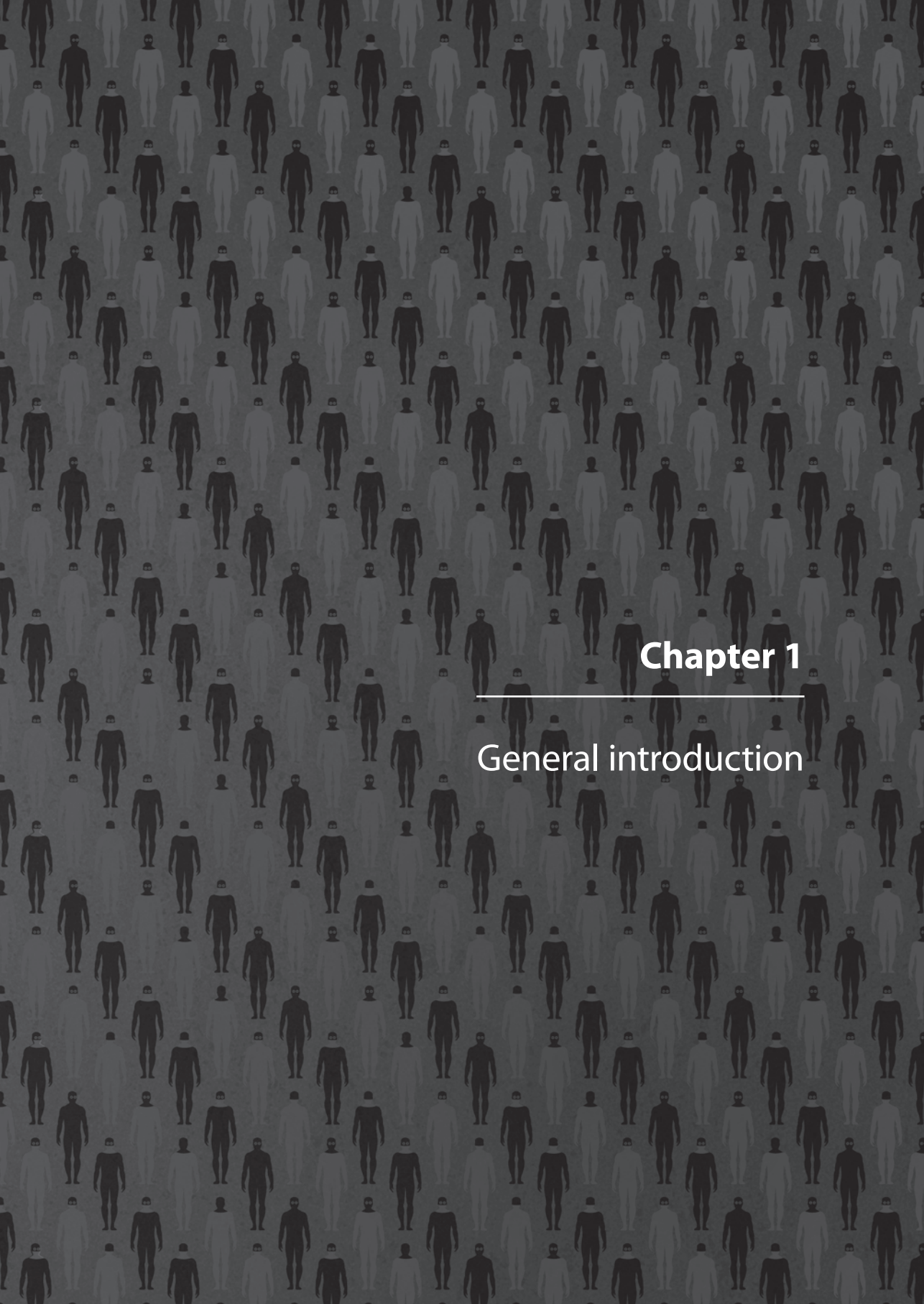
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INTRODUCTION





Chapter 1

General introduction

HISTORY OF MYASTHENIA GRAVIS' CONCEPTION

The first descriptions of a myasthenia gravis (MG) patient were made in the 17th century. One side of the Atlantic Ocean claimed that the first described MG patient was native American Chief Opechancanough in 1644: "The excessive fatigue he encountered wrecked his constitution; his flesh became macerated; his sinews lost their tone and elasticity; and his eyelids were so heavy that he could not see unless they were lifted up by his attendants . . . he was unable to walk; but his spirit rising above the ruins of his body directed from the litter on which he was carried by his Indians".^{1, 2} According to the other side of the Ocean, the first description of MG was made in England by Thomas Willis in 1672: "She speaks freely and readily enough for a while, but after a long period of speech . . . she is not able to speak a word and is as mute as a fish. Her voice does not return for one or two hours".^{3, 4} Interestingly, the first two descriptions of MG patients express two different patterns of muscle weakness. The former description highlights ocular and generalized muscle weakness but gives no mention of bulbar muscle weakness, whilst for the latter description the reverse is true. Heterogeneous patterns of muscle weakness were observed from the start; however, the factors causing this heterogeneity are still largely unknown.

In the second half of the 19th century, Erb and Goldflam gave a first detailed description of the disease that was then referred to as Erb's or Erb–Goldflam disease.^{5, 6} In 1895, Jolly showed that during repeated stimulation of a nerve, decreased contraction of the innervated muscle is observed. This neurophysiological proof of fatigability in MG was in accordance with the first clinical observations of fatigable muscle weakness.⁷ As MG could be distinguished from 'true' paralyzes, Jolly coined the term 'myasthenia gravis pseudoparalytica' (myo, muscle; asthenia, weakness; gravis, severe). A possibly pathophysiological mechanism involving unknown intrinsic factors was first hypothesized by Buzzard In 1905: "the symptoms of the disease are best explained by assuming the presence of some toxic, possibly autotoxic, agent." Even though at the time, MG was already commonly believed to be a disease of the motor system, he argued that non-motor symptoms occur as well: "myasthenia gravis is a disease in which the symptoms are not always confined to the motor system, but may include other sensory, mental or other origin".⁸ However, as later research would indicate the neuromuscular junction as the localization of the neurological deficit, MG to date is conceived as a disease with only motor symptoms.

In the 20th century, the auto-immune disorder underlying MG became apparent by observations such as the presence of lymphocyte aggregates in muscle biopsies and thymic abnormalities in a proportion of MG patients. In 1960, Simpson proposed the hypothesis that MG was caused by an antibody-mediated auto-immune disorder, mainly supported by the observation that neonates of mothers with MG had a transient form of myasthenia.⁹ This hypothesis was further supported in 1976 by Pinching et al. who found that plasma exchange resulted in strong clinical improvement of MG patients.¹⁰ The paradigm of the

pathophysiology in MG was set after the presence of antibodies directed towards the acetylcholine receptor (AChR), a postsynaptic protein playing a crucial role in neuromuscular transmission, was shown in 85% of MG patients by Lindstrom et al.¹¹ One of the major areas of research focused on the identification of additional antibody targets in the remaining 15% 'seronegative' MG patients. In the last two decades, several new antibodies to synaptic proteins were found, among which the voltage-gated calcium channel (VGCC), muscle-specific kinase (MuSK), low-density lipoprotein receptor-related protein 4 (LRP4) and agrin.¹²⁻¹⁵ Interestingly, the pattern of muscle weakness in MuSK MG is different from that in AChR MG. In MuSK MG, bulbar and respiratory weakness is more predominant and ocular weakness often resolves early in the disease course. In AChR MG, on the other hand, ocular weakness is often the first symptom and frequently remains present to some extent during the disease course.^{16, 17} It has been hypothesized that differences in the relative importance of the AChR and MuSK proteins in various muscles might explain the distinct muscle weakness patterns.¹⁸ The predominant presence of proximal leg weakness and less frequent involvement of oculobulbar muscles in Lambert-Eaton Myasthenic Syndrome (LEMS), with the presynaptic Cav2.1 voltage-gated calcium channel (VGCC) as antigen, is another example of a strikingly different pattern of muscle weakness in an antibody mediated disease of the neuromuscular synapse. Several factors have been suggested to contribute to the distinct involvement of muscle groups in MG and other neuromuscular disorders: differences in embryological origin of muscles, adult or fetal AChR isoform expression, innervation patterns, the neuromuscular safety factor, the firing frequencies of motor neurons, the sensitivity to complement mediated membrane damage or differences in the mitochondrial content between extraocular and skeletal muscles. All these factors are proposed as explanations for the differences in involvement of extraocular, bulbar or limb-girdle muscles in MG.¹⁹⁻²¹

However, aside from the heterogeneity of muscle weakness patterns between AChR MG, MuSK MG and LEMS, there is also a remarkable variation *within* these disorders, such as ocular versus generalized AChR MG, rare occasions of distal weakness in AChR MG, or pure ocular weakness in LEMS.^{19, 22}

HETEROGENEITY AND SHIFTS IN DISTRIBUTION OF MUSCLE WEAKNESS

Although the exposure of the target antigens to circulating serum antibodies theoretically should be similar for all muscles, different patterns of muscle weakness are observed within the group of AChR MG patients. Acknowledging different phenotypes in MG might be of importance in treatment choices and clinical trials as some studies show that ocular or generalized weakness responds distinctly to different therapies.²³⁻²⁵ Moreover, the sensitivity of outcome scales for changes in muscle weakness could depend on the muscles involved.^{26, 27}

To identify different MG phenotypes and to quantify the extent of heterogeneity between patients and shifts of muscle weakness pattern within patients, we systematically analyzed the distribution of muscle weakness in AChR MG patients on three points in time. **(CHAPTER 2)**

Ocular muscles are the most frequently involved muscles in MG.²⁸ The affected ocular muscles can be subdivided in muscles that move the eyeball (extraocular muscles; EOM), the muscle that elevates the upper eyelid (m. levator palpebrae superioris; LPS) and the muscle involved in closure of the eye (m. orbicularis oculi; OO). Although MG is a systemic disease, ptosis is reported to be typically asymmetric. In addition, the (more pronounced) ptosis has been reported to shift from one eye to the other during the disease course, but no quantitative data of this phenomenon are reported.²⁹ Detailed knowledge of the patterns and fluctuations of ocular weakness in MG could be helpful for neurologists treating patients with neuromuscular disorders: for diagnosis, to understand its pathophysiology and to establish the relevance of current commonly used outcome measures.^{26, 30} To investigate patterns of ptosis, diplopia and eye closure weakness (ECW) in MG and the sensitivity of the most frequently used clinical MG outcome measures for EOM weakness, we systematically analyzed ocular weakness in a large prospective cohort of MG patients. **(CHAPTER 3)**

OUTCOME MEASURES IN MG

The clinical course of MG is highly variable, ranging from stable disease or remission to the occurrence of several exacerbations over time. Previous studies have mainly focused on prognostic factors for remission. However, prognostic factors for exacerbations, myasthenic crises and emergency treatments could also be useful to the clinician for predicting the course of the disease. These outcome measures can be used in order to better predict which patients are at risk for a more severe and debilitating disease course. We investigated the association between baseline clinical features and the risk of exacerbations and emergency treatments in 96 MG patients. **(CHAPTER 4)**

The Quantitative Myasthenia Gravis (QMG) score can be used to measure ocular, bulbar, and generalized muscle fatigability.³¹ Several other measures have been developed to assess quality of life or impairments in activities of daily living (ADL) in patients with MG.³²⁻³⁸

However, impairments in ADL have never been compared between MG patients and patients with other NMD. The systematic use of a quantitative measure of activity limitations for different NMD may be useful to compare the burden they impose on ADL and establish clinically relevant outcome measures for future clinical trials. In addition, it is not known how fluctuations of the severity of muscle weakness affect the limitations that MG patients experience in ADL. The ACTIVLIM (acronym of 'ACTIVITY LIMitations') questionnaire is a validated measure of daily activity limitations for patients with NMD in general focusing

on generalized weakness.³⁹ We measured the ACTIVLIM score in MG patients to estimate their ADL limitations. To identify which factors contribute to limitations in daily activities, we investigated the relationship between several clinical variables and the ACTIVLIM score. We also analyzed how changes in muscle strength and fatigability, as measured by the QMG, affected the ACTIVLIM score over a longer period of time. **(CHAPTER 5)**

MG-ADL (Myasthenia Gravis Activities of Daily Living) is a commonly used questionnaire in MG trials.⁴⁰⁻⁴² Of the 8 questions, only 3 query generalized weakness. These questions concern respiratory function, the ability to brush teeth or comb hair and the ability to rise from a chair. Alongside this ADL scale, the QMG score is often used in clinical trials as an objective physician-reported scale. The QMG, however, includes more items on generalized weakness (8 of 13). In general, the MG-ADL score correlates well with the QMG score,³⁸ but in a recently published trial on the effect of eculizumab in generalized MG patients, there was a non-significant change in MG-ADL whereas QMG improved significantly.^{26, 41} This raises the question whether MG-ADL is equally sensitive to changes in generalized weakness and oculobulbar weakness and whether adding questions on generalized weakness would improve sensitivity. To investigate whether the sensitivity of MG-ADL for generalized weakness could be improved, we analyzed whether ACTIVLIM has an additional value on top of the MG-ADL in the prediction of the generalized domain of the QMG score in individual patients. **(CHAPTER 6)**

The studies described in chapter 6 indicated that MG-ADL had a lower sensitivity for changes in generalized weakness.²⁷ In addition, a prior study reported that patients considered limb weakness as most invalidating, further stressing the importance of a high sensitivity for generalized weakness.⁴³ The recently developed Myasthenia Gravis Impairment Index (MGII) is a promising measure as it has less floor effects and a higher relative efficiency in its responsiveness to treatment effect compared to other MG measures.^{44, 45} Moreover, MGII has 10 out of 28 items reflecting generalized weakness. We validated the MGII in a Dutch cohort of MG patients and analyzed the sensitivity of the MGII for changes in generalized weakness. **(CHAPTER 7)**

UNMET DIAGNOSTIC NEED IN OCULAR MG

Early recognition and treatment is of great importance for patients' quality of life.¹⁶ Currently, the diagnosis of MG is made by a combination of clinically confirmed fluctuating muscle weakness supported by either the presence of serum autoantibodies, abnormal findings during neurophysiological testing or a positive neostigmine test. Current neurophysiological tests have several limitations. The greatest limitation is that the most commonly affected muscles, the extra-ocular muscles, cannot be tested. Therefore, repetitive nerve stimulation (RNS) has a very low sensitivity (0.29) in ocular MG (OMG) patients. In this

group, antibodies are found in only 44% of patients and therefore there is a strong need for a test to support the diagnosis.⁴⁶ Single-fiber EMG (SFEMG) has been reported to have a relatively high sensitivity in OMG (62-97%), however it does not directly assess EOMs, requires a skilled neurophysiologist to reliably perform this test, and is time-consuming and operator-dependent. In summary, the lack of neurophysiological tests measuring the neuromuscular transmission of EOMs complicates the diagnosis in OMG patients.⁴⁷

A possible solution to this problem was found by a recent study by Valko et al. that shows the possibility of measuring fatigability in the ocular muscles by using ocular vestibular evoked myogenic potentials (oVEMP).⁴⁸ The stimulation of the semicircular canals with bone vibrations administered to the skull cause a vestibulo-ocular reflex, currently used for diagnoses in vestibular disease. In vestibular disorders, a single oVEMP potential is elicited and analyzed. Valko et al. showed that NMJ function can be investigated by applying a train of ten repetitive oVEMPs. In MG patients, a decrement in the n2-p2 amplitude can be observed, analogous to the decrement observed in RNS. We will refer to this technique as the Repetitive oVEMP (RoVEMP) test. To further investigate the diagnostic value of the RoVEMP test, we investigated its diagnostic yield compared to a control group of patients with a neuromuscular disorder other than MG. We also analyzed the effect of pyridostigmine use on RoVEMP results. Furthermore, we included a larger cohort of MG patients in order to further analyze the sensitivity and specificity of the RoVEMP in diagnostically challenging subgroups (OMG and seronegative MG patients). (**CHAPTER 8**)

SCOPE OF THE THESIS

Before the discovery of the auto-immune dysfunction underlying the manifestations of MG, the phenomenological description of MG was the starting point for understanding the disease. Both the heterogeneity in the patterns of muscle weakness and the possible minor involvement of non-motor systems were disease manifestations being closely described to find clues for the pathophysiology underlying this disease. The name Jolly coined for this disease, 'myasthenia gravis pseudoparalytica,' indicates the open and broad clinical and scientific approach towards this disorder. After the discovery of antibodies against the components of the neuromuscular junction, the pathophysiological paradigm of auto-antibodies causing the disease became the starting point for further MG research. Different MG antibody subgroups were found to have different distributions of muscle weakness. Several hypotheses were formed to explain why ocular muscles showed weakness more often than other muscles. However, changes in weakness patterns within patients and differences in weakness patterns between patients with the same antibody status were not considered to be 'challenges' to the paradigm that disease expression in MG is solely explained by the systemic autoimmune disorder underlying it. The focus on antibody status

over phenomenological disease expression was also found in inclusion criteria of clinical trials. Antibody status often formed a hard criterion, whereas the expression of disease was only subdivided between 'isolated ocular' and 'generalized'. Even though outcome measures take into account different forms of muscle weakness, the 'unidimensional' score following from these scales shows that there is no regard for the possibly different responses of ocular, bulbar or limb-girdle muscle weakness to the intervention that is studied in therapy trials.

The main scope of this thesis is to elucidate the heterogeneity of muscle weakness patterns found in MG and to investigate how outcome measures and diagnostic tests cope with different distributions of muscle weakness.

The specific main objectives of the different parts (I-II) and chapters (2-8) in this thesis are:

- I. To describe heterogeneity and fluctuations in muscle weakness patterns
 2. To identify different phenotypes in AChR MG and to quantify the extent of heterogeneity and the frequency of shifts in muscle weakness pattern
 3. To investigate patterns of ptosis, diplopia and eye closure weakness in MG and to analyze the sensitivity of QMG for EOM weakness
- II. To analyze the quantification of distinct forms of myasthenic weakness in outcome measures and a new diagnostic test
 4. To investigate the association between baseline clinical features and the risk of exacerbations and emergency treatments
 5. To identify factors contributing to limitations in daily activities and to analyze how changes in muscle weakness affected the ACTIVLIM score
 6. To investigate the sensitivity of MG-ADL for generalized weakness
 7. To validate the MGII in a Dutch cohort of MG patients and to analyze the sensitivity of the MGII for changes in generalized weakness
 8. To investigate the diagnostic value of the RoVEMP test in diagnostically challenging subgroups.

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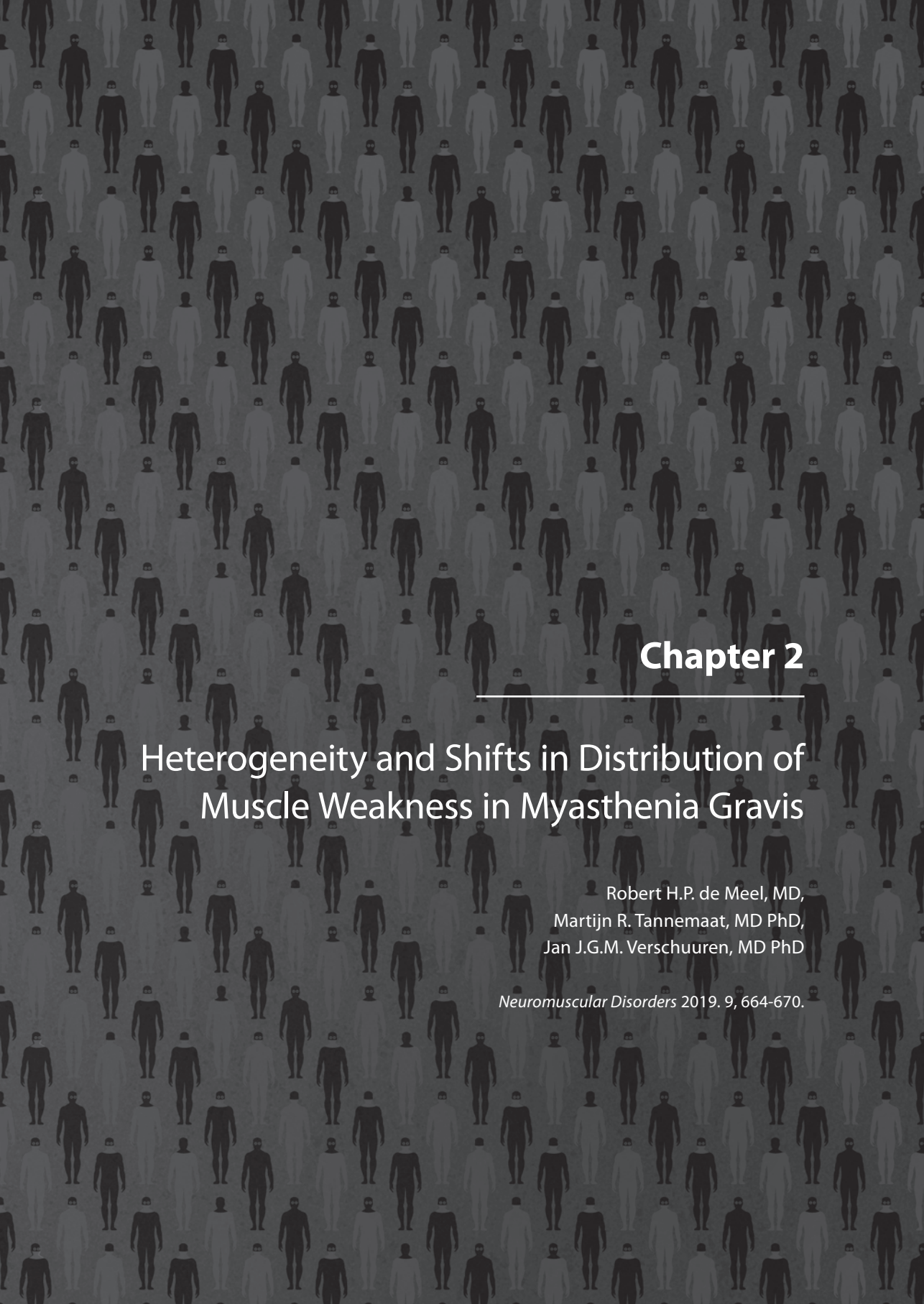
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PART I

Heterogeneity and fluctuations in muscle weakness





Chapter 2

Heterogeneity and Shifts in Distribution of Muscle Weakness in Myasthenia Gravis

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Neuromuscular Disorders 2019. 9, 664-670.

ABSTRACT

Introduction

The distribution of muscle weakness in myasthenia gravis (MG) patients with acetylcholine receptor (AChR) antibodies is highly variable. As muscle groups respond differently to therapeutic interventions, it is important to acknowledge this variability. We analysed the distribution of muscle weakness in AChR MG patients over time and the relation with treatment.

Methods

We systematically investigated the distribution of muscle weakness in 225 AChR MG patients using questionnaires. Symptoms were inquired for three consecutive periods: baseline, time of maximum severity and last follow-up. On the basis of combinations of muscle weakness, seven phenotypes were defined: 'ocular' (O), 'bulbar' (B), 'neck/limbs/respiratory' (NLR), or a combination (O+B, O+NLR, B+NLR and O+B+NLR).

Results

Throughout the disease course MG was restricted to ocular weakness in 5%, whereas 7% never had any form of ocular weakness. At last follow-up, ocular or bulbar weakness had resolved more frequently than NLR weakness (40%, 38% and 25%; $p = 0.003$, respectively). Patients with O, B or OB phenotype at baseline had a higher age at onset and were more frequently male than patients with NLR, ONLR, BNLR or OBNLR phenotype (52.7 ± 17.5 vs. 44.0 ± 18.9 ; $p = 0.007$ and 64% vs. 37%; $p = 0.002$, respectively).

Discussion

MG patients have a large heterogeneity in the distribution of muscle weakness and frequently shift between phenotypes. The phenotypic variations found in AChR MG suggest that also other factors aside from the AChR antibody mediated immune response are of importance in determining the disease expression in MG.

INTRODUCTION

Myasthenia gravis (MG) is an autoimmune disease, mediated by antibodies to a number of well characterized autoantigens.¹ However, current knowledge on the involved autoantigens does not provide conclusive answers for the large heterogeneity that is observed in distribution of muscle weakness and the shifts in this distribution within individual MG patients. The most frequently involved autoantigen is the acetylcholine receptor (AChR), against which antibodies are present in about 85% of MG patients.² Although the exposure of the target antigens to circulating serum antibodies should be similar for all muscles, different patterns of muscle weakness are observed in individual patients. Acknowledging different phenotypes in MG might be of importance in treatment choices and clinical trials as some studies show that ocular or generalized weakness responds distinctly to different therapies.³⁻⁵ Moreover, the sensitivity of outcome scales for changes in muscle weakness depends on the muscles involved.^{6,7} In this study, we therefore systematically analysed the distribution of muscle weakness in AChR MG patients and shifts in this distribution within patients.

METHODS

Patients

We included a prospective cohort of MG patients in the Netherlands and Belgium that participated in the Dutch-Belgian Myasthenia Registry between 2016 and 2018 (website address: <https://www.lumc.nl/org/neurologie/research/myasthenie-register/>). The diagnosis of AChR MG was based on a combination of clinically confirmed fluctuating muscle weakness and the presence of serum autoantibodies to AChR.⁸ This study was approved by the Medical Ethics Boards of the Leiden University Medical Center. All patients provided informed, written consent prior to study participation.

Clinical data

To allow the systematic inclusion of a large number of patients we used questionnaires to investigate the localization of muscle weakness. We asked for the presence of the following forms of myasthenic weakness: ptosis, diplopia, dysarthria, chewing weakness, swallowing weakness, facial weakness, neck weakness, respiratory weakness, hand weakness, arm weakness and leg weakness. These questions are available as a supplement 1. The presence of symptoms was inquired for three consecutive periods: baseline (initial weakness during the first six months from the onset of MG), time of maximum severity (defined as the period in which patients experienced the highest burden of disease) and last follow-up (last three months). In addition, the use of pyridostigmine, prednisone and other immunosuppressants (azathioprine, mycophenolate mofetil, rituximab, cyclosporine, methotrexate or

cyclophosphamide) in the last three months was inquired. All patients gave permission to access the clinical reports and results of additional electrophysiological and laboratory investigations of their treating physicians. This information was used to validate the information provided by the patient.

Phenotypes

Muscle weakness was categorized into three groups: ocular (ptosis or diplopia), bulbar (dysarthria, chewing weakness, swallowing weakness or facial weakness) and neck/limbs/respiratory (neck weakness, respiratory weakness, hand weakness, arm weakness or leg weakness; abbreviated 'NLR'). The combination of muscles included in these groups were based on prior clinical subdivision of muscle weakness in MG and the allotype to which these muscles belong.⁹⁻¹² Based on the presence of muscle weakness in one or more of these groups, seven phenotypes were formulated. 'ocular' (O), 'bulbar' (B) or 'neck/limbs/respiratory' (NLR) are phenotypes in which weakness of only one group is present. Alongside these three phenotypes with 'isolated' weakness, four phenotypes entail combinations of these forms of muscle weakness: O+B (OB), O+NLR (ONLR), B+NLR (BNLR) and O+B+NLR (OBNLR). We classified all patients in phenotypes using two different classifications: a 'point in time' phenotype and a 'cumulative' phenotype. The 'point in time' phenotype of each patient was based on the distribution of muscle weakness at baseline, time of maximum severity or last follow-up. The cumulative phenotype was based on the involvement of all affected muscle categories at any time up until that point in time.

As an example, a patient with only ocular weakness throughout the disease course is classified as an O phenotype in both phenotype classifications. A patient starting with ocular weakness, who later on develops bulbar and NLR weakness will be classified as O at the start of disease, but as OBNLR in the cumulative phenotype classification, even if the three forms of weakness are present at different points in time.

Statistical Analysis

We used a chi-squared test to assess differences in baseline characteristics and differences in the frequency of phenotypes at different points in time. We used a unpaired t-test to analyze differences in mean age at onset. *P*-values < 0.05 were considered significant. Statistical analyses were performed using SPSS version 23 (IBM Corp., Armonk, NY) and GraphPad Prism version 7.00 (GraphPad Software Inc., San Diego, CA).

RESULTS

We included 225 AChR MG patients. Source data was used to check antibody status and age at onset. 168 (75%) of patients correctly indicated AChR as their antibody status in the

questionnaire, the rest of the patients indicated that they did not know their antibody status. Baseline characteristics, symptoms at baseline, at maximum severity and at last follow-up are shown in table 1.

Table 1. Baseline characteristics of 225 patients with AChR MG included in this study

Demographic characteristics			
Age, y	59.4 ± 15.1		
Age at onset, y	45.6 ± 19.0		
Gender (% male)	94 (42)		
Disease duration	13.7 ± 13.6		
Clinical characteristics			
Medication use	Baseline	Max. Severity	Last FU
Pyridostigmine			166 (74)
Prednisone			110 (49)
Other immunosuppressants			105 (47)
Symptoms			
Ptosis	175 (78)	166 (74)	98 (44)
Diplopia	162 (72)	160 (71)	82 (36)
Dysarthria	144 (64)	152 (68)	75 (33)
Chewing weakness	139 (62)	151 (67)	64 (28)
Swallowing weakness	142 (63)	152 (68)	77 (34)
Facial weakness	141 (63)	143 (64)	71 (32)
Neck weakness	108 (48)	132 (59)	71 (32)
Respiratory weakness	94 (42)	124 (55)	88 (39)
Hand weakness	127 (56)	142 (63)	93 (41)
Arm weakness	130 (58)	162 (72)	94 (42)
Leg weakness	128 (57)	151 (67)	114 (51)

Baseline characteristics, medication use at last follow-up, symptoms at baseline, at maximum severity and at last follow-up are shown. Data are presented as number of patients (%) for categorical variables and as mean ± SD for continuous variables.

Abbreviations: Baseline = initial weakness during the first six months from the onset of MG, Max. Severity = time of maximum severity, Last FU = last three months

At baseline, 86% of patients reported ocular weakness, 76% bulbar weakness and 81% NLR weakness. Ocular or bulbar weakness had resolved more frequently at last follow-up than NLR weakness (40%, 38% and 25%; $p = 0.003$, respectively). Clinical remission occurred more frequently in patients without NLR weakness at baseline (O, B or OB phenotypes) than in patients with NLR weakness (NLR, ONLR, BNLR or OBNLR) (33% and 13%; $p = 0.002$, respectively). The former group (O, B or OB at baseline) had a higher age at onset and had a higher proportion of male patients than the latter group (NLR, ONLR, BNLR or OBNLR at baseline) (52.7 ± 17.5 vs. 44.0 ± 18.9 ; $p = 0.007$ and 64% vs. 37%; p

= 0.002, respectively). The same differences were seen for cumulative phenotypes at last follow-up: patients without NLR weakness throughout the disease course, in whom the weakness never developed beyond the O, B or OB phenotype, had a higher age at onset and had a higher proportion of male patients than patients with NLR weakness at some point in the disease course (cumulative NLR, ONLR, BNLR or OBNLR at last follow-up) (55.1 ± 17.6 vs. 44.6 ± 18.9 ; $p = 0.016$ and 67% vs. 39% ; $p = 0.020$, respectively). Characteristics of MG patients subdivided by phenotype are shown in figure 1 and table 2.

Table 2. Characteristics of MG patients subdivided by phenotype

A. Baseline phenotypes							
Baseline phenotypes	O N=25	B N=4	OB N=13	NLR N=9	ONLR N=19	BNLR N=18	OBNLR N=137
Age, y	63.7 ± 12.8	53.0 ± 16.1	64.9 ± 10.4	55.6 ± 13.7	59.2 ± 15.9	57.4 ± 13.7	58.7 ± 15.9
Age at onset, y	51.5 ± 20.2	42.3 ± 17.5	58.2 ± 8.7	42.7 ± 15.4	49.0 ± 19.5	42.9 ± 17.9	43.5 ± 19.3
Gender (% male)	17 (68)	2 (50)	8 (62)	3 (33)	6 (32)	6 (33)	52 (38)
Disease duration	12.2 ± 15.4	10.8 ± 2.2	6.7 ± 5.8	12.9 ± 7.3	10.3 ± 8.8	14.6 ± 12.2	15.2 ± 14.8
<i>Medication use</i>							
Pyridostigmine	15 (60)	4 (100)	9 (69)	6 (67)	16 (84)	10 (56)	106 (77)
Prednisone	16 (64)	1 (25)	6 (46)	2 (22)	11 (58)	7 (39)	67 (49)
Other immunosuppressants	6 (24)	4 (100)	5 (39)	1 (11)	5 (26)	13 (72)	71 (52)
B. Cumulative phenotypes at last FU							
Cumulative phenotypes (Last FU)	O N=12	B N=1	OB N=8	NLR N=1	ONLR N=17	BNLR N=13	OBNLR N=173
Age, y	64.4 ± 13.6	61.0 ± 0	64.3 ± 9.5	70.0 ± 0	60.1 ± 16.5	55.1 ± 16.2	58.9 ± 15.2
Age at onset, y	52.4 ± 22.3	51.0 ± 0	59.5 ± 8.3	60.0 ± 0	51.6 ± 19.1	43.6 ± 16.1	43.9 ± 19.0
Gender (% male)	9 (75)	0 (0)	5 (63)	1 (100)	6 (35)	4 (31)	69 (40)
Disease duration	12.0 ± 19.2	10 ± 0	4.8 ± 5.2	10.0 ± 0	8.5 ± 8.2	11.5 ± 7.4	15.0 ± 14.1
<i>Medication use</i>							
Pyridostigmine	7 (58)	1 (100)	6 (75)	0 (0)	15 (88)	6 (46)	131 (76)
Prednisone	5 (42)	1 (100)	3 (38)	1 (100)	9 (53)	4 (31)	87 (50)
Other immunosuppressants	1 (8)	1 (100)	2 (25)	0 (0)	3 (18)	10 (77)	88 (51)

Characteristics of MG patients subdivided by phenotype are shown. Table A shows data for phenotypes at baseline. Table B shows data for cumulative phenotypes at last follow-up. ‘cumulative phenotypes’ represent all the weakness the patients had experienced up until that point in time (i.e. last follow-up). Data are presented as number of patients (%) for categorical variables and as mean $\pm$ SD for continuous variables.

Abbreviations: O = ocular, B = bulbar, OB = oculobulbar, NLR = neck/limbs/respiratory, ONLR = O+NLR, BNLR = B+NLR, OBNLR = O + B + NL, Baseline = initial weakness during the first six months from the onset of MG, Max. Severity = time of maximum severity, Last FU = last three months

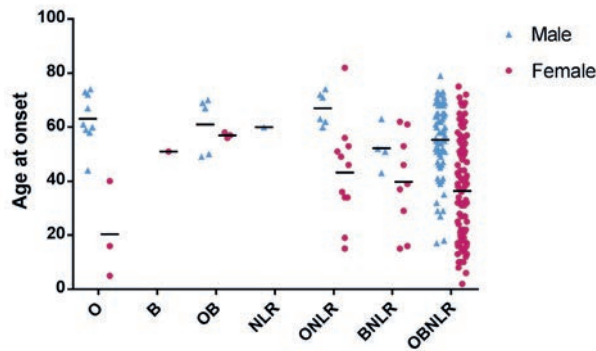


Figure 1. Age at onset and gender subdivided by cumulative phenotypes

Dot plots showing the age at onset and gender of MG patients subdivided by phenotype. The mean is shown by lines.

Abbreviations: O = ocular, B = bulbar, OB = oculobulbar, NLR = neck/limbs/respiratory, ONLR = O+NLR, BNLR = B+NLR, OBNLR = O + B + NLR, Baseline = initial weakness during the first six months from the onset of MG, Max. Severity = time of maximum severity, Last FU = last three months

At baseline, 25 of patients had a pure ocular phenotype. Twelve of these patients (48%) remained purely ocular throughout their disease course. The appearance of NLR weakness (secondary generalization) was reported by 12 of these patients (48%), and one patient developed an oculobulbar cumulative phenotype. Non-ocular phenotypes (B, NLR, BNLR) were reported by 31 patients (14%) at baseline. Of these patients, 15 (48%) never had any ocular weakness throughout their disease course. Figure 2 shows the frequency of “point-in-time” phenotypes and transitions between these phenotypes. Figure 3 gives an overview of the “cumulative” phenotypes over time.

DISCUSSION

In a cohort of 225 AChR MG patients, we found a high heterogeneity in the distribution of muscle weakness and a frequent occurrence of shifts between phenotypes in individual patients. Ocular or bulbar weakness disappeared more frequently than NLR weakness, suggesting that the latter form of weakness responds less well to therapy or that oculobulbar muscles are more capable of adapting to the chronic exposure to autoantibodies. Clinical remission occurred more often in patients that did not have any form of NLR weakness in the first 6 months of disease. This suggests that the initial phenotype in AChR MG patients is of prognostic value.

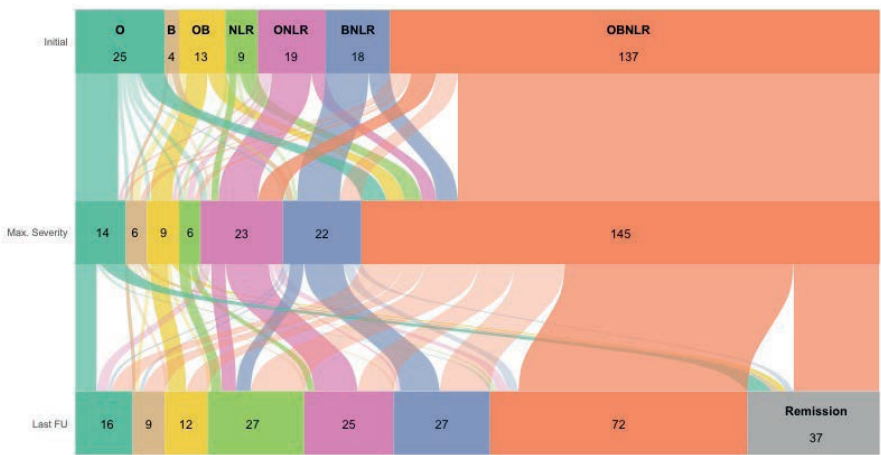


Figure 2. Point-in-time phenotypes and shifts over time
The frequency of point-in-time phenotypes within AChR MG and transitions between these phenotypes are shown. The two most frequent transitions for each phenotype are shown more prominently (darker colour). The frequencies of the phenotypes are shown in their respective boxes. At last follow-up, an additional category is added: remission (the absence of any form of myasthenic weakness).
Abbreviations: O = ocular, B = bulbar, OB = oculobulbar, NLR = neck/limbs/respiratory, ONLR = O+NLR, BNLR = B+NLR, OBNLR = O + B + NLR, Baseline = initial weakness during the first six months from the onset of MG, Max. Severity = time of maximum severity, Last FU = last three months

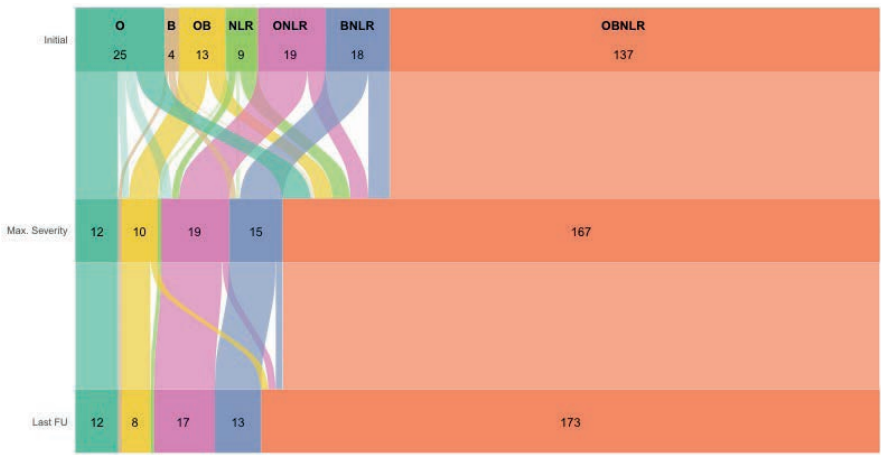


Figure 3. Cumulative phenotypes and shifts over time
The frequency of cumulative phenotypes within AChR MG and transitions between these phenotypes are shown. ‘cumulative phenotypes’ represent all the weakness the patients had experienced up until that point in time. The two most frequent transitions for each phenotype are shown more prominently (darker colour). The frequencies of the phenotypes are shown in their respective boxes. At Max. Severity and Last FU only one patient had phenotype B and only one patient had phenotype NLR.
Abbreviations: O = ocular, B = bulbar, OB = oculobulbar, NLR = neck/limbs/respiratory, ONLR = O+NLR, BNLR = B+NLR, OBNLR = O + B + NLR, Baseline = initial weakness during the first six months from the onset of MG, Max. Severity = time of maximum severity, Last FU = last three months

The appearance of NLR weakness in priorly ocular MG patients, occurred in 48% of our AChR MG patients. Earlier reported rates of secondary generalization vary greatly. In a Chinese cohort only 26% of patients with ocular MG developed generalized MG during a 13-year follow-up period.¹³ On the other hand, Grob et al. found that generalized weakness emerged in 80% of initially ocular MG patients and in 88% generalization occurred within one year from disease onset.¹⁴ Other studies reported secondary generalization rates between of these extremes: Schlezinger et al. (56%), Wirtz et al. (58%), Li et al. (61%), Oosterhuis (69%).^{9, 15-17} One explanation underlying this variation may be caused by differences in the use of the term ‘generalization’. For example, Wirtz et al. defined ‘generalized weakness’ as “ocular or bulbar plus limb muscle weakness”, but in the definition of Li et al. the presence of limb muscle weakness was not necessary and bulbar weakness alone was sufficient (“symptoms other than ocular symptoms, such as dysarthria, dysphagia, dyspnea or weakness of the jaw, neck or arms and legs”).^{9, 17} For this reason we avoided the term ‘generalized weakness’ in our phenotype classification and opted to describe different muscle regions separately.

We do expect that the phenotypes with NLR weakness (NLR, ONLR, BNLR, OBNLR) would mostly be recognized as ‘generalized MG’. These four ‘generalized’ phenotypes all occurred in significant frequencies and were observed throughout the disease course. We did find a relatively low secondary generalization rate in patients without NLR weakness at baseline. This is likely explained by the broader period which we considered to be the baseline phenotype. Oosterhuis, for example, looked at initial signs within three months from onset, as opposed to six months from onset in our study.¹⁶

A more precise classification based on muscle weakness patterns may be useful to gain a more detailed understanding of the effect of treatment. Currently, trials generally regard both patients with NLR weakness and patients with OBNLR symptoms as belonging to the same subgroup (‘generalized’). However, this broad classification could affect the outcome in clinical trials as the latter group will have the possibility to respond to ‘oculobulbar’ as well as ‘generalized’ items in outcome measures whereas the former will only respond to ‘generalized’ items.

Interestingly, in 12 patients (5%) MG was restricted to ocular weakness, whereas 15 other patients (7%) never had any form of ocular weakness throughout their disease course. These phenotypic extremes suggest that other factors, such as characteristics of individual muscles and their resistance against the antibody mediated attack, are of importance in determining the disease expression in MG. The O phenotype group with pure ocular MG consisted mainly of male patients with an age at onset higher than 60 and the 3 female patients had a much lower age at onset (mean around 20). This distribution of age at onset and gender was similar to that found in a previous study.¹⁸ The B and OB phenotype group was characterised by late age at onset. Some case reports also suggest that MG presenting with bulbar weakness is mainly found in late-onset patients.^{19, 20} In the phenotype groups with NLR

weakness (NLR, ONLR, BNLR and OBNLR), male patients had a mean age at onset around 60 whereas female patients had a mean age at onset around 40. This distribution was comparable to earlier studies on ‘generalized’ MG.⁸ The phenotype groups without any ocular or oculobulbar weakness (BNLR and NLR, respectively) were mainly constituted by female patients with early-onset of disease. The predominance of female patients was also found in an earlier study on limb-girdle MG (comparable to our NLR phenotype).²¹ It is likely that the final phenotype in MG patients is the result of a complex interplay between a set of immunological parameters, among which the fine specificity of the AChR antibodies, the capacity for complement activation, the frequency of AChR blocking antibodies and HLA allotype on one hand, and a set of the muscle characteristics on the other hand, including regenerative capacity, complement resistance, or innervation patterns.²²⁻³³

Limitations of this study include recall bias as patients were asked to report symptoms from an early stage of disease and the categorization of muscles in groups that may be considered somewhat arbitrary (O, B and NLR). The purpose of this categorization, however, was not to introduce a new classification system for MG, but to elucidate the heterogeneity of muscle weakness and shifts during the disease course in a standardized way. Our categorization, which was based on prior clinical subdivisions and on the allotype to which these muscles belong⁹⁻¹², served this purpose well. Furthermore, including one type of muscle weakness in another category (e.g. including neck weakness in the B phenotype instead of the NLR phenotype) would not affect the main conclusions of this paper.

In this study, we found a high heterogeneity in the distribution of muscle weakness and a frequent occurrence of shifts between phenotypes in individual patients. The phenotypic extremes found in AChR MG suggest that other factors than solely AChR antibodies are of importance in determining the disease expression in MG. Exploring these factors may help in developing a ‘personalized medicine’ approach in MG by taking patient phenotypes into account in future clinical trials and in day-to-day treatment decisions.

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Supplemental material

SUPPLEMENT 1

Questionnaire Dutch-Belgian Myasthenia Registry

Abbreviated version translated from the original Dutch questionnaire

1. Which symptoms of myasthenia did you experience at the beginning of the disease? (*within the first 6 months of disease*)

- ☐ Weak or falling eyelids (droopy eyelids)
- ☐ Double vision
- ☐ Hand weakness (difficulty opening jars, squeezing or grabbing)
- ☐ Arm weakness (difficulty combing hair, washing or taking something out of a high cabinet)
- ☐ Leg weakness (difficulty walking stairs or walking long distances)
- ☐ Neck weakness (difficulty keeping your head straight up)
- ☐ Facial weakness (difficulty to whistle or to smile)
- ☐ Difficulty with speaking (bad articulation, unclear speech)
- ☐ Difficulty with chewing (difficulty to chew food)
- ☐ Difficulty with swallowing (difficulty to swallow food and fluids)
- ☐ Difficulty with breathing (deep breathing during minimal exercise)

2. Which symptoms of myasthenia did you experience when the disease was at its worst?

- ☐ Weak or falling eyelids (droopy eyelids)
- ☐ Double vision
- ☐ Hand weakness (difficulty opening jars, squeezing or grabbing)
- ☐ Arm weakness (difficulty combing hair, washing or taking something out of a high cabinet)
- ☐ Leg weakness (difficulty walking stairs or walking long distances)
- ☐ Neck weakness (difficulty keeping your head straight up)
- ☐ Facial weakness (difficulty to whistle or to smile)
- ☐ Difficulty with speaking (bad articulation, unclear speech)
- ☐ Difficulty with chewing (difficulty to chew food)
- ☐ Difficulty with swallowing (difficulty to swallow food and fluids)
- ☐ Difficulty with breathing (deep breathing during minimal exercise)

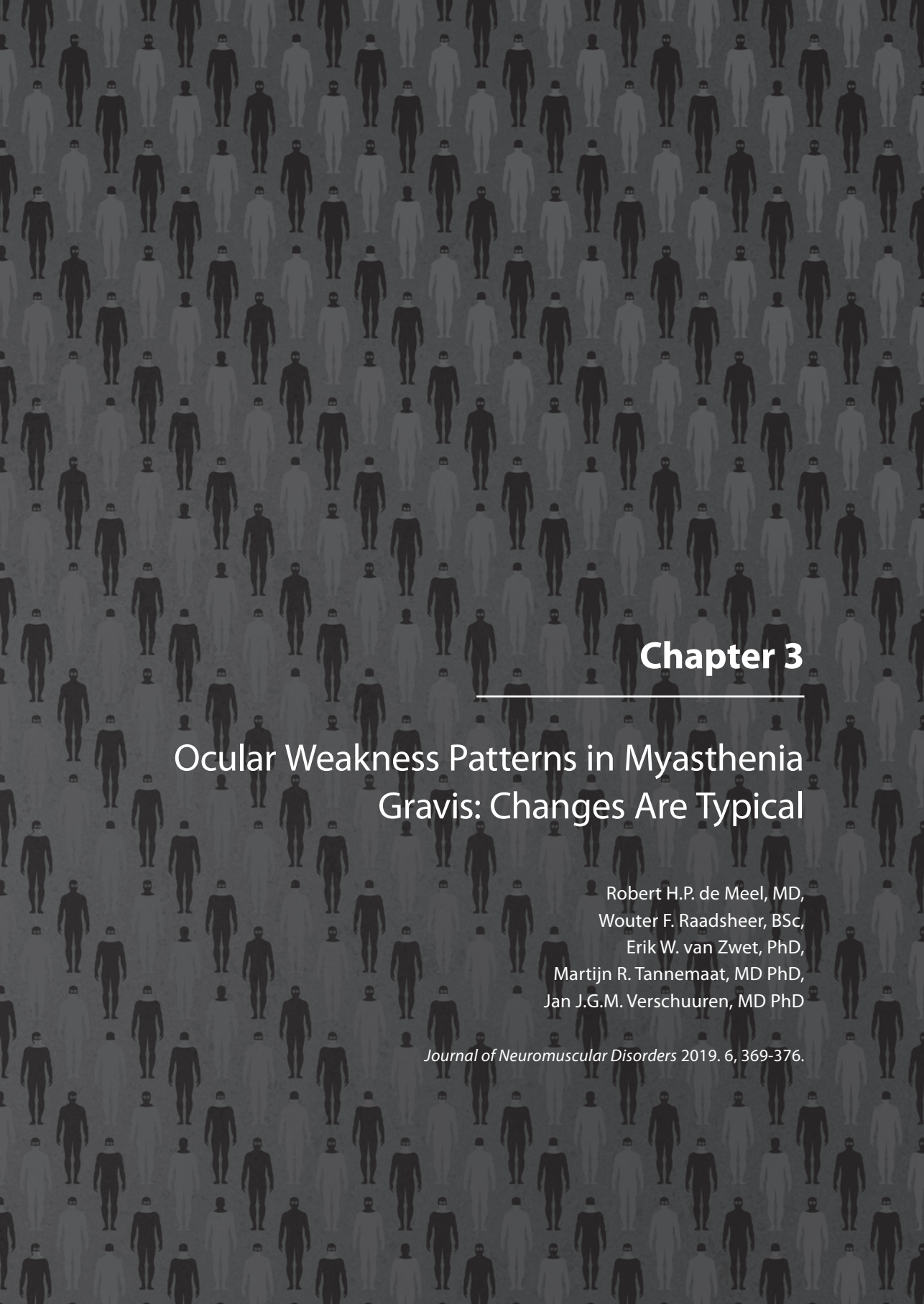
3. Which symptoms of myasthenia have you experienced in the last three months?

- ☐ Weak or falling eyelids (droopy eyelids)
- ☐ Double vision
- ☐ Hand weakness (difficulty opening jars, squeezing or grabbing)
- ☐ Arm weakness (difficulty combing hair, washing or taking something out of a high cabinet)
- ☐ Leg weakness (difficulty walking stairs or walking long distances)
- ☐ Neck weakness (difficulty keeping your head straight up)
- ☐ Facial weakness (difficulty to whistle or to smile)
- ☐ Difficulty with speaking (bad articulation, unclear speech)
- ☐ Difficulty with chewing (difficulty to chew food)
- ☐ Difficulty with swallowing (difficulty to swallow food and fluids)
- ☐ Difficulty with breathing (deep breathing during minimal exercise)

4. Which of the following medications or therapies did you use or undergo in the last three months?

- ☐ Pyridostigmine (Mestinon)
- ☐ Prednisolone or prednisone
- ☐ Azathioprine (Imuran)
- ☐ Mycophenolate mofetil (Cellcept)
- ☐ Rituximab (MabThera)
- ☐ Ciclosporine (Neoral, Sandimmune)
- ☐ Methotrexate (Metoject, Emthexate)
- ☐ Cyclophosphamide (Endoxan)





Chapter 3

Ocular Weakness Patterns in Myasthenia Gravis: Changes Are Typical

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ABSTRACT

Introduction

In this study we quantitatively describe ocular weakness patterns in myasthenia gravis (MG) to help neurologists in making the clinical diagnosis and to investigate how the current outcome measures reflect ocular weakness in MG.

Methods

We investigated ptosis and diplopia patterns in a retro- and prospective cohort of 306 MG patients. Diplopia was systematically examined by testing extra-ocular muscle (EOM) fatigability in eight gaze directions for 60 seconds.

Results

Of patients with initial symmetric ptosis, 40% developed asymmetric ptosis at the second visit. Changes in type of ptosis occurred less often in seronegative MG patients (50%) than in patients with acetylcholine receptor (AChR) antibodies (70%) or muscle-specific kinase (MuSK) antibodies (69%) ($p = 0.038$). Of patients with diplopia on the first visit, EOMs of both eyes were affected in 75% and double vision contained both a vertical and horizontal component in 95%. At the second visit, 83% manifested weakness of other EOMs. The mean time (in seconds) to diplopia was 11.6 ± 14.0 and the mean time to ptosis was 27.6 ± 19.8 . Diplopia or ptosis manifested within 30 seconds in 87% and 58%, respectively. Patients who manifested diplopia after 30 seconds, reported no limitations due to diplopia.

Discussion

Changes in ptosis side or affected EOMs occur frequently in MG. In diagnostically challenging cases, we recommend testing ptosis and diplopia in all gaze directions for 60 seconds during at least two follow-up visits to maximize the chance of observing diplopia or changes in diplopia patterns observed earlier.

INTRODUCTION

Myasthenia gravis (MG) is an autoimmune disease characterized by fatigability and fluctuating muscle weakness that usually begins in ocular muscles.¹ The affected ocular muscles can be subdivided in muscles that move the eyeball (extraocular muscles; EOM), the muscle that elevates the upper eyelid (m. levator palpebrae superioris; LPS) and the muscle involved in closure of the eye (m. orbicularis oculi; OO). Weakness of these muscles results in diplopia, ptosis, and dry eyes, respectively. Weakness of the OO is less frequent and often occurs later on in the disease course.² Although MG is a systemic disease, ptosis is reported to be typically asymmetric. In addition, the (more pronounced) ptosis has been reported to shift from one eye to the other during the disease course, but no quantitative data of this phenomenon are reported.³ Furthermore, different reports describe distinct patterns of diplopia in MG.^{4,5} Unfortunately, only data from small retrospective cohorts is available and fatigability of EOMs has not been tested systematically. Detailed knowledge of the patterns and fluctuations of ocular weakness in MG is essential for neurologists treating patients with neuromuscular disorders: for diagnosis, to understand its pathophysiology and to establish the relevance of current commonly used outcome measures.^{6,7} In this study, we therefore aimed to investigate patterns of ptosis, diplopia and eye closure weakness (ECW) in MG patients and the sensitivity of the most frequently used clinical MG outcome measures for EOM weakness in a large prospective cohort of MG patients.

METHODS

Study design and participants

We included a prospective cohort of MG patients under treatment at the Leiden University Medical Center between 2016 and 2017 for systematic analysis of ptosis, EOM weakness and ECW. Healthy controls were also included to validate our tests for EOM weakness. In addition, patient records were included of patients under treatment at our hospital retrospectively alongside the prospective cohort to study the occurrence and symmetry of ptosis and ECW over time. As the collection of ptosis and ECW data occurred in the same way for both the prospective and the retrospective cohort (by using the appropriate items from quantitative myasthenia gravis (QMG) scores), the data from both cohorts could be pooled. Retrospective analysis of EOM weakness from patient records is more complex, as associating diplopia with a certain gaze direction to weakness of a specific EOM requires a detailed evaluation that is usually not performed during routine clinical follow-up. Moreover, subtle EOM weakness can be easily missed if not tested systematically. EOM weakness was therefore only tested in a prospective cohort. The diagnosis of MG was based on a combination of clinically confirmed fluctuating muscle weakness and the presence

of serum autoantibodies to the acetylcholine receptor (AChR) or muscle-specific kinase (MuSK). Seronegative myasthenia gravis (SNMG) was defined as fatigable muscle weakness in combination with abnormal decrement (at least 10%) during low-frequency repetitive nerve stimulation, increased jitter in single-fiber electromyography testing or a positive neostigmine test.¹

Standard protocol approvals, registrations, and patient consents.

The Medical Ethics Boards of the Leiden University Medical Center approved the study and its use of human subjects. All patients provided informed, written consent prior to study participation.

Outcome variables

In the prospective cohort of MG patients and healthy controls, EOM fatigability was systematically examined by asking patients to avert eye gaze in eight directions for 60 seconds. The angles in which eyes were averted were fixed, as the patients were seated in a chair with a fixed position and were instructed to look at dots on a wall. Angles were chosen according to normal ranges of eye movement and with respect to ranges decreasing with age.⁸⁻¹⁰ Horizontal angles were 30 degrees and the angles of elevation and depression were 23 and 46 degrees, respectively. These angles were within the normal ranges of elderly persons and were used for all participants. We always ensured dots were seen with both eyes at the start of the test for each particular gaze direction. The provocation time at which diplopia occurred was recorded in seconds. After manifestation of diplopia, patients were asked to cover one eye and asked which image disappeared. With this information, we could determine which EOM caused diplopia. In addition, ptosis was tested by asking patients to look upwards for 60 seconds. Patients were asked not to use pyridostigmine for at least five hours prior to testing. In the combined pro- and retrospective cohort, the presence of ptosis and ECW was investigated by collecting data from patient records. Moreover, we analyzed QMG and Myasthenia Gravis Activities of Daily Living (MG-ADL) scores in all MG patients. In addition, the diplopia items of the QMG (first item) and the MG-ADL (seventh item; MG-ADLdip) were independently investigated to assess sensitivity for overall diplopia or the relation between physician-reported EOM weakness and patient-reported limitations of EOM weakness. The relation between physician-reported ptosis (second QMG item) and patient-reported limitation due to ptosis (eighth MG-ADL item; MG-ADLpto) was also investigated. Additional clinical data collected in this study were sex, age, age at onset of first symptoms and presence of one or more additional autoimmune disease(s) (AID) and thymectomy with or without the presence of thymoma.

Statistical analysis

Kaplan-Meier survival curves were plotted to illustrate the differences in changes of ptosis type over time between MG patients with initial asymmetric or symmetric ptosis. To evaluate the differences between the curves a log-rank test was performed. A chi-squared test was performed to analyse the association of MG-ADLdip with the presence of diplopia and to analyse differences in the occurrence of changes in ptosis between different serological groups (AChR, MuSK or SNMG). Pearson correlation coefficients were used to investigate the correlation between patient-reported burden of diplopia or ptosis (MG-ADLdip or MG-ADLpto) and the shortest time to diplopia (of all eight gaze directions) or ptosis. *P*-values < 0.05 were considered significant. Statistical analyses were performed using SPSS version 23 (IBM Corp., Armonk, NY) and GraphPad Prism version 7.00 (GraphPad Software Inc., San Diego, CA).

RESULTS

Baseline characteristics

Ptosis and ECW were studied in 306 MG patients, 162 in a retrospective cohort and 144 in a prospective cohort, and 1402 visits were analyzed. Diplopia was investigated in a prospective cohort of 144 MG patients and 20 healthy controls and 210 visits were analyzed. Baseline characteristics of all patients are shown in Table 1.

Table 1. Baseline characteristics of 306 patients with MG included in this study

	Prospective cohort N=144	Retrospective cohort N=162
Age, y	58.0 ± 16.7	57.1 ± 19.6
Age at onset, y	45.5 ± 19.6	43.6 ± 20.5
Gender		
Male	58 (40)	51 (32)
Female	86 (60)	111 (68)
Antibodies		
AChR+	120 (83)	104 (64)
MuSK+	8 (6)	14 (9)
Seronegative	16 (11)	44 (27)
Immunotherapy	84 (58)	54 (33)
Thymectomy		
Yes, with thymoma	11 (8)	17 (11)
Yes, without thymoma	26 (18)	24 (15)
No	107 (74)	121 (75)
MG-ADL	4.1 ± 3.5	3.3 ± 1.7
QMG	6.6 ± 4.7	9.7 ± 5.5

Baseline characteristics, including MG-ADL and QMG scores at the first visit of 306 patients with MG included in this study are shown. Data are presented as number of patients (%) for categorical variables and as mean ± SD for continuous variables.

Ocular weakness patterns

In our complete cohort ptosis was present on the first visit in 66% and ECW in 28%. In our prospective cohort, diplopia was present in 64%. In 18%, no ocular weakness was manifest. The most frequent pattern of ocular weakness was isolated diplopia (42%) or ptosis with diplopia (22%). Ptosis was asymmetric in 92% and ECW in 9%. EOM weakness occurred in both eyes in 75%. Ocular weakness patterns at the first visit are reported in more detail in Table 2. None of the 20 healthy controls manifested diplopia during EOM fatigability testing.

Table 2. Baseline ocular weakness patterns

	MG patients N=306
Ptosis (P)	
Absent	104 (34)
Right eye	102 (33)
Left eye	84 (28)
Symmetric	16 (5)
ECW	
Absent	221 (72)
Right eye	2 (1)
Left eye	6 (2)
Symmetric	77 (25)
	MG patients N=144 ^a
Diplopia (D)	
Absent	52 (36)
Right eye	8 (6)
Left eye	15 (10)
Both eyes	69 (48)
No ocular weakness	26 (18)
P only	10 (7)
D only	50 (35)
ECW only	3 (2)
P + D	26 (18)
P + ECW	3 (2)
D + ECW	12 (8)
P + D + ECW	14 (10)

The frequency of the occurrence of ptosis (P), eye closure weakness (ECW) and diplopia (D) at the first visit are shown. In addition, the frequency of simultaneously present ocular symptoms is reported. Data are presented as number of patients (%).

^a Diplopia and combinations of ocular symptoms is shown of the prospective cohort of 144 MG patients.

EOMs were affected unilaterally in 74 %. The most commonly affected EOMs were the m. rectus lateralis (65%) and the m. obliquus superior (53%). Purely horizontal diplopia was present in 5 patients, in whom solely the rectus lateralis was affected. Purely vertical diplopia did not occur. An overview of the frequency of the weakness of the 6 EOMs at the first visit is shown in Figure 1. In 82% of patients, more than one EOM was affected at the first visit.

Changes in ocular weakness patterns over time

146 patients had multiple visits during which ptosis and ECW were investigated. Forty percent of patients with initial symmetric ptosis developed asymmetric ptosis at the second visit (Figure 2). The change from symmetric ptosis to asymmetric ptosis occurred significantly more often than the other way around ($p = 0.0002$). Only 1% of patients with initial asymmetric ptosis developed symmetric ptosis at the second visit. In 10% of MG patients, the side of ptosis changed at the second visit. Changes in type of ptosis occurred less often in SNMG patients (50%) than in AChR (70%) or MuSK patients (69%) ($p = 0.038$).

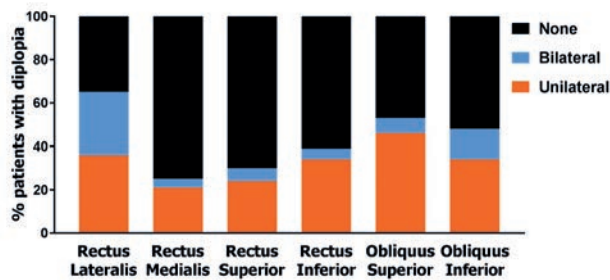


Figure 1. Overview of affected EOMs

Bars showing the distribution of involved EOMs in patients with diplopia at the first visit. For each EOM, the frequency of uni- and bilateral weakness is shown.

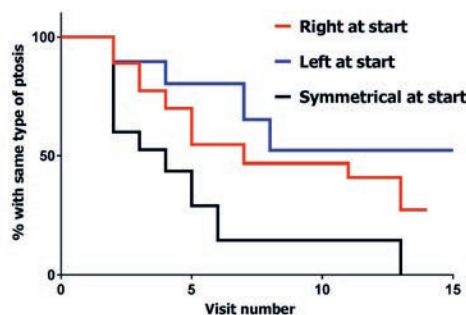


Figure 2. Changes in type of ptosis

Kaplan-Meier survival curves indicate the change of the type of ptosis within 146 MG patients, subdivided by initial type of ptosis. Only changes from one side to another or from symmetric to asymmetric or vice versa were considered changes, the disappearance of ptosis was not considered as a change of type of ptosis. Both retro- and prospective data was used for these curves.

Eighty-three percent of patients with EOM weakness at the first visit manifested weakness of other EOMs at the next visit. In 33% of patients with initial diplopia caused by unilateral EOM weakness, both eyes were affected at the next visit. In 8% of patients, initial diplopia caused by bilateral EOM weakness became confined to one eye at the next visit. Figure 3 provides an overview of the affected EOM on the first and second visit in 3 MG patients that showed substantial changes. The time between visits is also shown in this figure and mostly was around 4 weeks. Five percent of patients with initial symmetric ECW developed (temporary) asymmetric ECW at some point within their whole disease course.

Antibody status / sex / age	Disease duration	Affected EOMs						Time between visits	
AChR ♂ 66y	1y	V1	R	R	R	R	R	R	4w
			L	L	L	L	L	L	
		V2	R	R	R	R	R	R	
			L	L	L	L	L	L	
		RL RM RS RI OS OI							
AChR ♀ 54y	47y	V1	R	R	R	R	R	R	4w
			L	L	L	L	L	L	
		V2	R	R	R	R	R	R	
			L	L	L	L	L	L	
		RL RM RS RI OS OI							
AChR ♂ 74y	51y	V1	R	R	R	R	R	R	4w
			L	L	L	L	L	L	
		V2	R	R	R	R	R	R	
			L	L	L	L	L	L	
		RL RM RS RI OS OI							

Figure 3. Changes of EOM weakness in individual MG patients
Baseline characteristics and an overview of affected EOMs of three AChR MG patients that had a substantial change of diplopia pattern at the second visit. Red indicates that the EOM is affected and green indicates that the EOM is not affected.
Abbreviations: R = right eye, L = left eye, RL = m. rectus lateralis, RM = m. rectus medialis, RS = m. rectus superior, RI = m. rectus inferior, OS = m. obliquus superior, OI = m. obliquus inferior

Provocation time required to develop ptosis or diplopia

The mean time (in seconds) to diplopia was 11.6 ± 14.0 (Figure 4) and the mean time to ptosis was 27.6 ± 19.8 (Figure 5). Diplopia or ptosis manifested within 30 seconds in 87% and 58%, respectively. The correlation between the lowest individual times to diplopia or

ptosis and their respective MG-ADL items were $r = -0.340$; $p = 0.001$ and $r = 0.0407$; $p = 0.132$, respectively. In other words, diplopia usually manifested within 30 seconds and a longer time to onset during examination correlated significantly with a lower patient-reported disease burden. In contrast, ptosis occurred almost as frequently within 0-30 seconds as within 30-60 seconds and the time to ptosis during examination did not show a correlation to patient-reported limitations. Figure 6 shows that diplopia that manifested after 30 seconds was not associated with limitations in MG patients. The MG-ADLdip was significantly correlated to the presence of diplopia ($p < 0.001$). However, 50 patients with diplopia during examination (56%) did not report limitations due to diplopia.

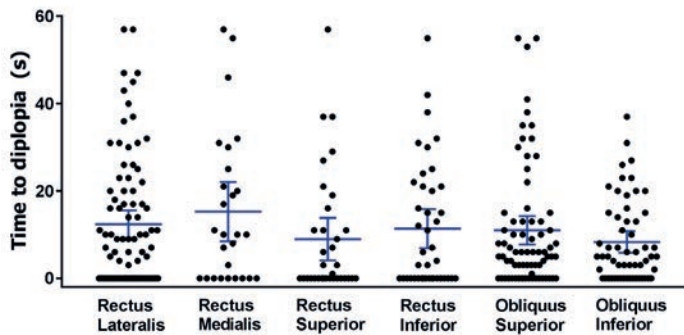


Figure 4. Time to diplopia for all EOMs

Scatter dot plots showing the time within which diplopia manifested after provocation for each EOM. Mean and 95% CI are shown by lines and error bars. Dots at the bottom (0), indicate spontaneous diplopia. Data of patients without diplopia have been omitted.

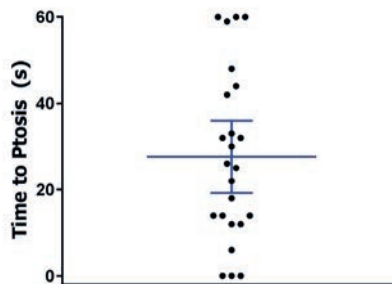


Figure 5. Time to ptosis

Scatter dot plot showing the time within which ptosis manifested after provocation. The eye with the shortest time to ptosis is plotted here. Mean and 95% CI are shown by lines and error bars. Dots at the bottom (0), indicate spontaneous ptosis. Data of patients without ptosis have been omitted.

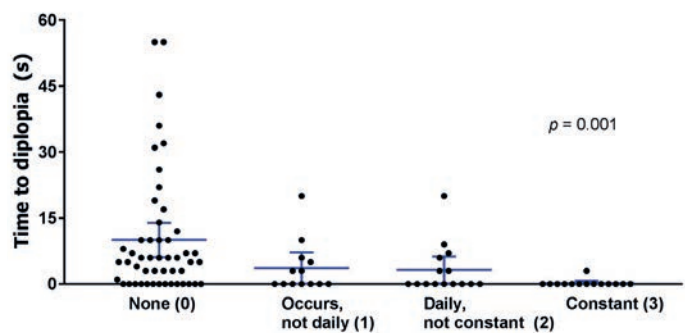


Figure 6. Correlation time to diplopia and MG-ADL diplopia item
Scatter dot plots showing the time within which diplopia manifested after provocation, grouped according to the MG-ADL diplopia score of each MG patient (x-axis). An MG-ADL diplopia item score of 0 indicated that no subjective symptoms of diplopia were present in the patient. Eight gaze directions were tested per patient, the gaze direction with the shortest time to diplopia is plotted here. Mean and 95% CI are shown by lines and error bars. Dots at the bottom (0), indicate spontaneous diplopia. Data of patients without diplopia are not shown.

Clinical diplopia and the diplopia item of the QMG

Of the 92 patients with diplopia, 29 (32%) did not manifest diplopia in the horizontal right gaze direction. Thus, the current diplopia item of the QMG would be considered normal in 32% of patients with diplopia. As we previously found that diplopia occurring after 30 seconds was both rare and of no major burden for the patient, we investigated which additional gaze directions gave the highest yield within 30 seconds of testing. By additionally testing the top left gaze direction for 30 seconds, 14 additional patients manifested diplopia (15%). By also testing the bottom right gaze direction for 30 seconds, another 8 patients (9%) manifested diplopia, thereby missing only 7 patients with overall diplopia (8%), of which only 3 with relevant diplopia (manifesting within 30 seconds).

DISCUSSION

This is the first detailed study of fluctuations in ocular weakness in a large group of 306 MG patients. We found that ocular symptoms are very common, that diplopia is usually caused by EOM weakness in both eyes and that ptosis is most commonly asymmetrical or becomes so over time. Both EOM weakness and the side of ptosis frequently change over the course of the disease. The most commonly affected EOMs are the m. rectus lateralis and the m. obliquus superior. Diplopia usually occurs within 30 seconds and time to diplopia is strongly correlated with MG-ADL-dip. Ptosis takes longer to develop and is not correlated with patient-reported measures (MG-ADLpto).

Eighty-two percent of the MG patients in our prospective cohort had ocular symptoms at the first visit. This is in line with previous studies reporting that approximately 85% of MG

patients had ocular symptoms at disease onset, while only 10-20% of patients had weakness of arms or legs initially.^{2,11} The fact that ocular muscles are predominantly affected clinically in MG may be caused by differences in neuromuscular junction (NMJ) morphology and physiology. Several features of the NMJs of EOMs may cause their increased susceptibility: less prominent synaptic folds, fewer postsynaptic AChRs, smaller motor units (around 4-12 muscle fibers compared to 400-600 in limb muscles), the 6-8 times higher innervation rate and the low expression of complement regulators.^{3,12-17} In addition to differences at the level of the NMJ, EOMs have distinct functional properties that may also play a role in the increased vulnerability of these muscles. Compared to limb muscles, EOMs are continuously active, have limited peak activity and due to the equal innervation of EOMs, a minor deficit in innervation can lead to diplopia as no compensation can occur.³ However, an explanation for the frequent occurrence of changes in involved EOMs is currently lacking. In contrast to EOMs (or LPS), fluctuations in generalized weakness often affect muscles on both sides in a similar degree.¹ Future research investigating the differences between muscles should also focus on clarifying why changes in affected EOMs occur more frequently than changes in side of affected limbs.

In our prospective cohort of MG patients, 64% manifested diplopia during EOM fatigability testing. None of the 20 healthy controls manifested diplopia during this test. This was in line with earlier studies showing that EOMs are relatively fatigue resistant in healthy subjects.^{18,19} Furthermore, our findings show that the specific EOMs involved frequently change between visits, in 83% of MG patients at least one other EOM was involved at the second visit. In patients who developed diplopia, it usually manifested within 30 seconds. When diplopia was present, EOMs of both eyes were usually affected (75%) and double vision contained both a vertical and horizontal component in most cases (95%). Supplement 1 gives an overview of EOM involvement in MG patients in earlier studies.

Diplopia manifested within 30 seconds in 87% and a strong correlation was found between time to diplopia and MG-ADLdip, suggesting that time to diplopia is a good marker for clinical severity. However, 54% of patients with observed diplopia (in 48% occurring within 30 seconds) did not report limitations due to diplopia (MG-ADLdip = 0). We hypothesize that the discrepancy between physician-reported diplopia by testing EOM fatigability and the patient-reported burden due to diplopia is caused by adaptive mechanisms such as turning the head into the desired direction straight away without (first) moving the eyes in that direction.

When ptosis is present, it is usually asymmetric. In those patients who present with symmetric ptosis, it usually becomes asymmetric within a few visits. In patients with asymmetric ptosis, the worst affected side frequently changes. These features should be helpful in the distinction from other causes of ptosis, such as cranial nerve palsy or chronic progressive external ophthalmoplegia, which are not expected to show similar fluctuations. Interestingly, changes in form of ptosis occurred less often in SNMG patients than in AChR or MuSK MG

patients. To our knowledge, it has not earlier been described that SNMG patients show less disease expression. In contrast to diplopia, ptosis manifested in only a minority of patients (42%) within 30 seconds, suggesting that longer testing is needed for an optimal evaluation of ptosis. Our findings differ from that of a study by Burns et al. on the Myasthenia Gravis Composite (MGC) scale, who reported that <1% of ocular score times were between 46 and 60 seconds compared to 21% in our cohort.²⁰

In contrast to diplopia, no significant correlation was found between time to ptosis and the clinical burden as quantified in the MG-ADLpto. We hypothesize that the reason for this lack of correlation is that in daily life, the provocation of ptosis (by looking upward for a prolonged time) occurs less frequently and could be diminished by adaptive mechanisms, such as watching television with the eyes in a slightly depressed position. In addition, subtle forms of ptosis are less likely to affect quality of life as long as the upper eyelid does not impair vision.

The strength of this study is the systematic evaluation of ocular weakness patterns in a large cohort of clinically well-defined MG patients. However, the study has a number of limitations. Patients were included from a single center and as this is a tertiary referral center, our study may not fully reflect the total MG population due to referral bias. Moreover, our testing method for EOM weakness did not provide quantitative data on the deviation of the eye axes (i.e. angle between the axes), as can be measured by ophthalmologic tools such as the Hess chart. However, the Hess chart does not provide data on the time required to develop diplopia. As muscle fatigability rather than weakness is the hallmark of MG, we focused on ‘provocation time’ instead of the quantitative deviation of the eye axes at a single time point. Indeed, our data show that time to diplopia is strongly correlated with clinical, patient-reported severity of diplopia. In addition, the testing method described here is applicable by neurologists that treat MG patients without the use of advanced equipment. Finally, the retrospective analysis of the occurrence and symmetry of ptosis and ECW over time is a limitation of this study. However, retrospective analyses allowed us to study a far larger number of patients and patient visits and provided us with a longer time period over which changes in ptosis and ECW could be investigated.

In conclusion, ocular symptoms are very common in MG and in 10-17% of patients, muscle weakness remains clinically localized to the ocular muscles during the entire disease course (ocular MG; OMG).^{2,3} As both laboratory and neurophysiological tests have a lower sensitivity and specificity in OMG, the diagnosis is more difficult to make in this group of patients and often relies solely on clinical grounds.²¹ Here, we described typical ocular weakness patterns to aid clinical recognition of MG. In diagnostically challenging cases, we recommend testing ptosis and diplopia in all gaze directions for 60 seconds during at least two follow-up visits to maximize the chance of observing diplopia or changes in diplopia patterns observed earlier. The involvement of multiple EOMs in both eyes and changes in affected EOMs or ptosis side will aid the neurologist in clinically diagnosing MG.

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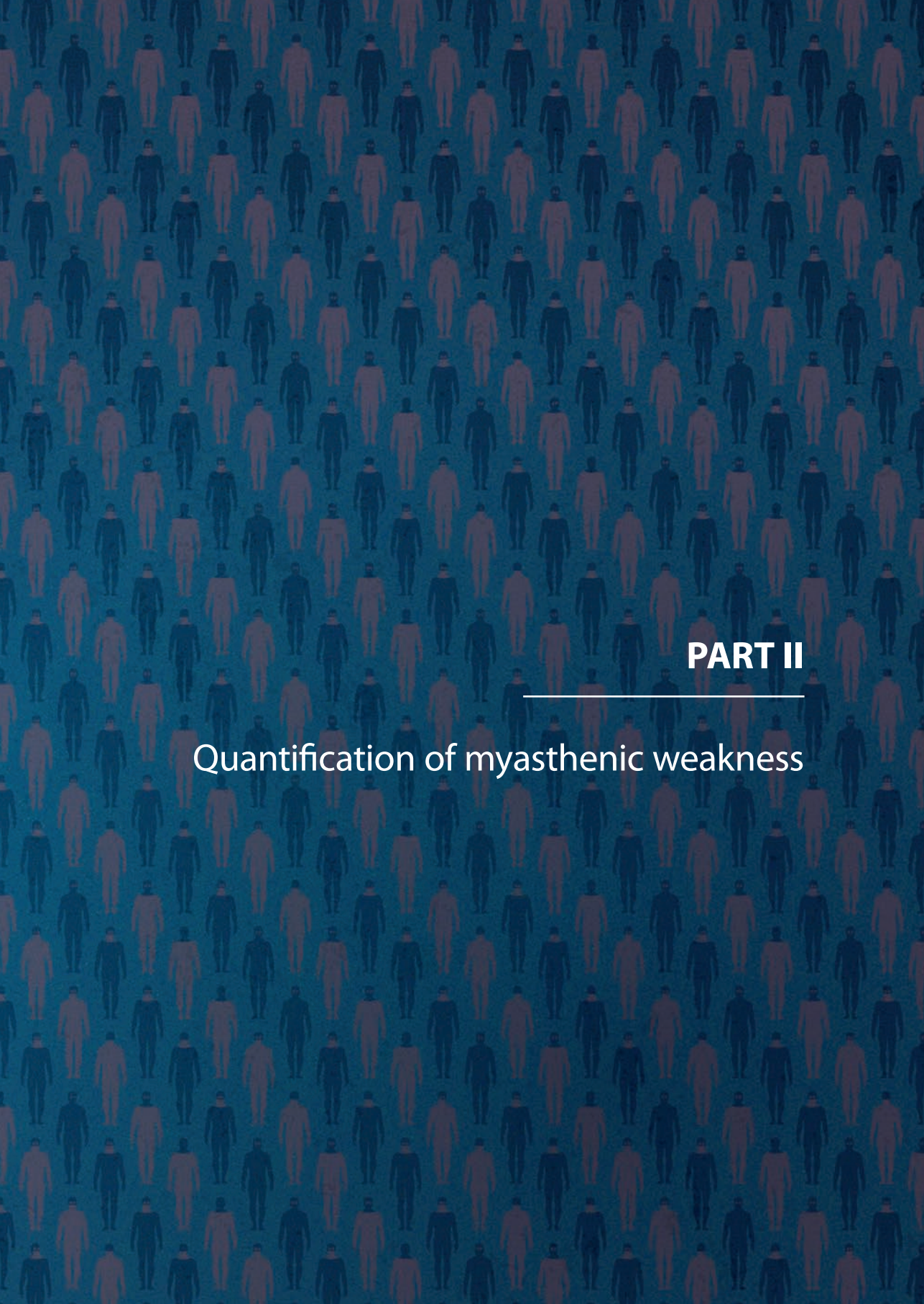
Supplemental material

SUPPLEMENT 1

Overview EOM involvement in different studies

EOM	Oosterhuis ²⁸	Cleary et al. ⁷³	Almog et al. ⁷⁴	Current study
Rectus Lateralis	-	59%	30%	65%
Rectus Medialis	“most commonly affected”	56%	13%	25%
Rectus Superior	“followed by the elevators with a preference for the superior recti”	82%	13%	30%
Rectus Inferior	-	41%	20%	39%
Obliquus Superior	-	39%	10%	53%
Obliquus Inferior	“followed by the elevators with a preference for the superior recti”	67%	63%	48%

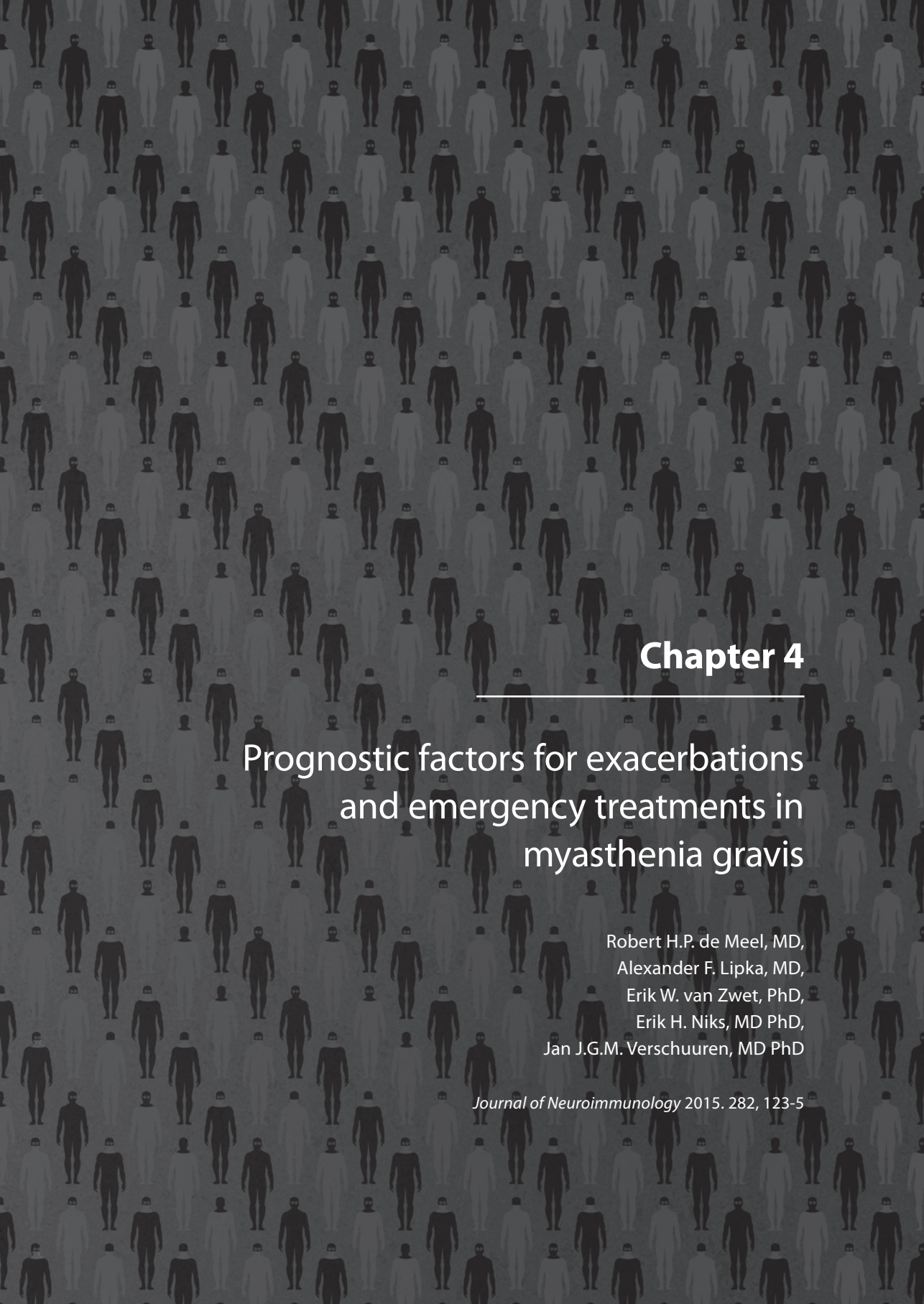
Overview of the frequency of EOM involvement in different studies. In the publication of Oosterhuis, no exact frequencies were reported and therefore the relevant passages of the article are quoted.



PART II

Quantification of myasthenic weakness





Chapter 4

Prognostic factors for exacerbations and emergency treatments in myasthenia gravis

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ABSTRACT

Background

Disease course in myasthenia gravis (MG) patients is highly variable. Prognostic factors that identify patients at risk for a more severe disease course would be helpful in clinical practice.

Methods

We investigated MG patients under treatment at our university medical center between 1993 and 2013. The impact of baseline characteristics on the occurrence of exacerbations and the necessity of emergency treatments were investigated using multiple logistic regression analysis and log-rank tests for our Kaplan-Meier survival curves.

Results

We included 96 MG patients. Late age at onset (LOMG, ≥ 50 years) was associated with a higher risk of an exacerbation (odds ratio [OR] = 9.33, 95% confidence interval [CI] = 2.43-35.87; $p = 0.001$) and the necessity of an emergency treatment within 3 years (OR = 5.25, 95% CI = 1.21-22.80; $p = 0.027$). The presence of additional autoimmune diseases (AID) in the patient was associated with a higher risk of an exacerbation (OR = 4.03, 95% CI = 1.09-14.85; $p = 0.036$). Patients with both LOMG and an additional AID had a markedly higher risk of an exacerbation (OR = 47.00, 95% CI = 6.49-340.65; $p < 0.001$) and the necessity of an emergency treatment (OR = 26.11, 95% CI = 4.12-165.55; $p = 0.001$) compared to early-onset patients without additional autoimmune diseases.

Conclusions

Late-onset MG and the presence of additional autoimmune diseases were associated with a higher risk of exacerbations of MG and the necessity of emergency treatments.

INTRODUCTION

Myasthenia gravis (MG) is characterized by fatigability and fluctuating muscle weakness of extraocular and skeletal muscles. The clinical course of MG is highly variable, ranging from stable disease or remission to the occurrence of several exacerbations over time. Previous studies have mainly focused on prognostic factors for remission. However, prognostic factors for exacerbations, myasthenic crises and emergency treatments could also be useful to the clinician for predicting the course of the disease. These outcome measures can be used in order to better predict which patients are at risk for a more severe and debilitating disease course. We investigated the association between baseline clinical features and the risk of exacerbations and emergency treatments in 96 MG patients.

METHODS

Patients

We investigated MG patients under treatment at our university medical center between 1993 and 2013 for whom follow-up of at least 3 years was available. Myasthenia gravis was defined as the combination of clinically confirmed fluctuating muscle weakness with autoantibodies to the acetylcholine receptor (AChR MG) or muscle-specific kinase (MuSK MG). Seronegative myasthenia gravis (SNMG) was defined as a similar clinical presentation in combination with abnormal decrement (at least 10%) during low-frequency repetitive nerve stimulation, increased jitter in single-fiber EMG testing or a positive neostigmine test.¹ All patients included in this study gave informed consent.

Baseline Characteristics

Baseline variables included in this study were sex, age at onset of first symptoms below 50 years or older, MGFA clinical classification at the first visit in our center, presence of one or more additional autoimmune disease(s) (AID), presence of acetylcholine receptor (AChR) antibodies, thymectomy and presence of thymoma in the patient history. These variables were chosen based on previous prognostic studies, potential clinical relevance and reliability with which these data could be analyzed retrospectively.¹⁻⁶

Endpoints

Primary endpoint was the occurrence of one or more exacerbation(s) within 3 years of follow-up. An exacerbation was defined as a clinical deterioration of MG that required starting with or an increase of at least 20 mg of prednisone or led to an emergency treatment. An emergency treatment was defined as the administration of intravenous immunoglobulins, plasmapheresis or mechanical ventilation. In order to define the most severe subgroup of

MG patients that had an exacerbation, the necessity of one or more emergency treatment(s) within 3 years of follow-up was also investigated as a separate secondary endpoint.

Statistical Analysis

A multiple logistic regression analysis was performed to assess the impact of baseline variables on all endpoints. We used Firth logistic regression to assess the significance of the variable 'AChR antibodies' for the secondary endpoint, since none of the AChR antibody-negative patients had required an emergency treatment, prohibiting the inclusion of this variable in a standard multiple logistic regression model.⁷ We omitted this variable from further consideration, as it was not significant in the Firth logistic regression and had no impact on the primary endpoint.

The variables that were shown to be significant in the first analyses were included in a second multiple regression model in which their two-way interactions were included. Kaplan-Meier survival curves were plotted to illustrate the differences in course of the disease over time. To evaluate the differences between the curves a log-rank test was performed. Results are expressed as odds ratios (OR) with 95% confidence intervals (CI). *P*-values lower than 0.05 were considered significant. Statistical analyses were performed using SPSS version 20 (IBM Corp., Armonk, NY) and GraphPad Prism version 6.02 (GraphPad Software Inc., San Diego, CA).

RESULTS

We included 96 patients with MG. Baseline characteristics of the patients, as well as the number of patients with an exacerbation or an emergency treatment within 3 years are shown in table 1.

Late-onset MG (LOMG) was independently associated with a higher risk of an exacerbation within 3 years (odds ratio [OR] = 9.33, 95% confidence interval [CI] = 2.43-35.87; *p* = 0.001). Presence of another AID, in addition to MG, was also independently associated with a higher risk of an exacerbation (OR = 4.03, 95% CI = 1.09-14.85; *p* = 0.036). The most frequently observed additional autoimmune diseases were thyroid disease (*n* = 8), psoriasis (*n* = 4) and SLE (*n* = 3). Some patients had more than one additional autoimmune disease. Outcomes for the first endpoint are reported in table 2.

Table 1. Baseline characteristics of 96 patients with MG and the number of patients with an exacerbation and the necessity of an emergency treatment within 3 years after presentation in our university medical center are shown. Data are presented as number of patients (%) for categorical variables and as mean $\pm$ SD for continuous variables.

		Exacerbations <3 years	Emergency treatments <3 years
Age, y	56.4 $\pm$ 19.7		
Age at onset, y	41.6 $\pm$ 20.1		
<50	62 (65)	5 (8)	4 (7)
$\geq$ 50	34 (35)	16 (47)	10 (29)
Sex			
Female	61 (64)	9 (15)	5 (8)
Male	35 (36)	12 (34)	9 (26)
Disease duration, y	14.6 $\pm$ 11.7		
Antibodies			
AChR antibodies	85 (89)	20 (24)	14 (17)
MuSK antibodies	1 (1)	0 (0)	0 (0)
Seronegative MG	10 (10)	1 (10)	0 (0)
Additional AID			
Yes	20 (21)	8 (40)	6 (30)
No	76 (79)	13 (17)	8 (11)
MGFA at baseline			
0	11 (11)	2 (18)	2 (18)
1	34 (35)	7 (21)	2 (6)
2	32 (33)	4 (13)	3 (9)
3	14 (15)	5 (36)	5 (36)
4	3 (3)	1 (33)	0 (0)
5	2 (2)	2 (100)	2 (100)
Thymectomy			
Yes	23 (24)	4 (17)	4 (17)
No	73 (76)	17 (23)	10 (14)
Histology			
No abnormalities	15 (65)	2 (13)	2 (13)
Thymus hyperplasia	2 (9)	0 (0)	0 (0)
Thymoma	6 (26)	2 (33)	2 (33)

Abbreviations: AChR = acetylcholine receptor; MuSK = muscle-specific kinase; AID = autoimmune disease(s)
MGFA = Myasthenia Gravis Foundation of America.

Table 2. Results of the first and second, subgroup-based, logistic regression for the occurrence of an exacerbation within 3 years are shown. The subgroups were defined by the variables age at onset and additional AID. Data are presented as number of patients (%) and OR (95% CI). The category or subgroup with OR = 1 was used as reference group.

Exacerbations	No. of patients	No. of patients with ≥ 1 exacerbation(s)	OR (95% CI)	<i>p</i> Value
Sex				
Female	61	9 (15)	1	
Male	35	12 (34)	1.78 (0.54-5.89)	0.345
Age at onset, y				
<50	62	5 (8)	1	
≥ 50	34	16 (47)	9.33 (2.43-35.87)	0.001
MGFA at first visit				
≤ 1	43	10 (23)	1	
>1	53	11 (21)	1.56 (0.48-5.12)	0.463
Additional AID				
No	76	13 (17)	1	
Yes	20	8 (40)	4.03 (1.09-14.85)	0.036
AChR antibodies				
No	11	1 (9)	1	
Yes	85	20 (24)	2.02 (0.21-19.58)	0.543
Thymectomy				
No	73	17 (23)	1	
Yes	23	4 (17)	0.77 (0.10-5.99)	0.802
Thymoma				
No	90	19 (21)	1	
Yes	6	2 (33)	1.64 (0.10-26.12)	0.728
Subgroups				
EOMG & AID –	50	3 (6)	1	
EOMG & AID +	12	2 (16)	3.13 (0.46-21.27)	0.242
LOMG & AID –	26	10 (39)	9.79 (2.39-40.08)	0.002
LOMG & AID +	8	6 (75)	47.00 (6.49-340.65)	< 0.001

Abbreviations: EOMG = early-onset MG; LOMG = late-onset MG; MGFA = Myasthenia Gravis Foundation of America; AID = autoimmune disease(s); AID – = no additional autoimmune disease(s); AID + = additional autoimmune disease(s); AChR = acetylcholine receptor; OR = odds ratio; CI = confidence interval.

LOMG also independently increased the risk of the necessity of an emergency treatment within 3 years (OR = 5.25, 95% CI = 1.21-22.80; $p = 0.027$). The relationship between an additional AID and the occurrence of an emergency treatment showed a similar OR as for an exacerbation, although this failed to reach statistical significance (OR = 3.64, 95% CI = 0.94-14.11; $p = 0.062$). For the other baseline variables no significant differences were found. All outcomes for the second endpoint are reported in supplement 1.

LOMG in combination with an additional AID was strongly associated with a higher risk of an exacerbation within 3 years as compared to early-onset MG without an additional AID (OR = 47.00, 95% CI = 6.49-340.65; $p < 0.001$). The combination also strongly increased the risk of an emergency treatment (OR = 26.11, 95% CI = 4.12-165.55; $p = 0.001$). Four subgroups can be defined using combinations of these two variables. Survival curves for the time courses without exacerbations and emergency treatments of these four subgroups are illustrated in figure 1.

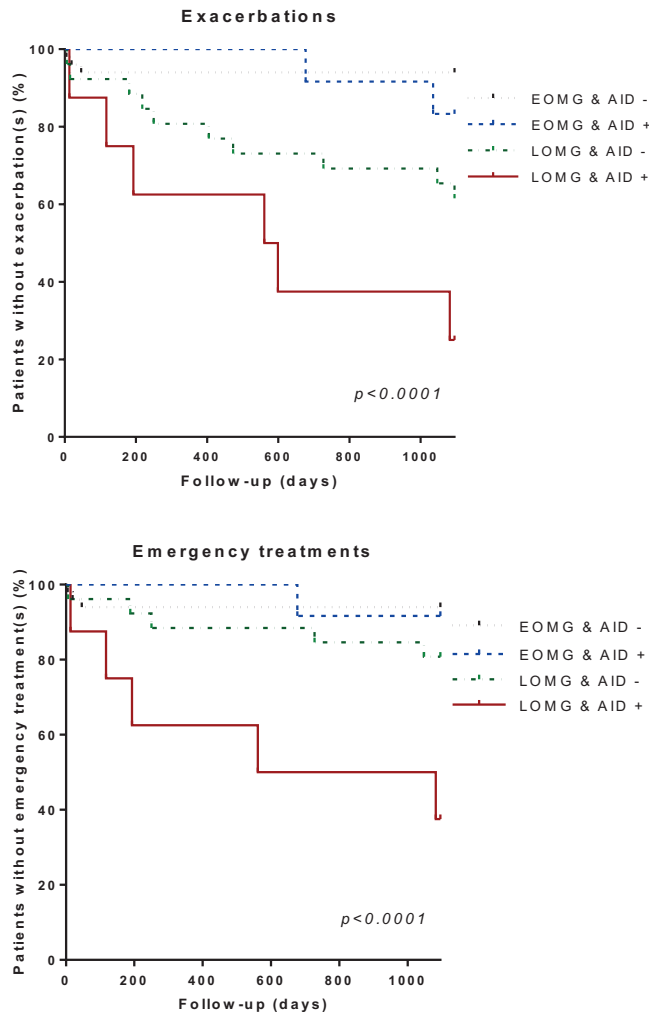


Figure 1. Kaplan-Meier survival curves for the time courses without exacerbations and emergency treatments of MG patients, subdivided by age at onset and presence of additional AID, are shown. To evaluate the differences between the curves a log-rank test was performed. The p -values are shown in the figure.

Abbreviations: EOMG = early-onset MG; LOMG = late-onset MG; AID - = no additional autoimmune disease(s); AID + = additional autoimmune disease(s).

DISCUSSION

LOMG patients had a higher risk of an exacerbation and an emergency treatment within 3 years. This is in line with the reported association of LOMG with a lower remission rate and a higher frequency of progression to more severe disease, although the effect size on exacerbations in our study was considerably higher than those found for remission and progression of disease severity in previous studies.^{3-6,8,9} The presence of an additional AID was also associated with a higher risk of an exacerbation. Moreover, patients with both LOMG and an additional AID had a markedly higher risk of an exacerbation and an emergency treatment.

The variables sex, MGFA, AChR antibodies, thymectomy and thymoma showed no significant associations with exacerbations. In contrast, studies focusing on remission found associations with thymectomy, clinical stage at onset and maximal worsening.^{2,4-6}

Limitations of our study include the single center of inclusion, a relatively small sample size, definition of our primary endpoint and retrospective analysis. We tried to minimize these by choosing variables that could reliably be analyzed afterwards. The current study is tertiary clinic-based and may not fully reflect the total MG population due to a referral bias. As for our primary endpoint, there is currently no consensus regarding a definition for exacerbation.¹⁰ For this study we defined an exacerbation as a clinical deterioration of MG that required starting with or an increase of at least 20 mg of prednisone or led to an emergency treatment. The cut-off point of 20 mg was based on clinical experience and was chosen to exclude clinically non-significant fluctuations, which result in small adaptations of the dose but do not represent genuine exacerbations of the disease.

Late-onset of symptoms and the presence of additional autoimmune diseases were shown to be prognostic factors for exacerbations and the necessity of emergency treatments in MG. More stringent control of care and treatment could help prevent exacerbations and emergency treatments in these high-risk patients. Besides remission, exacerbations and emergency treatments could also be considered as endpoints in future studies.

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Supplemental material

SUPPLEMENT 1

Logistic regression for the occurrence of emergency treatments

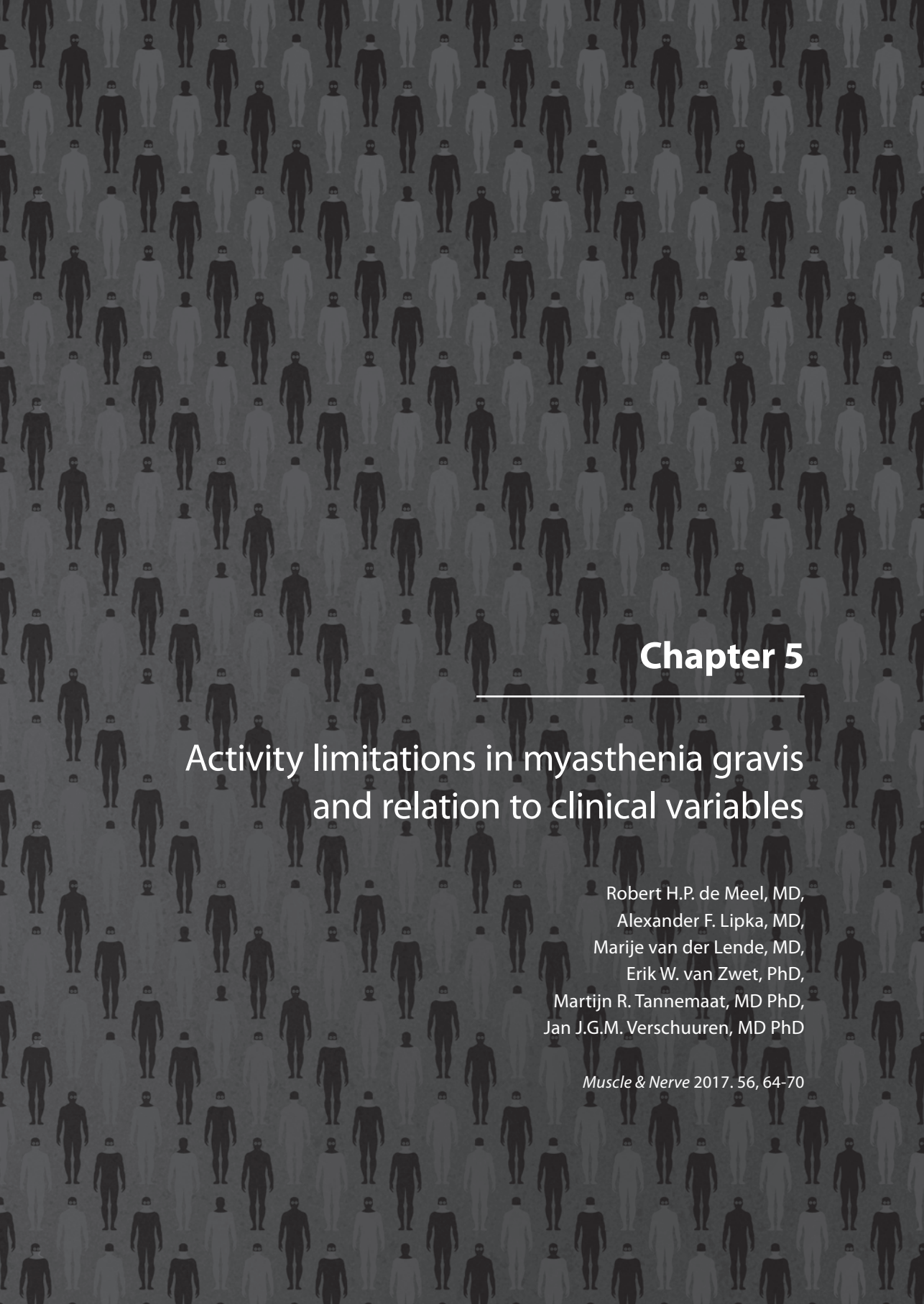
Emergency Treatments	No. of patients	No. of patients with ≥ 1 emergency treatment(s)	OR (95% CI)	<i>p</i> Value
Sex				
Female	61	5 (8)	1	
Male	35	9 (26)	2.24 (0.59-8.54)	0.237
Age at onset, y				
<50	62	4 (7)	1	
≥ 50	34	10 (29)	5.25 (1.21-22.80)	0.027
MGFA at first visit				
≤ 1	43	8 (19)	1	
>1	53	6 (11)	0.86 (0.23-3.15)	0.818
Additional AID				
No	76	8 (11)	1	
Yes	20	6 (30)	3.64 (0.94-14.11)	0.062
AChR antibodies ^a				
No	11	0 (0)	-	
Yes	85	14 (17)	-	-
Thymectomy				
No	73	10 (14)	1	
Yes	23	4 (17)	1.51 (.20-11.45)	0.690
Thymoma				
No	90	12 (13)	1	
Yes	6	2 (33)	1.85 (0.13-26.93)	0.653
Subgroups				
EOMG & AID -	50	3 (6)	1	
EOMG & AID +	12	1 (8)	1.42 (0.14-15.03)	0.769
LOMG & AID -	26	5 (19)	3.73 (0.82-17.07)	0.090
LOMG & AID +	8	5 (63)	26.11 (4.12-165.55)	0.001

Results of the first and second, subgroup-based, logistic regression for the occurrence of an emergency treatment within 3 years are shown. The subgroups were defined by the variables age at onset and additional AID. Data are presented as number of patients (%) and OR (95% CI). The category or subgroup with OR = 1 was used as reference group.

^a For statistical reasons it was impossible to include the serum AChR antibodies variable in our multiple logistic regression analysis for the secondary endpoint (see methods).

Abbreviations: EOMG = early-onset MG; LOMG = late-onset MG; MGFA = Myasthenia Gravis Foundation of America; AID = autoimmune disease(s); AID – = no additional autoimmune disease(s); AID + = additional autoimmune disease(s); AChR = acetylcholine receptor; OR = odds ratio; CI = confidence interval.





Chapter 5

Activity limitations in myasthenia gravis and relation to clinical variables

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ABSTRACT

Introduction

It is unknown how fluctuations in muscle weakness affect activity limitations in myasthenia gravis patients and how the severity of these limitations compares with published data on other neuromuscular disorders (NMD).

Methods

We analyzed ACTIVLIM (acronym of 'ACTIVity LIMitations') and Quantitative Myasthenia Gravis (QMG) scores. We assessed the impact of QMG and other clinical variables on ACTIVLIM, using B coefficients.

Results

The mean ACTIVLIM score in 118 MG patients was 3.3. There was a correlation between QMG and ACTIVLIM (B coefficient = -0.206; $p < 0.001$) and between changes in both scores (B coefficient = -0.175; $p = 0.002$). Men and patients without another autoimmune disease had a better ACTIVLIM score (B coefficient = 0.785; $p = 0.015$ and B coefficient = 0.998; $p = 0.008$, respectively).

Discussion

The ACTIVLIM score in MG is higher than in other NMD. Fluctuations in QMG correlated significantly with changes in ACTIVLIM.

INTRODUCTION

Myasthenia gravis (MG) is an autoimmune disease characterized by fatigability and fluctuating muscle weakness. In contrast to many other neuromuscular disorders (NMD) that are irreversibly progressive, the disease severity in many MG patients may change markedly over time with both improvements and deteriorations. Furthermore, the clinical spectrum of MG is broad, ranging from mild ptosis to severe generalized weakness.¹ The Quantitative Myasthenia Gravis (QMG) test can be used to measure ocular, bulbar, and generalized muscle fatigability.² Several other measures have been developed to assess quality of life or impairments in activities of daily living (ADL) in patients with MG.³⁻⁹ However, impairments in ADL have never been compared between MG patients and patients with other NMD. The systematic use of a quantitative measure of activity limitations for different NMD may be useful to compare the burden they impose on ADL and establish clinically relevant outcome measures for future clinical trials. In addition, it is not known how fluctuations of the severity of muscle weakness affect the limitations that MG patients experience in ADL. The ACTIVLIM (acronym of 'ACTIVity LIMitations') questionnaire is a validated measure of daily activity limitations for patients with NMD in general.¹⁰ Clinically, it may be practical to use a generic outcome measure of ADL for all NMD to provide insight into the impact of different NMD on daily activities for individual patients. We measured the ACTIVLIM score in MG patients to estimate their ADL limitations. This enabled a comparison between the scores of our MG patients and previously published data from patients with other NMD.¹¹⁻¹⁴ We then investigated the relationship between several clinical variables and the ACTIVLIM score to identify which factors contribute to limitations in daily activities. Finally, we analyzed how changes in muscle strength and fatigability, as measured by the QMG test, affected the ACTIVLIM score over a longer period of time.

METHODS

Patients

We included MG patients under treatment at the Leiden University Medical Center between 2007 and 2013. The QMG and ACTIVLIM scores were recorded in all MG patients who visited the outpatient clinic as part of routine clinical care. Inclusion in this study required a combination of clinically confirmed fluctuating muscle weakness and the presence of serum autoantibodies to the acetylcholine receptor (AChR MG). Patients who were seronegative or had autoantibodies to muscle-specific kinase (MuSK MG) were excluded. Patients were classified as either oculobulbar or generalized on the basis of clinical characteristics retrieved from their charts. All data were gathered according to the same clinical protocol that is used for all MG patients who visit our outpatient clinic for routine clinical care. This study was approved by the Medical Ethics Boards of the Leiden University Medical Center.

Clinical variables

The primary endpoint was the ACTIVLIM score, which is a measure of self-reported activity limitations. The questionnaire assesses the perceived difficulty patients have in performing daily activities on a 3-level ordinal scale (0=impossible, 1=difficult, or 2=easy). It contains 22 daily activities designed for both children and adults with NMD. The ordinal total score was transformed into an interval level measure of activity limitations using a Rasch model. The Rasch model estimates the item difficulty and the patient activity level so that the resulting measure is linear and can therefore be treated as a continuous variable. A higher score indicates fewer activity limitations.¹⁰ We performed a literature search to compare the ACTIVLIM scores in other NMD with our findings.

The other clinical variables included in this study were QMG score, age, age at onset of first symptoms below age 50 or older, presence of one or more additional autoimmune disease(s) (AID), thymectomy with or without the presence of thymoma, and exacerbations or emergency treatments within the last year. These variables were chosen based on previous studies, potential clinical relevance, and reliability with which these data could be analyzed retrospectively.^{1, 15-20} For the analysis of the correlation of changes in QMG and ACTIVLIM we also subdivided the QMG into an oculobulbar (first 5 items of the QMG) and a generalized domain (remaining 8 items). An exacerbation was defined as a clinical deterioration of MG that required starting with or an increase of at least 20 mg of prednisone daily or led to an emergency treatment. An emergency treatment was defined as the administration of intravenous immunoglobulin, plasmapheresis, or mechanical ventilation.¹⁸

Statistical Analysis

We assessed the impact of multiple clinical variables on the ACTIVLIM score by analyzing only the first visit in all patients. In addition, we investigated the correlation between changes in QMG and ACTIVLIM scores by taking into account multiple measurements in the same patient. We used generalized estimating equations (GEE) with an unstructured correlation model to account for the dependence between repeated measurements in the same individual. To account for missing values in clinical variables, we used a 5-fold multiple imputation (for which we included all clinical variables). Results are expressed as B coefficients, which represent the slopes of the regression lines, and 95% confidence intervals (CI). For the association between changes in QMG and ACTIVLIM, these regression lines are shown in figures. *P*-values < 0.05 were considered significant. Statistical analyses were performed using SPSS version 23 (IBM Corp., Armonk, NY).

RESULTS

We included 118 consecutive patients with AChR MG. Of this group, 63 underwent multiple assessments, ranging up to 6 assessments per patient. Baseline characteristics of all patients are shown in table 1. The mean ACTIVLIM score in our cohort was 3.3 (standard deviation $[\pm]$ 2.2), and subdivided by weakness pattern 3.6 ± 2.1 for oculobulbar MG patients and 2.7 ± 2.3 for generalized MG patients. Before missing data imputation, the pattern of weakness was missing on 21 of 252 measurements and the presence of an exacerbation or emergency treatment in the last year was unknown on 47 occasions. An overview of mean ACTIVLIM scores in MG patients and patients with other NMD is shown in table 2.

Table 1. Baseline characteristics of 118 patients with MG included in this study

	All patients N=118	Oculobulbar N=84	Generalized N=34
Age, y	51.9 $\pm$ 18.9	54.1 $\pm$ 19.7	46.2 $\pm$ 15.6
Age at onset, y	41.5 $\pm$ 20.3	44.7 $\pm$ 20.9	33.8 $\pm$ 17.0
<50	73 (62)	49 (58)	24 (71)
≥ 50	45 (38)	35 (42)	10 (29)
Gender			
Male	43 (36)	37 (44)	6 (18)
Female	75 (64)	47 (56)	28 (82)
Additional AID			
Yes	18 (15)	14 (17)	30 (88)
No	100 (85)	70 (83)	4 (12)
Thymectomy			
Yes, with thymoma	15 (13)	10 (12)	5 (44)
Yes, without thymoma	25 (21)	11 (13)	14 (41)
No	78 (66)	63 (75)	15 (15)
QMG	9.2 $\pm$ 5.3	8.5 $\pm$ 5.3	10.9 $\pm$ 4.7
ACTIVLIM	3.3 $\pm$ 2.2	3.6 $\pm$ 2.1	2.7 $\pm$ 2.3

Baseline characteristics of 118 patients with MG included in this study are shown. Row 3 and 4 show the data subdivided by oculobulbar and generalized weakness patterns. Data are presented as number of patients (%) for categorical variables and as mean $\pm$ SD for continuous variables.

QMG was strongly and inversely associated with ACTIVLIM (B coefficient = -0.206, 95% CI = -0.250; -0.163; $p < 0.001$). Thus, high performance on quantitative muscle tests and therefore a low QMG score was associated with fewer activity limitations, which is reflected in a high ACTIVLIM score. In addition, men had a higher ACTIVLIM score than women (B coefficient = 0.785, CI = 0.153; 1.416; $p = 0.015$), and patients without another autoimmune disease had fewer limitations in ADL (B coefficient = 0.998, CI = 0.262; 1.733; $p = 0.008$). Outcomes of the multivariate analysis for ACTIVLIM are shown in table 3.

Table 2. Overview of ACTIVLIM scores in MG and other NMD

NMD	N	Age	Gender (% male)	ACTIVLIM
CMD				
Meilleur et al. ⁹²	24 ^a	11.4 (5-22)	41	0.6 ± 3.0
Vuillerot et al. ⁹⁵	42 ^b	10.8 (5-19)	55	-1.1 ± 2.9
HN				
Vandervelde et al. ⁹⁴	44	(8-80)	100	2.0 ± 2.1
MD				
Vandervelde et al. ⁹⁴	37	(16-72)	-	2.7 ± 2.1
ALS				
Vandervelde et al. ⁹⁴	18	(46-80)	-	0.4 ± 2.31
SMA				
Vandervelde et al. ⁹⁴	14	(9-61)	-	-0.5 ± 3.4
FSHD				
Vandervelde et al. ⁹⁴	12	(12-67)	-	1.6 ± 2.0
Other				
Vandervelde et al. ⁹³	132 ^c	31 (6-80)	67	1.2 ± 2.7
Vandervelde et al. ⁹⁴	45 ^d	(6-72)	-	-1.1 ± 3.1
AChR MG				
Current study	118	51.9 (13-88)	36	3.3 ± 2.2

Overview of mean ACTIVLIM scores in MG patients (this study) and in patients with other neuromuscular diseases (other studies). Data are shown subdivided by neuromuscular disease. Age (range), % male and ACTIVLIM ± SD are shown for each study separately.

^a Within the group of CMD patients, 53% had COL6-RD and 47% had LAMA2-RD.

^b Group of 42 CMD patients included patients with COL6-RD, LAMA2-RD or unclassified CMD. The exact distribution of these subgroups has not been specified.

^c Group consisted of 27 DMD patients, 17 MD patients, 20 CMT patients and 68 patients with another neuromuscular disease.

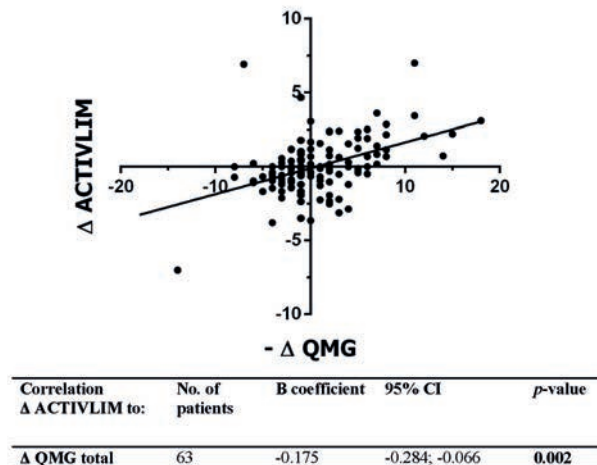
^d Group consisted of DMD, BMD and LGMD patients. The exact distribution of these subgroups has not been specified.

In individual patients who underwent repeated assessments, changes in QMG (Δ QMG) and ACTIVLIM (Δ ACTIVLIM) were strongly and inversely correlated (B coefficient = -0.175, CI = -0.284; -0.066; $p = 0.002$). Thus, improvement in quantitative muscle tests was associated with fewer activity limitations (figure 1). Generalized MG patients had a strong correlation between Δ QMG and Δ ACTIVLIM on both the generalized items of Δ QMG (B coefficient = -0.353, CI = -0.505; -0.202; $p < 0.001$) and total Δ QMG (B coefficient = -0.222, CI = -0.334; -0.109; $p < 0.001$). In patients with oculobulbar MG there also was a correlation between Δ QMG and Δ ACTIVLIM on both the generalized items of Δ QMG (B coefficient = -0.199, CI = -0.337; -0.062; $p = 0.005$) and total Δ QMG (B coefficient = -0.137, CI = -0.252; -0.021; $p = 0.020$). In both oculobulbar and generalized MG patients there was no significant correlation between the oculobulbar items of Δ QMG and Δ ACTIVLIM. Results of the analysis of the correlation between Δ QMG and Δ ACTIVLIM divided by subgroups are shown in figure 2.

Table 3. Association of clinical factors with ACTIVLIM score

ACTIVLIM	No. of patients	B coefficient	95% CI	p-value
Age, y	118	-0.020	-0.044; 0.005	0.118
Age at onset, y				
<50	66	0.561	-0.296; 1.418	0.200
≥50	52	0		
Gender				
Male	41	0.785	0.153; 1.416	0.015
Female	77	0		
Additional AID				
Yes	14	0		
No	104	0.998	0.262; 1.733	0.008
Thymectomy				
Yes, with thymoma	23	0		
Yes, without thymoma	19	-0.122	-0.896; 0.652	0.758
No	76	-0.258	-0.949; 0.432	0.463
Exacerbations <1 y				
Yes		0		
No		0.209	-0.465; 0.882	0.543
Emergency treatments <1 y				
Yes		0		
No		-0.061	-0.965; 0.843	0.894
QMG	118	-0.206	-0.250; -0.163	<0.001

Multivariate analysis of ACTIVLIM score according to clinical variables. Data are presented as B coefficient and 95% confidence interval (CI). Boldfaced p-values indicate significant differences ($p < 0.05$).

**Figure 1.** Correlation of Δ QMG with Δ ACTIVLIM in all MG patients

Correlation of Δ QMG with Δ ACTIVLIM in 63 MG patients. Data are shown as B coefficient (slope) and 95% confidence interval (CI). The Δ QMG score is shown inverted on the x-axis so that a higher value on both the y- and the x-axis represents an improvement on the scale.

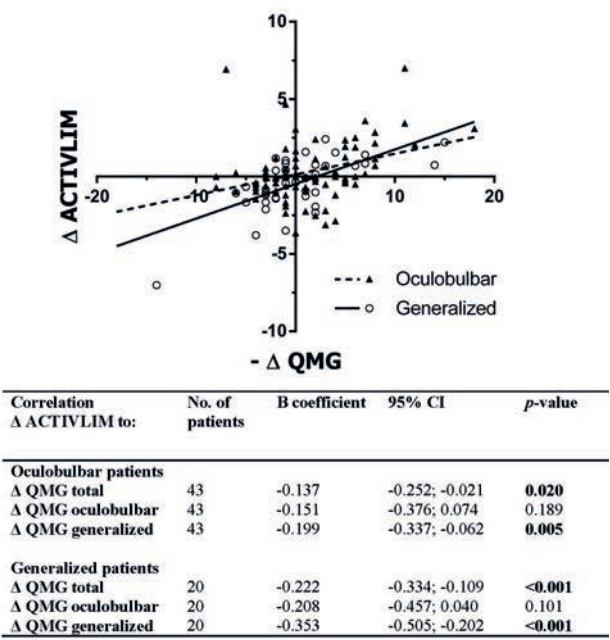


Figure 2. Correlations of Δ QMG with Δ ACTIVLIM in oculobulbar and generalized MG patients
Correlations of Δ QMG with Δ ACTIVLIM are shown for oculobulbar and generalized MG patients separately. Data are shown as B coefficient (slope) and 95% confidence interval (CI) for the correlations of the total Δ QMG and the oculobulbar and generalized items of the Δ QMG separately with Δ ACTIVLIM. Δ QMG score is shown inverted on the x-axis so that a higher value on both the y- and the x-axis represents an improvement on the scale.

DISCUSSION

In a series of 118 consecutive MG patients, we found that the mean ACTIVLIM score in MG patients is relatively high, indicating fewer limitations in daily activities in comparison with other NMD and that the ACTIVLIM score in MG patients is associated with QMG score, gender, and the presence of other autoimmune diseases. Fluctuations in QMG score were associated with changes in activity limitations in individual patients as well.

The mean ACTIVLIM score was higher in our cohort of MG patients (3.3) than in patients with other NMD in previous studies (-1.13 to 2.71).¹¹⁻¹⁴ MG appears to cause fewer activity limitations than a number of other NMD, such as hereditary neuropathy, congenital muscular dystrophy, myotonic dystrophy, and amyotrophic lateral sclerosis as reported in previous studies. This difference in ADL limitations is probably explained by a number of factors, including the availability of treatment options and the temporary and fluctuating nature of weakness in MG in comparison to NMD that are irreversibly progressive. The ability to quantify the activity limitations caused by different NMD on the same scale may

be useful for health care policy makers who want to compare the burden imposed by different NMD on ADL. Furthermore, our results may help establish clinically relevant outcome measures in future clinical trials involving MG and other NMD.

QMG was strongly and inversely associated with ACTIVLIM, which shows that quantified muscle testing as measured by the QMG is directly relevant for activity limitations in MG patients. We also found that men had a significantly higher ACTIVLIM. This finding is in agreement with several large international studies in which a higher health-related quality of life (HRQoL) was found in men with MG.^{5,7,21} Furthermore we found that patients with an additional AID had significantly more limitations in ADL. A potential explanation could be that the disease course of MG itself is more severe in patients with other AID. Indeed, the presence of an additional AID is associated with a higher risk of future exacerbations and emergency treatments.¹⁸ However, the most likely cause for this finding is that these AID have a negative impact on ADL independent of MG, as QMG scores between patients with additional AID and patients without additional AID did not differ significantly in our study population (mean scores were 8 and 9, respectively).

Changes in QMG and ACTIVLIM were strongly and inversely correlated in individual patients. Our findings also showed that both oculobulbar and generalized MG patients had changes in activity limitations that were most clearly associated with changes in the generalized items of the QMG score. This finding suggests that we might underestimate the effect of subclinical generalized muscle weakness in patients who are clinically diagnosed with pure oculobulbar MG. This is in line with a previous study that described subclinical generalized weakness in ocular MG patients.²² A previous study has shown that the ACTIVLIM score shows a good degree of responsiveness: in patients with a wide range of NMD undergoing repeated measurements, a self-reported deterioration in ACTIVLIM score corresponded to a decrease in functional status.¹² However, this study included progressive NMD in which only a deterioration of function was expected. Our study shows a good responsiveness of the ACTIVLIM score for both deteriorations and improvements in muscle strength.

Besides the ACTIVLIM described here, several measures for patient-oriented outcomes that assess ADL and quality of life (QoL) have been developed.²³ The MG-ADL is an ADL measure specifically for MG that correlates well with the QMG score, and improvements in QMG scores correlated well with changes in MG-ADL during a trial of mycophenolate mofetil.^{8,9} As the MG-ADL contains mainly oculobulbar items and only 2 extremity items, ACTIVLIM may be a useful addition for assessing generalized impairments in MG patients. It should be noted, however, that the ACTIVLIM score may underestimate the impact of oculobulbar symptoms on activity limitations. Indeed, of all the items on the MG-ADL, the items “chewing” and “swallowing” have the greatest impact on self-reported health, and these items are not assessed in the ACTIVLIM questionnaire.⁴

QoL in MG patients can be assessed with the Myasthenia Gravis Questionnaire and the MG-QOL15, in which the 60 item MG-QOL has been reduced to 15 items.^{3,6} A high cor-

relation was found between QMG score and MG-QOL15.³ Although these disease-specific outcomes can be very useful in clinical trials, a questionnaire for all NMD in general such as ACTIVLIM may be practical in routine clinical care and allow for comparison of multiple NMD as well.

The strengths of this study are the large cohort of consecutive AChR MG patients, the analysis of repeated measurements to determine within-patient fluctuations in disease severity, and the systematic assessment of validated, quantitative measures for both muscle weakness (QMG) and activity limitations (ACTIVLIM).

Limitations of this study include the single center of inclusion, no assessment of ocular and bulbar weakness in our ADL measure, and the retrospective analysis of data. The ACTIVLIM questionnaire has been validated for NMD in general, although not specifically for MG patients. In addition, our data on the MG patient cohort was compared to historical data from previous publications on NMD. Potential differences in the selection of patients and evaluation points between studies could limit the validity of this comparison. Future studies could combine or compare assessment of activity limitations with ACTIVLIM and MG-ADL to gain a more complete view of oculobulbar and generalized impairments. We aimed to minimize the limitation of retrospective analysis by only including variables that could reliably be analyzed afterwards. Finally, our study population is from a tertiary referral center and may not fully reflect the total MG population due to a referral bias.

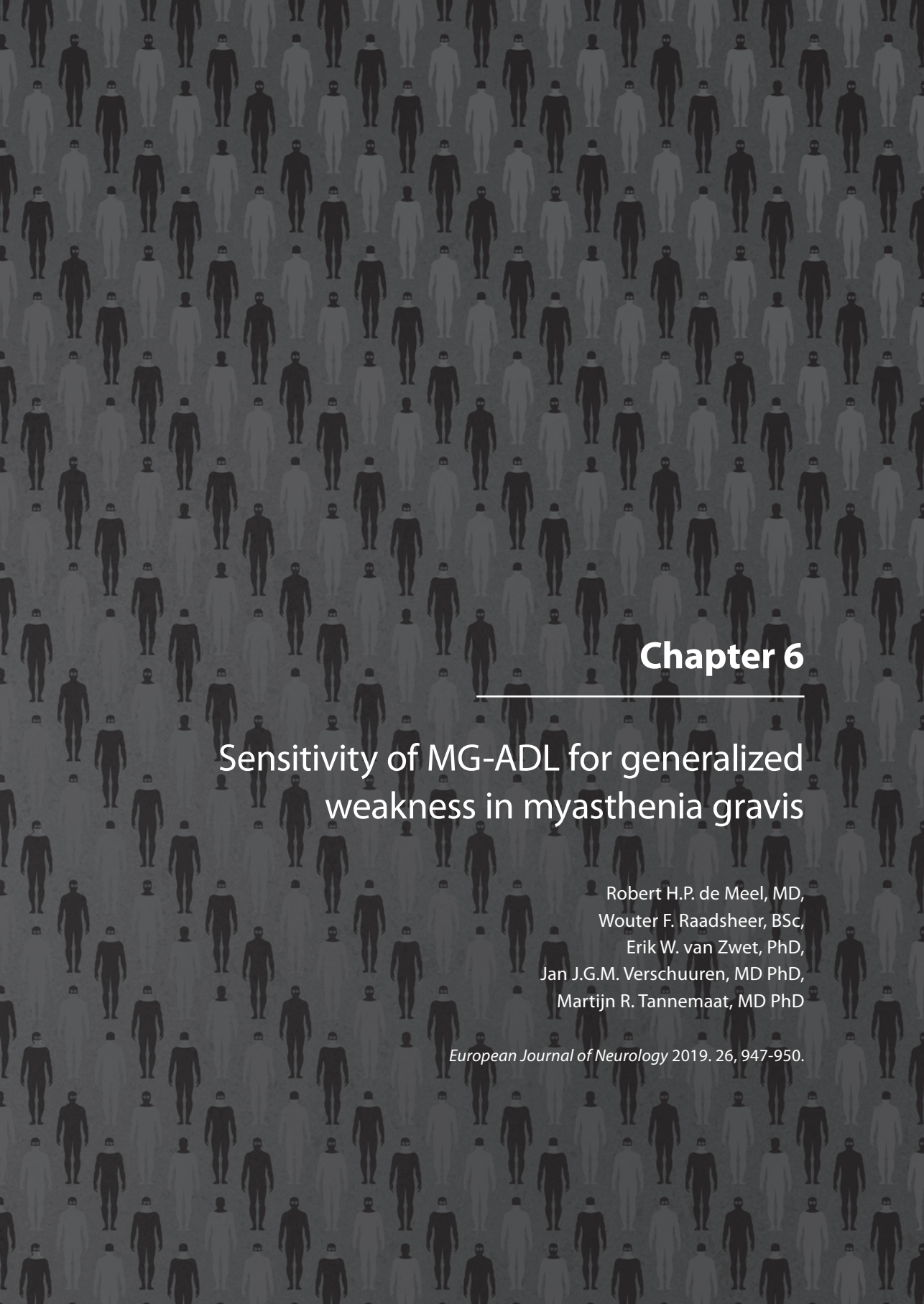
In conclusion, we have shown that MG patients report fewer activity limitations in comparison with other NMD, and that changes in the QMG score were associated with changes in activity limitations in individual MG patients. It may be useful to study the ACTIVLIM score and MG-ADL in the same group of MG patients to analyze whether the ACTIVLIM provides additional information or is more responsive to changes in generalized weakness in MG.

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Chapter 6

Sensitivity of MG-ADL for generalized weakness in myasthenia gravis

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ABSTRACT

Introduction

MG-ADL (Myasthenia Gravis Activities of Daily Living) is a commonly used questionnaire in MG trials. To investigate whether MG-ADL is equally sensitive to oculobulbar and generalized weakness, we analyzed its correlation with the oculobulbar and generalized domain of the quantitative myasthenia gravis (QMG) score (QMG_{ob} and QMG_{gen}, respectively). To test whether the sensitivity of MG-ADL for generalized weakness could be improved, we investigated whether adding questions on generalized weakness would increase its correlation with QMG_{gen}.

Methods

We analyzed MG-ADL, QMG and ACTIVLIM, an ADL questionnaire focusing on generalized weakness, in a prospective cohort of 112 MG patients. We used a generalized linear model to calculate the correlation of MG-ADL with QMG_{ob} and QMG_{gen} and to assess the additional value of ACTIVLIM on top of MG-ADL for its correlation with QMG_{gen}.

Results

MG-ADL had a higher correlation with QMG_{ob} than with QMG_{gen} ($B=0.68$; $p<0.001$ and $B = 0.38$; $p<0.001$, respectively). A similar trend was found for changes in the scores ($B = 0.68$; $p = 0.132$ and $B = 0.39$; $p = 0.492$, respectively). ACTIVLIM had a significant additional value on top of MG-ADL in the prediction of QMG_{gen}, both cross-sectionally ($B = -0.61$, $p < 0.001$) and for changes within individual patients ($B = -0.93$, $p = 0.041$).

Discussion

MG-ADL has a lower sensitivity for generalized weakness than for oculobulbar weakness. Adding questions on generalized weakness would improve the sensitivity of the MG-ADL for generalized weakness.

INTRODUCTION

Myasthenia gravis (MG) is an autoimmune disease which can affect extra-ocular, bulbar, limb and respiratory muscles.¹ The degree in which the different muscles are affected varies greatly between MG patients.² MG-ADL (Myasthenia Gravis Activities of Daily Living) is a commonly used questionnaire in MG trials.³⁻⁵ Of the 8 questions, 3 query generalized weakness. These questions concern respiratory function, the ability to brush teeth or comb hair and the ability to rise from a chair. Alongside this ADL scale, the QMG (Quantitative Myasthenia Gravis) score is often used in clinical trials as an objective physician-reported scale. The QMG, however, includes more items on generalized weakness (8 of 13). In general, the MG-ADL score correlates well with the QMG score,⁶ but in a recently published trial on the effect of eculizumab in generalized MG patients, there was a non-significant change in MG-ADL whereas QMG improved significantly.^{4,7} This raises the question whether MG-ADL is equally sensitive to changes in generalized weakness and oculobulbar weakness and whether adding questions on generalized weakness would improve sensitivity. There are no other MG-specific ADL measures with more questions on generalized weakness. In a prior study, we validated a general neuromuscular ADL measure focusing on generalized weakness, the ACTIVLIM (acronym of “ACTIVityLiMitations”), in a cohort of 118 MG patients.^{8,9} To investigate whether the sensitivity of MG-ADL for generalized weakness could be improved, we analyzed whether ACTIVLIM has an additional value on top of the MG-ADL in the prediction of the generalized domain of the QMG score in individual patients.

METHODS

Patients

We included a prospective cohort of MG patients under treatment at Leiden University Medical Center (LUMC) between 2016 and 2017. The QMG, MG-ADL and ACTIVLIM scores were recorded in all MG patients who visited the outpatient clinic as part of routine clinical care. The diagnosis of MG was based on a combination of clinically confirmed fluctuating muscle weakness and the presence of serum autoantibodies to the acetylcholine receptor (AChR) or muscle-specific kinase (MuSK). Seronegative myasthenia gravis (SNMG) was defined as fatigable muscle weakness in combination with abnormal decrement (at least 10%) during low-frequency repetitive nerve stimulation, increased jitter in single-fiber EMG testing or a positive neostigmine test.¹ This study was approved by the medical ethics boards of the LUMC.

Outcome variables

We compared two ADL measures and one physician-reported measure of muscle fatigability: MG-ADL, ACTIVLIM and QMG, respectively. We subdivided the QMG into an oculobulbar (first 5 items of the QMG; QMG_{Gob}) and a generalized domain (remaining 8 items; QMG_{gen}).

Statistical Analysis

We used a generalized linear model to calculate the correlation of MG-ADL with QMG_{Gob} and QMG_{gen} and to assess the additional value of ACTIVLIM on top of MG-ADL in the prediction of QMG_{gen} on the first visit. Furthermore, we analyzed this additional value for changes in the measures (Δ ACTIVLIM, Δ MG-ADL and Δ QMG_{gen}) within the same patient. First, we fitted a linear model for QMG_{gen} with MG-ADL as the single predictor. Next, we regressed the residuals from that model on ACTIVLIM. The residual is the difference between observed and predicted QMG_{gen} based on the MG-ADL score of that patient. Results are expressed as B coefficients with 95% confidence intervals (CI), which reflect the additional value of ACTIVLIM in the prediction of QMG_{gen} compared to MG-ADL alone. Regression lines show the degree in which ACTIVLIM can compensate for the mismatch between observed and predicted QMG_{gen}. *P*-values < 0.05 were considered significant. Statistical analyses were performed using SPSS version 23 (IBM Corp., Armonk, NY).

RESULTS

We included 112 consecutive patients with MG. Of this group, 14 patients had a second visit with all assessments. Baseline characteristics of all patients are shown in table 1. MG-ADL had a higher correlation with QMG_{Gob} than with QMG_{gen} ($B=0.68$; $p<0.001$ and $B = 0.38$; $p<0.001$, respectively). A similar trend was found for changes in the scores ($B = 0.68$; $p = 0.132$ and $B = 0.39$; $p = 0.492$, respectively).

The mean deviation of MG-ADL from the predicted values of QMG_{gen} was 2.6 ± 1.9 . ACTIVLIM had an additional value on top of MG-ADL in the prediction of QMG_{gen} ($B = -0.61$, $p < 0.0001$; Figure 1A). In addition, the mean deviation of Δ MG-ADL from the predicted values of Δ QMG_{gen} was 1.7 ± 1.3 . Δ ACTIVLIM had an additional value on top of Δ MG-ADL in the prediction of Δ QMG_{gen} ($B = -0.93$, $p = 0.041$; Figure 1B).

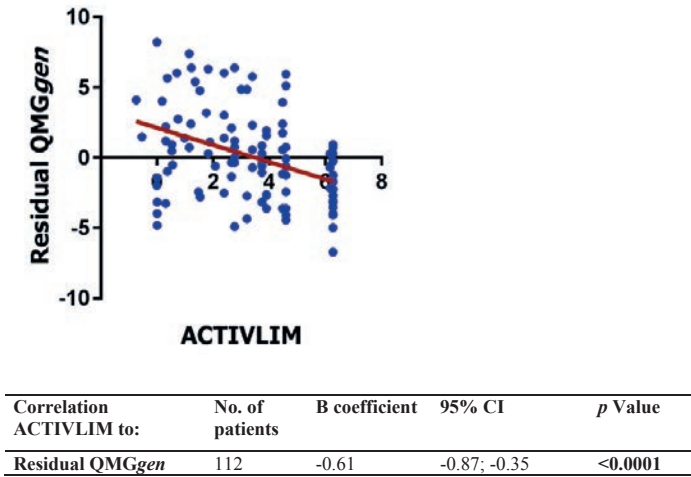


Figure 1A

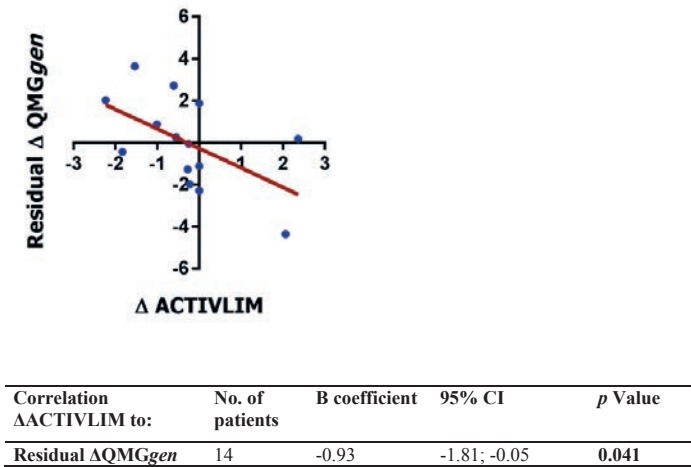


Figure 1B

Figure 1. Analysis of the (Δ)ACTIVLIM, (Δ)MG-ADL and (Δ)QMGgen scores in 112 individual patients and in 14 patients with a follow-up visit. The dots show the (Δ)QMGgen residual for each individual patient. The (Δ)QMGgen residual is the difference between observed and predicted (Δ)QMGgen based on the (Δ)MG-ADL score of that patient. The B coefficient (slope) shows the degree in which (Δ)ACTIVLIM correlates with the residual of (Δ)QMGgen. The significant correlation implies that (Δ)ACTIVLIM can (partly) compensate for the mismatch between observed and predicted (Δ)QMGgen and therefore has an additional value on top of the (Δ)MG-ADL.

Table 1.

	MG patients N=112
Age, y	57.1 ± 18.0
Age at onset, y	46.7 ± 20.0
Gender	
Male	42 (38)
Female	70 (62)
Antibodies	
AChR+	84 (75)
MuSK+	8 (7)
Seronegative	20 (18)
Thymectomy	
Yes, with thymoma	8 (7)
Yes, without thymoma	27 (24)
No	77 (69)
ACTIVLIM	3.5 ± 2.2
MG-ADL	4.5 ± 3.5
QMG	6.4 ± 4.5
QMG _{gen}	4.3 ± 3.6

Baseline characteristics of 112 patients with MG included in this study. Data are presented as number of patients (%) for categorical variables and as mean ± SD for continuous variables.

DISCUSSION

In a cohort of 112 MG patients we found that MG-ADL correlates better with QMG_{ob} than with QMG_{gen}. ACTIVLIM had a significant additional value on top of MG-ADL in the prediction of QMG_{gen}, suggesting that adding questions on generalized weakness could improve the sensitivity of the MG-ADL for generalized weakness. This additional value was even higher for changes in generalized weakness and was found to be significant, even in the small group of patients with a follow-up visit (n=14) in which this analysis could be performed.

Further research could focus on formulating and testing one or two additional questions on generalized weakness that could improve the MG-ADL score by balancing oculobulbar and generalized items without losing a major positive trait of the MG-ADL, time efficiency, as this minor addition would cost less than a minute. The importance of having a high sensitivity for changes in both oculobulbar and generalized weakness is shown by Barnett et al. who found that ocular weakness appeared to respond better to treatment with prednisone, while generalized weakness was more amenable to intravenous immunoglobulin or plasma exchange.¹⁰ Their newly developed outcome measure, the MG Impairment Index (MGII),

takes changes in generalized weakness into account and may be a good measure for future studies. As QMG and MG-ADL will probably remain the leading outcome measures in the coming years, we would suggest reporting the oculobulbar and generalized subscores of these outcome measures separately. This might help to distinguish potential preferential effects on oculobulbar or generalized weakness.

Limitations of this study include the single center of inclusion and our study population within a tertiary referral center that may not fully reflect the total MG population due to a referral bias. Moreover, it may be argued that ACTIVLIM is not a proper reference for generalized weakness in MG, as little is known about this measure in MG besides our earlier study.⁸ ACTIVLIM is a validated, Rasch-built ADL measure for neuromuscular diseases in general and has shown to be highly responsive to decreases in functional status.¹¹ Our previous study showed that ACTIVLIM is highly responsive to changes in generalized weakness in MG patients as well.⁸ We therefore found it appropriate to use ACTIVLIM instrumentally to investigate whether adding ADL questions on generalized weakness could improve the sensitivity of the MG-ADL for generalized weakness. Importantly, we do not propose using ACTIVLIM as a primary outcome measure in MG as it lacks oculobulbar items. However, ACTIVLIM might also be useful in the future in assessing the generalized component of new MG outcome measures.

In conclusion, the current study shows that MG-ADL has a lower sensitivity for generalized weakness than for oculobulbar weakness. Adding questions on generalized weakness would improve the sensitivity of the MG-ADL for generalized weakness, thereby increasing the overall performance of the MG-ADL in future clinical trials.

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DISTINCT REPRESENTATION OF MUSCLE WEAKNESS IN QMG AND MG-ADL (LETTER)

Robert H.P. de Meel, MD, Martijn R. Tannemaat, MD PhD, Jan J.G.M. Verschuuren, MD PhD

The Lancet Neurology 2018. 17, 204-205

In response to the published article “Safety and efficacy of eculizumab in anti-acetylcholine receptor antibody-positive refractory generalised myasthenia gravis (REGAIN): a phase 3, randomised, double-blind, placebo-controlled, multicentre study” by Howard et al., we submitted the letter below.

We read with interest the findings of Howard and colleagues¹ showing an apparent discrepancy between the primary (Myasthenia Gravis Activities of Daily Living; MG-ADL) and secondary (Quantitative Myasthenia Gravis test; QMG) endpoints for the effect of eculizumab in anti-acetylcholine receptor antibody-positive refractory generalised myasthenia gravis patients in a phase 3, randomised, double-blind, placebo-controlled multicentre study. The primary endpoint did not show a significant difference ($p = 0.0698$), whereas the secondary endpoint did ($p = 0.0129$). The prespecified responder analyses, shown in figure 2, also illustrated this difference between QMG and MG-ADL.

In an earlier article reporting the results of the phase 2 trial on eculizumab², the authors described a higher correlation between QMG and MG-ADL for changes from baseline to week 16 ($R = 0.726$) than for point-in-time disease status ($R = 0.552$). The higher correlation was ascribed to the diminished variability in the assessment scores due to treatment effect. However, this higher correlation of changes after treatment compared to point-in-time disease status was not found in a trial of mycophenolate mofetil in myasthenia gravis³. Furthermore, much smaller correlations between changes in QMG and MG-ADL were found in the mycophenolate mofetil trial ($R = 0.51$ and $R = 0.44$, from baseline to week 12 and week 36, respectively).

We would like to draw attention to the difference in quantitative representation of different muscle groups in QMG and MG-ADL as we believe it may provide an answer for the observed discrepancies. In QMG, 8 out of 13 items (62%) concern generalized weakness, whereas only 3 out of 8 items (38%) of MG-ADL are related to generalized weakness. Furthermore, ocular and generalized weakness appear to respond differently to various types of therapy: prednisone confers the largest benefit for ocular symptoms and intravenous immunoglobulin or plasma exchange for generalized symptoms⁴. We suspect that discrepancies

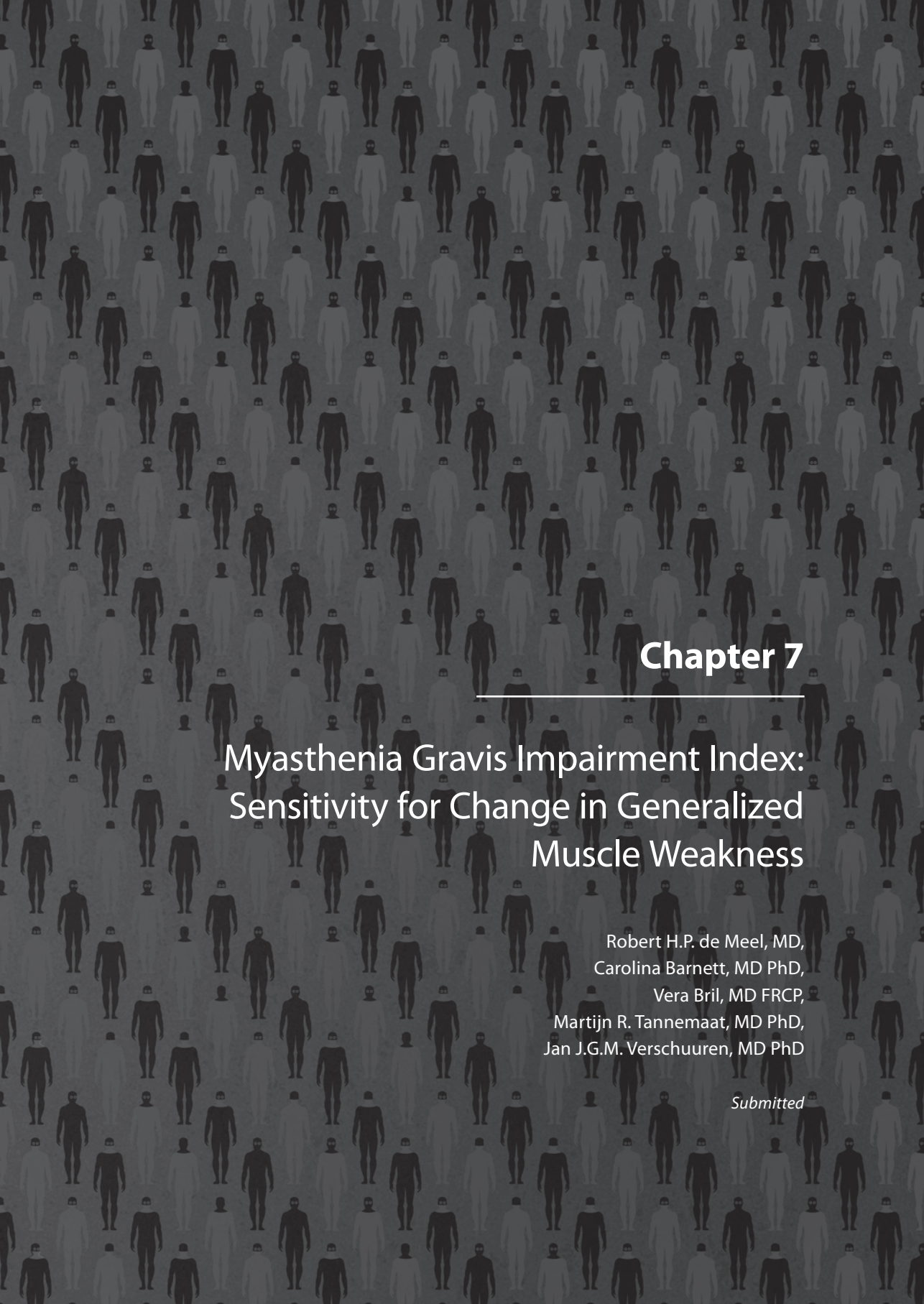
between QMG and MG-ADL in the current trial may be explained by a differential response of various muscle groups to eculizumab and the different quantitative representation of oculobulbar and generalized weakness in QMG and MG-ADL. Differences in treatment effects of eculizumab and mycophenolate mofetil on generalized weakness might also explain the different correlations in their respective trials.

It would be interesting to know the subscores of the oculobulbar and generalized items on both QMG and MG-ADL. With these data, it would be possible to determine whether there was a preferential effect of eculizumab on generalized weakness, which would explain the better response on QMG as compared to MG-ADL.

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Chapter 7

Myasthenia Gravis Impairment Index: Sensitivity for Change in Generalized Muscle Weakness

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Submitted

ABSTRACT

Introduction

The recently developed Myasthenia Gravis Impairment Index (MGII) is a promising measure as it has less floor effects and a higher relative efficiency in its responsiveness to treatment effect compared to other MG measures. This study aimed at validating the MGII in a Dutch cohort of MG patients and analyzing the sensitivity of MGII compared to MG-ADL for changes in generalized weakness.

Methods

We analyzed (generalized items of; *-gen*) MGII, quantitative myasthenia gravis (QMG), Myasthenia Gravis Activities of Daily Living (MG-ADL), EQ-5D visual analog, Myasthenia Gravis Composite (MGC) and ACTIVLIM (an ADL questionnaire focusing on generalized weakness) scores in a prospective cohort of 99 MG patients. We investigated correlations between MGII and other outcome measures. We used a generalized linear model to assess whether MGII_{gen} had an additional sensitivity on top of MG-ADL_{gen} for changes (Δ) in QMG_{gen} in individual patients.

Results

MGII had a lower floor effect (4%) compared to QMG (6%), MG-ADL (11%) and MGC (16%). MGII correlated well with QMG ($r = 0.68$), MG-ADL ($r = 0.83$) and MGC ($r = 0.74$). As expected, the correlations with EQ visual analog and ACTIVLIM were lower ($r = -0.57$ and -0.48). Δ MGII_{gen} had an additional value on top of Δ MG-ADL_{gen} in the prediction of Δ QMG_{gen} ($B = 0.54$, $p = 0.01$).

Discussion

The MGII score was cross-culturally validated in a Dutch cohort of MG patients. MGII had a higher sensitivity for generalized weakness than MG-ADL.

INTRODUCTION

Myasthenia gravis (MG) is an autoimmune disease characterized by heterogeneous patterns of muscle weakness. As earlier studies have shown that ocular, bulbar or generalized weakness respond differently to different therapies, an outcome measure's lack of sensitivity for weakness in either of these domains might result in failure to observe significant treatment effects.¹⁻⁴ In an earlier study, we found that Myasthenia Gravis Activities of Daily Living (MG-ADL) had a lower sensitivity for changes in generalized weakness.⁵ In addition, a prior study reported that patients considered limb weakness as most invalidating, further stressing the importance of a high sensitivity for generalized weakness.⁶ The recently developed Myasthenia Gravis Impairment Index (MGII) is a promising measure as it has less floor effects and a higher relative efficiency in its responsiveness to treatment effect compared to other MG measures.^{7,8} The MGII has 22 patient reported and 6 examination items, with 10 items reflecting generalized weakness. This study aimed at validating the MGII in a Dutch cohort of MG patients and analyzing the sensitivity of the MGII for changes in generalized weakness.

METHODS

Patients

We included a prospective cohort of MG patients under treatment at Leiden University Medical Center (LUMC) between 2016 and 2017. The MGII, Quantitative Myasthenia Gravis (QMG), MG-ADL, EQ-5D visual analog (EQ VAS), Myasthenia Gravis Composite (MGC) and ACTIVLIM (acronym of "ACTIVity LIMitations"; an ADL-scale focusing on generalized weakness) scores were recorded in all MG patients who visited the outpatient clinic as part of routine clinical care. The diagnosis of MG was based on a combination of clinically confirmed fluctuating muscle weakness and the presence of serum autoantibodies to the acetylcholine receptor (AChR) or muscle-specific kinase (MuSK). Seronegative myasthenia gravis (SNMG) was defined as fatigable muscle weakness in combination with abnormal decrement (at least 10%) during low-frequency repetitive nerve stimulation, increased jitter in single-fiber EMG testing or a positive neostigmine test.⁹

Standard protocol approvals, registrations, and patient consents.

The Medical Ethics Boards of the Leiden University Medical Center approved the study and its use of human subjects. All patients provided informed, written consent prior to study participation.

Cross-cultural Translation MGII

In order to translate the MGII from English to Dutch whilst taking into account cultural differences, we used the back-translation method. The original English version of the MGII (supplement 1) was translated to Dutch by a physician at our center. This Dutch version of the MGII was subsequently back-translated to English by independent translators, blinded to the original measure. The back-translation was then compared to the original, and items with major differences were revised.¹⁰

Subdomains Outcome Variables

QMG, MG-ADL and MGII scores were subdivided in oculobulbar domains (QMG_{ob}, MG-ADL_{ob} and MGII_{ob}) and generalized domains (QMG_{gen}, MG-ADL_{gen} and MGII_{gen}). See supplement 2 for the exact items included in the subdomains.

Statistical Analysis

To reproduce earlier reported construct validity findings of the MGII, we analyzed correlations between MGII and other outcome measures.⁸ We investigated whether there were significantly higher generalized scores (MGII_{gen}, MG-ADL_{gen} and QMG_{gen}) in patients with generalized MG compared to ocular MG patients with an unpaired t-test. We used a generalized linear model to assess whether MGII_{gen} had an additional sensitivity on top of MG-ADL_{gen} for changes (Δ) in QMG_{gen} in individual patients (see supplement 3 for details). *P*-values < 0.05 were considered significant. Statistical analyses were performed using SPSS version 23 (IBM Corp., Armonk, NY).

RESULTS

In the back translated version of the MGII, only the item assessing leg weakness with effort was different. The original item is phrased as weakness after walking a certain number of blocks. In the Netherlands, blocks are not commonly used, so blocks were converted to meters, and we kept this wording as it is culturally appropriate. All other items are a literal translation of the original.

We included 99 consecutive patients with MG. Of this group, 10 patients had a second visit with all assessments. Demographic and clinical baseline characteristics of all patients are shown in table 1.

Table 1.

	MG patients N=99
Age, y	57.4 ± 18.1
Age at onset, y	45.5 ± 20.0
Gender	
Male	34 (34)
Female	65 (66)
Antibodies	
AChR+	71 (72)
MuSK+	8 (8)
Seronegative	20 (20)
Thymectomy	
Yes, with thymoma	8 (8)
Yes, without thymoma	7 (7)
No	84 (85)
QMG	6.7 ± 4.8
QMG _{ob}	2.2 ± 2.5
QMG _{gen}	4.4 ± 3.7
MG-ADL	4.8 ± 3.5
MG-ADL _{ob}	3.1 ± 2.6
MG-ADL _{gen}	1.8 ± 1.7
MGII	19.5 ± 15.0
MGII _{ob}	12.0 ± 10.6
MGII _{gen}	7.5 ± 7.1
MGC	7.0 ± 6.5
EQ VAS	67.7 ± 19.4
ACTIVLIM	3.6 ± 2.1

Baseline characteristics of 99 patients with MG included in this study. Data are presented as number of patients (%) for categorical variables and as mean ± SD for continuous variables.

Construct validity

The mean total MGII score was 19.5 ± 15.0 , the mean oculobulbar score 12.0 ± 10.6 , and the mean generalized subscore 7.5 ± 7.1 . MGII had a lower floor effect (4%) compared to QMG (6%), MG-ADL (11%) and MGC (16%). Generalized MG patients ($N = 75$) had a significantly higher MGII_{gen} than oculobulbar MG patients ($N = 24$) (8.6 ± 6.9 and 4.3 ± 6.7 ; $p=0.01$, respectively). There were non-significant differences between generalized and oculobulbar MG patients when comparing QMG_{gen} (4.7 ± 3.9 and 3.6 ± 2.9 ; $p=0.14$, respectively) and MG-ADL_{gen} (1.7 ± 1.6 and 2.2 ± 2.0 ; $p=0.25$, respectively). Table 2 shows the correlations between MGII and other outcome measures.

Table 2.

Comparison measure	Pearson r (95% CI)	p Value
QMG	0.68 (0.56 to 0.77)	<0.001
MG-ADL	0.83 (0.76 to 0.88)	<0.001
MGC	0.74 (0.64 to 0.82)	<0.001
EQ VAS	-0.57 (-0.69 to -0.42)	<0.001
ACTIVLIM	-0.48 (-0.62 to -0.31)	<0.001

Correlations between MGII and other outcome measures are shown. With these correlations construct validity was tested. In ACTIVLIM and EQ VAS higher scores indicate a higher activity level or quality of life.

Responsiveness to change in generalized muscle weakness

The correlations between ΔMGII_{gen} and ΔQMG_{gen} or between $\Delta\text{MG-ADL}_{gen}$ and ΔQMG_{gen} were both significant ($B = 0.78$, $p < 0.001$, and $B = 1.50$, $p = 0.01$, respectively). The MGII_{gen} score has a range of 0-31, whereas the MG-ADL_{gen} has a range of 0-9, which explains why ΔMGII_{gen} has a more significant correlation compared to $\Delta\text{MG-ADL}_{gen}$ notwithstanding the lower B coefficient. The mean deviation of $\Delta\text{MG-ADL}_{gen}$ from the predicted values of ΔQMG_{gen} was 3.5 ± 2.5 . ΔMGII_{gen} had an additional value on top of $\Delta\text{MG-ADL}_{gen}$ in the prediction of QMG_{gen} ($B = 0.54$, $p = 0.01$; Figure 1).

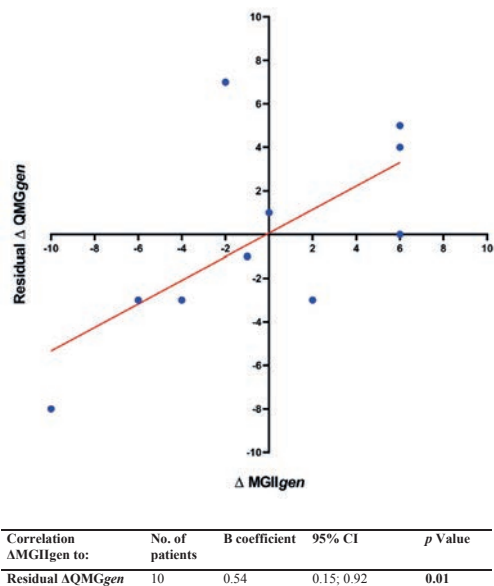


Figure 1. Analysis of the additional sensitivity of ΔMGII_{gen} on top of $\Delta\text{MG-ADL}_{gen}$ for ΔQMG_{gen} in 10 patients with two visits. The dots show the ΔQMG_{gen} residual for each individual patient. The ΔQMG_{gen} residual is the difference between observed and predicted ΔQMG_{gen} based on the $\Delta\text{MG-ADL}$ score of that patient. The B coefficient (slope) shows the degree in which ΔMGII correlates with the residual of ΔQMG_{gen} . The significant correlation implies that ΔMGII can to some degree compensate for the mismatch between observed and predicted ΔQMG_{gen} and therefore has an additional sensitivity for generalized muscle weakness on top of $\Delta\text{MG-ADL}$.

DISCUSSION

The MGII scale was cross-culturally validated in a cohort of 99 Dutch MG patients. Similarly to the findings of Barnett et al., the *MGIIgen* subscore was significantly higher in generalized patients compared to oculobulbar patients. In contrast, *MG-ADLgen* was not capable of differentiating oculobulbar from generalized patients. Moreover, Δ *MGIIgen* had a significantly higher sensitivity for changes in generalized weakness than Δ *MGIIgen*.

The correlation of MGII to both other MG-specific measures and non-specific measures were all within the range ($r = 0.6-0.8$ and $r = 0.4-0.7$, respectively) as hypothesized by the developers of the MGII. Only the MG-ADL was slightly above the hypothesized range (0.83), which also was the case in the previous study of Barnett et al. The floor effects found in this study and in that of Barnett et al. were (almost) identical for MGII (4% and 5%, respectively) and for MGC (16% in both studies). The floor effect of MG-ADL found in our study was lower (11%) than that found in the study of Barnett et al. (22%). Nonetheless, the floor effect of MGII was shown to be clearly lower than that of MG-ADL.

Limitations of this study include the single center of inclusion and our study population within a tertiary referral center that may not fully reflect the total MG population due to a referral bias. Moreover, it might be argued that *QMGgen* is not a 'gold standard' regarding changes in objective generalized muscle weakness. In this study we chose to use *QMGgen* as reference for generalized weakness as the QMG score is the best-known structured way of quantifying muscle weakness in MG and has a high focus on generalized weakness (8 out of 13 items).

Weakness in MG is not 'unidimensional' and muscle groups do not respond equally to different therapies. Outcome measures should therefore be sensitive to changes in both oculobulbar and generalized weakness. This study has shown that in addition to the high construct validity of the MGII in general, this outcome measure is more sensitive to changes in generalized weakness than the MG-ADL. We recommend including MGII in future clinical trials.

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Supplemental material

SUPPLEMENT 1

MG Impairment Index Questionnaire

MG Impairment Index (MGII)TM - Patient Questionnaire

Name: _____

Date: _____

INSTRUCTIONS:

Please answer the following questions regarding your symptoms. Only consider those that you think are related to myasthenia. Check the answer that best describes your symptoms over the past 2 weeks.

PROBLEMS WITH YOUR EYES: Please answer regarding the past 2 weeks.

1. Double vision throughout the day

Have you experienced episodes of double vision? If yes, at what time do they occur (on average)?	No Double Vision	Episodes only in the evenings	Episodes starting in the afternoons	Constant or present most of the day
	☐	☐	☐	☐
	0	1	2	3

2. Double vision with activities

Have you experienced double vision with activities such as reading, driving, watching TV or using a computer? If yes, how long does it take (on average) before the double vision occurs?	No Double Vision	After more than 1 hour	After less than 1 hour, but not immediately	Constant double vision or it starts immediately
	☐	☐	☐	☐
	0	1	2	3

3. Severity of double vision

Have you experienced double vision? If yes, how severe has it been (at your worst)?	No Double Vision	Mild: it doesn't affect my daily activities	It affects my activities but no need to cover one eye	I need to cover one eye to be able to function
	☐	☐	☐	☐
	0	1	2	3

4. Eyelid drooping throughout the day

Have you experienced drooping of your eyelid(s)? If yes, when does it occur (on average)?	No eyelid drooping	Only in the evenings	Drooping starts in the afternoons	Constant drooping or present most of the day
	☐	☐	☐	☐
	0	1	2	3

5. Eyelid drooping with activities

Have you experienced drooping of your eyelid(s) with activities such as reading, driving, watching TV or using a computer? If yes, how long does it take (on average) before the drooping occurs?	No eyelid drooping	After more than 1 hour	After less than 1 hour, but not immediately	Constant drooping or it starts immediately
	☐	☐	☐	☐
	0	1	2	3

6. Severity of eyelid drooping

Have you experienced drooping of your eyelids? If yes, how severe has it been (at your worst)?	No eyelid drooping	Mild: it doesn't affect my vision	It affects my vision but no need to lift my eyelid	I need to lift my eyelid or tilt my head to be able to see
	☐	☐	☐	☐
	0	1	2	3

MGII - Patient Questionnaire

Name: _____

PROBLEMS EATING: Please answer regarding the past 2 weeks.

7. Difficulty swallowing

Have you experienced difficulty swallowing? How severe has it been (at your worst) ?	No swallowing problems	Occasional episodes of choking/coughing with food or liquids	Liquids return through my nose, but no problems with solid food	Difficulty swallowing hard food, requiring a change in diet	Unable to swallow or using a feeding tube
	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4

8. Chewing different types of food

Have you experienced difficulty chewing? How severe has it been (at your worst) ?	No difficulty chewing	Difficulty chewing hard foods (e.g. steak, raw carrots)	Difficulty chewing soft foods (e.g. hard boiled egg)	Unable to chew (eating only liquids or feeding tube)
	☐ 0	☐ 1	☐ 2	☐ 3

9. Chewing tiredness/fatigue

At your worst , how long does it take to develop fatigue or tiredness in your jaw?	No difficulty chewing	Difficulty chewing at the end of the meal	Difficulty chewing from the beginning of the meal	Unable to chew (eating only liquids or feeding tube)
	☐ 0	☐ 1	☐ 2	☐ 3

PROBLEMS SPEAKING AND BREATHING: Please answer regarding the past 2 weeks.

10. Voice changes through the day

Have you experienced episodes of nasal, hoarse or weak voice? When do they occur on average ?	No voice changes	Voice changes only in the evenings	Voice changes starting in the afternoons	Constant voice changes or present most of the day
	☐ 0	☐ 1	☐ 2	☐ 3

11. Voice changes with prolonged conversation

How long can you talk (on average) , before developing voice changes, such as nasal, hoarse or weak voice? (Normal conversation, with pauses for other speakers)	No voice changes	Voice changes after more than 30 minutes	Voice changes after less than 30 minutes, but not immediately	Constant voice changes or they start immediately (less than 1 minute)
	☐ 0	☐ 1	☐ 2	☐ 3

12. Severity of voice changes

At your worst, how severe have your voice changes been? (Nasal, hoarse, weak voice)	No voice changes	Mild changes: my voice is mostly clear	Moderate changes: it can be hard to understand me	Severe changes: it is impossible to understand me
	☐ 0	☐ 1	☐ 2	☐ 3

MGII, V1.0.

MGII - Patient Questionnaire

Name: _____

PROBLEMS SPEAKING AND BREATHING (Cont.): Please answer regarding the past 2 weeks.

13. Speech clarity through the day

Have you experienced difficulty pronouncing words or slurred speech? When does it occur **on average**?

No episodes of slurred speech

☐
0

Slurred speech only in the evenings

☐
1

Slurred speech starting in the afternoons

☐
2

Constant slurring, or present most of the day

☐
3

14. Speech clarity with prolonged conversation

How long can you talk (**on average**), before developing slurred speech? (Normal conversation, with pauses for other speakers)

No episodes of slurred speech

☐
0

Slurred speech after 30 minutes

☐
1

Slurred speech after less than 30 minutes, but not immediately

☐
2

Constant slurring, or it starts immediately (less than 1 minute)

☐
3

15. Severity of speech changes

At your worst, how severe have your speech changes been? (Slurring, difficulty pronouncing words)

No episodes of slurred speech

☐
0

Mild slurring: It is easy to understand me

☐
1

Moderate slurring: There are some difficulties understanding me

☐
2

Severe slurring: it is impossible to understand me

☐
3

16. Difficulty breathing

Have you experienced shortness of breath that is caused by myasthenia? (i.e. not caused by asthma, or other lung/heart disease) If yes, when has it occurred (**at your worst**)?

No shortness of breath (except for strenuous exercise)

☐
0

With moderate effort (e.g. walking several blocks at my own pace)

☐
1

With minimal effort (e.g. getting dressed, walking inside the house)

☐
2

At rest or when lying on my back

☐
3

Requiring assisted ventilation

☐
4

GENERALIZED SYMPTOMS: Please answer regarding the past 2 weeks.

17. Overall physical tiredness

Have you experienced overall physical tiredness caused by myasthenia gravis? (i.e. not by sleeplessness, depression or other medical conditions)

No physical tiredness

☐
0

Overall physical tiredness in the evenings

☐
1

Overall physical tiredness starting in the afternoons

☐
2

Constant physical tiredness, or present most of the day

☐
3

MGII - Patient Questionnaire

Name: _____

GENERALIZED SYMPTOMS: Please answer regarding the past 2 weeks.

18. Arm weakness severity

Have you experienced weakness in your arms? If yes, how severe has it been **(at your worst)**?

No arm weakness

☐
0
Mild weakness
(e.g. difficulty lifting heavy objects)
☐
1
Moderate weakness
(e.g. difficulty lifting arms above the shoulders, but I can do it)
☐
2
Severe weakness
(unable to lift arms above the shoulders)
☐
3

19. Arm weakness with prolonged use

Have you experienced weakness in your arms after prolonged use? When does it happen **(on average)**?

No arm weakness

☐
0

Weakness when keeping arms up for long (e.g. washing or drying my hair)

☐
1

Weakness with prolonged activities at shoulder level (organizing objects on a shelf, holding a phone to the ear)

☐
2

Weakness with minimal effort (e.g. desk work, chopping vegetables)

☐
3

20. Leg weakness severity

Have you experienced weakness in your legs? If yes, how severe has it been **(at your worst)**?

No leg weakness

☐
0

Mild weakness (e.g. difficulty standing from a squat or from tying my shoes)

☐
1

Moderate weakness (e.g. difficulty standing from a chair, I need to push up with my arms)

☐
2

Severe weakness (e.g. unable to stand from a chair without assistance)

☐
3

21. Leg weakness with prolonged use

Have you experienced weakness in your legs after prolonged use? When does it happen **(on average)**?

No leg weakness

☐
0

Weakness when walking more than 10 blocks at my own pace

☐
1

Weakness when walking less than 10 blocks at my own pace

☐
2

Constant weakness or with minimal effort (standing, walking inside the house)

☐
3

22. Neck weakness

Have you experienced weakness in your neck? When does it happen **(on average)**?

No neck weakness

☐
0

Weakness only in the evenings

☐
1

Weakness starting in the afternoons

☐
2

Constant weakness or present most of the day

☐
3

MG Impairment Index (MGII) - Examination Manual

This measure evaluates disease severity in Myasthenia Gravis, incorporating measures of endurance and fatigability within each regional domain (Ocular, Bulbar and Generalized). It consists of two parts, one based on the clinical examination and a patient-reported questionnaire.

Below are the instructions for the items requiring physical examination. You only need a stopwatch. This part can take up to 8 minutes. You can score it directly on the scoring sheet, but please also record the actual time in seconds when appropriate.

Assessments:

- 1. Diplopia:** Ask the patient to follow your finger in all primary directions (up, down, right and left). Ask the patient if he/she has diplopia in any direction, including in primary gaze. Record the number of gaze directions where diplopia is present.
- 2. Ptosis:** Ask the patient to hold their gaze upwards, for up to 60 seconds, and record the time to develop ptosis (in seconds).
- 3. Lower Facial Strength:** Ask the patient to blow air into her/his cheeks and hold it against resistance. You can provide cues such as "like blowing a balloon".
- 4. Arm Endurance:** Ask the patient to hold both arms out-stretched at a 90° angle with forearms pronated (max 180 seconds). The endurance time (in seconds) of the best arm is recorded. If the arms are falling, you can encourage the patient to lift them up only once. If the arms fall again, the test is ended and that time is recorded. If the patient has shoulder pain (i.e. rotator cuff injury), only the arm not affected is tested.
- 5. Leg Endurance:** Ask the patient to lie flat on his/her back and raise the dominant leg at a 45° angle, with the knee straight and record the time (max. 90 seconds). If there are other conditions affecting the dominant leg (i.e. hip arthritis), the non-dominant leg will be tested. If the leg falls without touching the bed, you can encourage the patient to lift it up only once. If it falls again or if it touches the bed, the test is completed and that time is recorded.
- 6. Neck Endurance:** Ask the patient to lie flat on his/her back and hold their head up at a 45° angle. Record the endurance time (max. 60 seconds). If the head starts falling (without touching the bed), you can encourage the patient to lift it up only once. If it falls again or it touches the bed, the test is completed and that time is recorded.
- 7. Neck weakness:** Test neck flexion and extension with the patient flat on his/her back. Ask the patient to push with their head towards the back/front against your hand. Record the result from the **weakest** muscle group (flexors or extensors).

MGI² - Examination

Name: _____

Date: _____

This sheet refers to the physical examination. Detailed instructions are found in the instruction manual. You will need a stopwatch.

	0	1	2	3	Score
E1. Diplopia	No Diplopia	Diplopia in only 1 direction.	Diplopia in 2 directions.	Diplopia in ≥ 3 directions OR in primary gaze.	
E2. Ptosis	No ptosis	Ptosis between 10-60 seconds.	Spontaneous ptosis or in less than 10 seconds.		
E3. Lower Facial Strength	Normal strength	Can resist with cheeks, but air escapes through lips.	Unable to seal lips or provide resistance with cheeks.		
E4. Arm Endurance	Holds arms for 180 seconds.	Holds arms for 91-179 seconds.	Holds arms for 30-90 seconds.	Holds arms for < 30 seconds.	
E5. Leg Endurance	Holds leg for 90 seconds.	Holds leg for 40-89 seconds.	Holds leg for 16-39 seconds.	Holds leg for ≤ 15 seconds.	
E6. Neck Endurance	Holds head for 60 seconds.	Holds head for 35-59 seconds.	Holds head for 11-34 seconds.	Holds head for ≤ 10 seconds.	

SUPPLEMENT 2

Subdomains Outcome Variables (extended description of subdomain items)

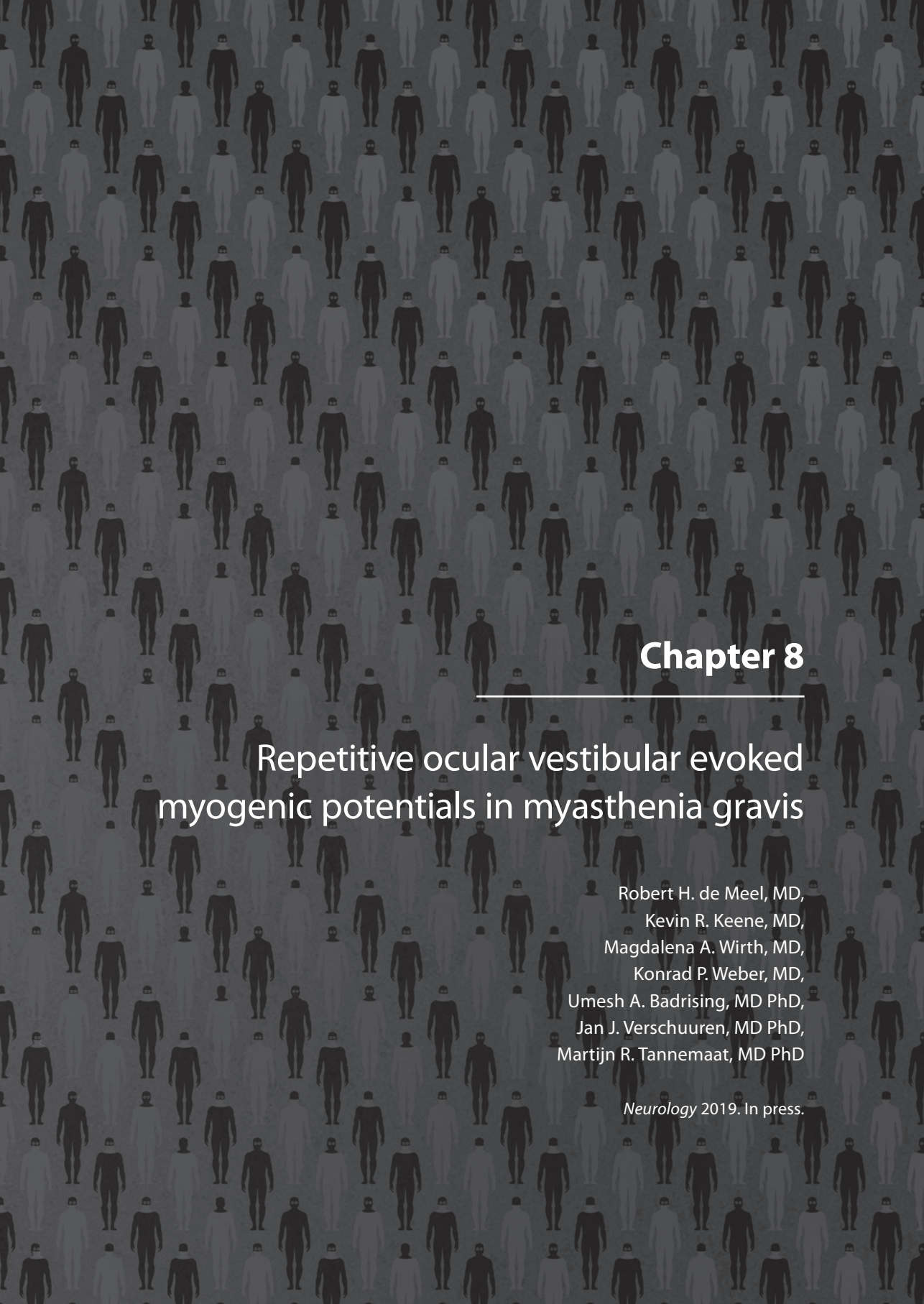
The first 5 items of the QMG constitute the oculobulbar domain (QMGob) and the remaining 8 items constitute the generalized domain (QMGgen). We considered QMGgen to be representative of objective generalized muscle weakness. The first 3 and last 2 items of the MG-ADL constitute the oculobulbar domain (MG-ADLob) and the 4th until the 6th items constitute the generalized domain (MG-ADLgen). The first 15 items of the questionnaire and the first 3 physical examination items of MGII constitute the oculobulbar domain (MGIIob) and the last 7 items of the questionnaire and the last 3 physical examination items constitute the generalized domain (MGIIgen).

SUPPLEMENT 3

Statistical Analysis (extended description of generalized linear model)

We used a generalized linear model to assess whether *MGIIgen* had an additional sensitivity on top of *MG-ADLgen* for changes (Δ) in *QMGgen* in individual patients. First, we fitted a linear model for *QMGgen* with *MG-ADLgen* as the single predictor. Next, we regressed the residuals from that model on *MGIIgen*. The residual is the difference between observed and predicted *QMGgen* based on the *MG-ADLgen* score of that patient. Results are expressed as B coefficients with 95% confidence intervals (CI), which reflect the additional value of *MGII* in the prediction of *QMGgen* compared to *MG-ADLgen* alone. The regression line shows the degree in which *MGIIgen* can compensate for the mismatch between observed and predicted *QMGgen*.





Chapter 8

Repetitive ocular vestibular evoked myogenic potentials in myasthenia gravis

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ABSTRACT

Objective:

To validate the repetitive ocular vestibular evoked myogenic potentials (RoVEMP) test for diagnostic use in myasthenia gravis (MG) and to investigate its value in diagnostically challenging subgroups.

Methods

The RoVEMP test was performed in 92 MG patients, 22 healthy controls, 33 patients with a neuromuscular disease other than MG (neuromuscular controls), 4 Lambert-Eaton Myasthenic Syndrome (LEMS) patients and 2 Congenital Myasthenic Syndrome (CMS) patients.

Results

Mean decrement was significantly higher in MG patients (28.4 ± 32.2) than in healthy controls (3.2 ± 13.9 ; $p < 0.001$) or neuromuscular controls (3.8 ± 26.9 ; $p < 0.001$). With neuromuscular controls as reference, a cut-off value of $\geq 14.3\%$ resulted in a sensitivity of 67% and a specificity of 82%. The sensitivity of the RoVEMP test was similar in ocular MG (80%) and generalized MG (63%). Only one of 7 seronegative MG (SNMG) patients with ocular weakness had a negative RoVEMP test. Of all SNMG patients with negative repetitive nerve stimulation (RNS) results, 73% had an abnormal RoVEMP test. Magnitude of decrement was correlated with the time since the last intake of pyridostigmine ($B = 5.40$, $p = 0.019$).

Discussion

The RoVEMP test is a new neurophysiological test that provides an objective measurement of the function of extra-ocular muscles, which are the most frequently affected muscles in MG. Especially in diagnostically challenging patients, with isolated ocular muscle weakness, negative antibody tests and negative RNS results, the RoVEMP test might be helpful for establishing the diagnosis of MG.

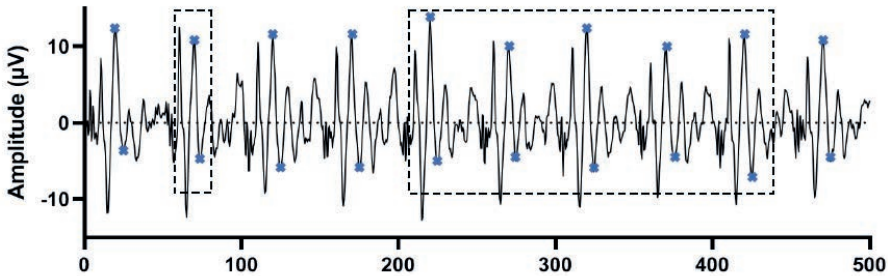
INTRODUCTION

Myasthenia gravis (MG) is a disease characterized by fatigable muscle weakness, caused by auto-antibodies most frequently directed against the acetylcholine receptor (AChR) and muscle specific tyrosine kinase (MuSK). Ocular muscles are involved at the onset of the disease in approximately 85% of patients, causing diplopia and ptosis.^{1,2} Many patients will also develop weakness of the bulbar muscles and proximal muscles of the limbs. Involvement of the respiratory muscles can cause a myasthenic crisis, characterized by respiratory failure. Early recognition and treatment are of great importance for patients' quality of life.³ Currently, the diagnosis of MG is made by a combination of clinically confirmed fluctuating muscle weakness supported by either the presence of serum autoantibodies, abnormal findings during neurophysiological testing or a positive neostigmine test.

Current neurophysiological tests have several limitations. The greatest limitation is that the most commonly affected muscles, the extra-ocular muscles (EOMs), cannot be measured. Moreover, repetitive nerve stimulation (RNS) has a low sensitivity (0.29) in the patient subgroup in which the diagnosis is most challenging: ocular MG (OMG) patients.⁴ In this group, antibodies are found in only 44% of patients, hence there is a strong need for an additional test to make the diagnosis. Single-fiber EMG (SFEMG) has been reported to have a relatively high sensitivity in OMG (62-97%)⁴, however it does not directly measure EOMs, is time-consuming and operator-dependent and requires a skilled neurophysiologist. In summary, the lack of neurophysiological tests measuring the neuromuscular transmission of EOMs complicates the diagnosis in OMG patients.⁵

Measurement of fatigability in the EOMs by using ocular vestibular evoked myogenic potentials (oVEMP) might be a solution to this problem.⁶ The stimulation of the otoliths with bone-conducted vibration administered to the skull cause a vestibulo-ocular reflex, currently used for diagnoses in vestibular disease. The oVEMP is a multiphasic wave with the first peak at a latency of 10 milliseconds after stimulation, measured with electrodes under the eyes to record myogenic activity from the inferior oblique muscle bilaterally (Fig 1).⁷⁻¹⁰ For vestibular disorders, a single oVEMP potential is elicited and analyzed. Valko et al. showed that the function of the neuromuscular junction (NMJ) can be investigated by applying a train of ten repetitive oVEMPs. In MG patients, a decrement in the n2-p2 amplitude was observed, analogous to the decrement observed in RNS.⁶ We will refer to this technique as the Repetitive oVEMP (RoVEMP) test.

A. Neuromuscular control



B. MG patient

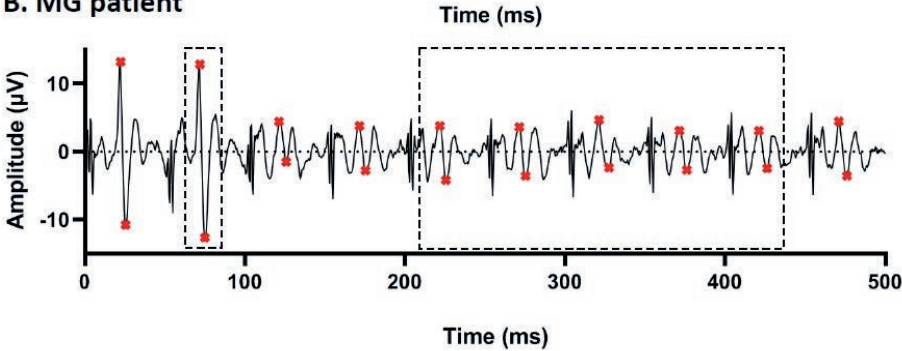


Figure 1. RoVEMP test results of a neuromuscular control (A) and an MG patient (B) are shown. The oVEMP response is a multiphasic wave. For the RoVEMP test, the amplitude between the second peak (n2) and the second trough (p2) of the oVEMP response is used (n2 and p2 are indicated by colored asterixes). Decrement is calculated by comparing the n2-p2 amplitude after the second stimulation with the mean of the fifth to ninth amplitudes.

We aim to validate and further investigate the diagnostic yield of RoVEMP compared with a control group of patients with a neuromuscular disease other than MG (neuromuscular controls) as well a group of healthy controls. We also analyzed the effect of pyridostigmine use on RoVEMP results. Furthermore, we included a large cohort of MG patients to analyze the sensitivity and specificity of the RoVEMP in diagnostically challenging subgroups (OMG and SNMG patients).

METHODS

Participants

We included a consecutive cohort of MG patients who visited the outpatient clinic of Leiden University Medical Center (LUMC) between 2018 and 2019. In addition, we included patients with Lambert-Eaton myasthenic syndrome (LEMS) and genetically confirmed

congenital myasthenic syndrome (CMS). We also included a group of healthy controls and a group of patients with a neuromuscular disease other than MG (neuromuscular controls). We considered the following diseases: inclusion-body myositis (IBM), facioscapulohumeral muscular dystrophy (FSHD), myotonic dystrophy (DM), myopathy, oculopharyngeal muscular dystrophy (OPMD), chronic inflammatory demyelinating polyneuropathy (CIDP), cranial nerve palsies (III, IV, VI), mechanical diplopia, and Graves' ophthalmopathy (GO). Patients with GO were included from the Ophthalmology Department of the LUMC. GO patients form an important control group as the ocular symptoms in these patients may resemble those of OMG and may cause diagnostic confusion and delay.¹¹⁻¹⁶ MG patients who were being treated with pyridostigmine were not asked to refrain from taking pyridostigmine before the RoVEMP test. All participants were examined for the presence of diplopia prior to RoVEMP testing. We recorded the time between the RoVEMP test and the last intake of pyridostigmine. We retrieved all relevant clinical information or RNS results of MG patients and neuromuscular control patients from their patient records.

The diagnosis of MG was based on a combination of clinically confirmed fluctuating muscle weakness and the presence of serum autoantibodies to AChR or MuSK. Seronegative myasthenia gravis (SNMG) was defined as fatigable muscle weakness in combination with abnormal decrement (at least 10%) during RNS, increased jitter in SFEMG testing or a positive response to an acetylcholinesterase inhibitor.¹⁷

Standard protocol approvals, registrations, and patient consents

The Medical Ethics Review Committee of the Leiden University Medical Center approved the study and its use of human subjects. All patients provided informed, written consent prior to study participation.

RoVEMP test

In collaboration with the group of Valko et al., we reproduced the measurement setup to perform the RoVEMP test. The placement of the surface electrodes was the same as described in the article of Valko et al., with two surface electrodes directly under each eye and a ground electrode on the forehead.⁶ We used a stimulation frequency of 20 Hz and applied vibrations in trains of 10 stimuli with a handheld mini-shaker (type 4810, manufacturer: Brüel & Kjær) to the skull. We repeated these trains 40 times to improve the signal-to-noise ratio. The signals were sampled with a rate of 2000 per second and saved using Nim Eclipse software and a Nim Eclipse recording device (Medtronic Xomed, inc).

Signal analysis

The recorded signals were analyzed for outliers using a median absolute deviation algorithm. The median absolute deviation of the signal between the stimulus artifacts is a measure for baseline fluctuations and other high voltage artifacts, which are most commonly caused

by eye-blinking during the measurement. Measurements with a median absolute deviation higher or lower than two standard deviations from the mean were considered outliers and were excluded. The remaining recorded signals were averaged. The average was high-pass filtered at 20Hz with a butterworth filter to correct for additional baseline fluctuations.

Decrement calculation

After averaging all measurements, the peaks and troughs of the oVEMP signal were analyzed and the amplitude of the oVEMP response was calculated using an in-house developed Matlab script (MATLAB 2016a, The Mathworks of Natick, Massachusetts, USA). Per measurement all peaks and troughs were automatically analyzed (thresholding using a minimal peak prominence of 0.8μV) and the correct peaks and troughs were automatically selected, using subject-specific latency values based on visual inspection of the averaged signals. The amplitudes from all second negative peaks (n2) to the second positive peaks (p2) were calculated. The N2P2 amplitude was used to calculate decrement as this 'reference' (instead of N1P1 for example) was shown to result in the highest diagnostic yield. As a measure of neuromuscular transmission, the RoVEMP decrement was calculated for each eye as described previously by Valko et al. using the following formula:

$$\text{Decrement} = 100\% - \frac{\text{Average of } n2p2\text{Amplitude}_{5th-9th}}{n2p2\text{Amplitude}_{2nd}} * 100\%$$

Of the two decrements found in the two eyes of the subject, the highest was considered as the RoVEMP decrement in that subject.

Statistical Analysis

For comparison of decrement and other numerical variables between groups, we used an unpaired t-test. To compare categorical variables between groups we used a chi-square test. To determine optimal oVEMP cut-off values, we created receiver operating characteristics (ROC) curves. Data are presented as number of patients (%) for categorical variables and as mean ± SD for continuous variables. P-values < 0.05 were considered significant. Statistical analyses were performed using SPSS version 23 (IBM Corp., Armonk, NY).

Data availability

Anonymized data presented in this article will be made available at the request of a qualified investigator. Requests should be made to dr. Tannemaat (m.r.tannemaat@lumc.nl).

RESULTS

Participant characteristics

We included 92 MG patients, 22 healthy controls, 33 neuromuscular controls, 4 LEMS patients and 2 CMS patients. Two MG patients had a follow-up visit in which the RoVEMP test was performed for a second time. The mean age and percentage of males was comparable between MG patients (57 ± 18 ; 48%), healthy controls (51 ± 14 ; 46%) and neuromuscular controls (58 ± 12 ; 46%). Diplopia upon examination prior to RoVEMP testing was found in 58% of MG patients and 67% of neuromuscular controls. Thirty-five MG patients used pyridostigmine ≤ 4.5 hours before the RoVEMP test. The RoVEMP results of one MG patient, 2 healthy controls and 2 neuromuscular controls were not analyzable due to excessive blink artifacts and were excluded. In one neuromuscular control, increment was truncated at 100%. Demographic and clinical baseline characteristics of all MG patients subdivided by antibody status are shown in table 1.

Table 1. Baseline characteristics of 92 patients with MG included in this study. Data are presented as number of patients (%) for categorical variables and as mean $\pm$ SD for continuous variables.

	AChR MG N=71	MuSK MG N=7	SNMG N=14	<i>p</i> Value
Age, y	57.1 $\pm$ 18.3	54.0 $\pm$ 20.3	57.1 $\pm$ 14.4	0.908
Age at onset, y	46.3 $\pm$ 18.7	46.4 $\pm$ 17.6	46.0 $\pm$ 15.1	0.998
Gender				
Male	36 (51)	4 (57)	4 (29)	0.278
Female	35 (49)	3 (43)	10 (71)	
Phenotype				
Ocular	18 (25)	0 (0)	7 (50)	0.040
Generalized	53 (75)	7 (100)	7 (50)	
Thymectomy				
Yes, with thymoma	7 (10)	0 (0)	0 (0)	0.138
Yes, without thymoma	21 (30)	1 (14)	1 (7)	
No	43 (60)	6 (86)	13 (93)	
Medication use				
Pyridostigmine	49 (69)	1 (14)	10 (71)	0.013
Prednisone	38 (54)	5 (71)	7 (50)	0.622
Other immunosuppressants	33 (47)	4 (57)	5 (36)	0.622
Repetitive nerve stimulation				
Abnormal decrement	24/49 (49)	2/6 (33)	1/11 (9)	0.048
RoVEMP				
Decrement	27.5 $\pm$ 33.0	29.7 $\pm$ 42.2	32.6 $\pm$ 23.2	0.861
Decrement $\geq$ 11.3%	50 (70)	5 (71)	10 (71)	0.996
Decrement $\geq$ 14.3%	47 (66)	5 (71)	10 (71)	0.904

oVEMP decrement in MG and other groups

Mean decrement was significantly higher in MG patients (28.4 ± 32.2) than in healthy controls (3.2 ± 13.9 ; $p < 0.001$) or neuromuscular controls (3.8 ± 26.9 ; $p < 0.001$), see figure 2 A. Mean decrement in LEMS patients (41.8 ± 40.4) and CMS patients (29.1 ± 16.2) was elevated, but due to the low numbers in these groups, statistical comparisons were not feasible. The results in neuromuscular controls shown for each disease separately are shown in supplement 1. Using ROC analysis we identified optimal decrement cut-offs for distinguishing between MG patients and healthy controls ($\geq 11.3\%$; sensitivity 71%, specificity 86%, area under the curve (AUC) 0.78), and between MG patients and neuromuscular controls ($\geq 14.3\%$; sensitivity 67%, specificity 82%, AUC 0.74), see figure 3.

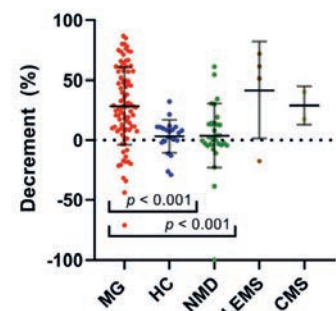


Figure 2A. Scatter dot plots showing RoVEMP decrements in the five groups that participated in this study. Mean and 95% CI are shown by lines and error bars.

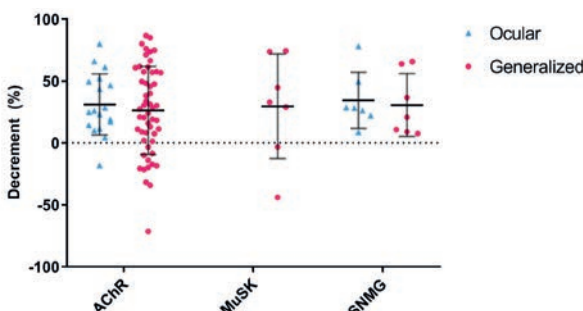


Figure 2B. Scatter dot plots showing RoVEMP decrements in AChR, MuSK and SNMG patients, subdivided by ocular and generalized phenotype. Mean and 95% CI are shown by lines and error bars.

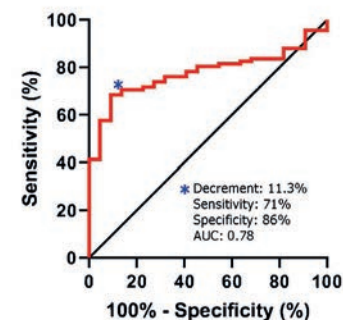


Figure 3A. ROC analysis with healthy controls as reference. Optimal cut-off value, the sensitivity and specificity and area under the curve (AUC) are shown.

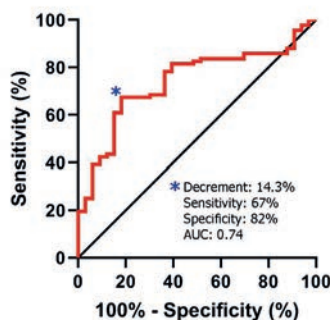


Figure 3B. ROC analysis with neuromuscular controls as reference. Optimal cut-off value, the sensitivity and specificity and area under the curve (AUC) are shown.

oVEMP decrement in MG subgroups

Mean decrement was comparable in AChR (27.5 ± 33.0), MuSK (29.7 ± 42.2) and SNMG patients (32.6 ± 23.2). The mean decrement in OMG (32.1 ± 23.7) and GMG patients (27.1 ± 34.9) was also comparable (figure 2 B). With a cut-off of $\geq 11.3\%$, a non-significant trend towards a higher occurrence of an abnormal RoVEMP test was found in OMG (84%) compared to GMG patients (66%, $p = 0.086$). With the cut-off of $\geq 14.3\%$, a similar difference was found (80% vs. 63%, $p = 0.115$). The RoVEMP was abnormal in similar frequencies in AChR, MuSK or SNMG patients (see table 1). Only one of 7 SNMG patients with ocular weakness had a decrement below the cut-off value of $\geq 11.3\%$, whilst the other 6 patients had decrements above 22%.

Comparison RoVEMP and RNS

Abnormal RNS findings occurred significantly more often in seropositive MG patients (47%) than in SNMG (9%; $p = 0.019$) patients and more often in GMG (51%) than in OMG patients (16%; $p = 0.008$). The percentage of patients with an abnormal RoVEMP test and a negative RNS was significantly higher in SNMG patients (73%) compared to seropositive (AChR or MuSK) MG patients (40%; $p = 0.047$). The results were identical with cut-off values $\geq 11.3\%$ and $\geq 14.3\%$.

Pyridostigmine effect on oVEMP decrement

Mean decrement was significantly higher in MG patients who did not use pyridostigmine before the RoVEMP test (33.2 ± 35.4), compared to MG patients that did use pyridostigmine $\leq 4,5$ hours before the RoVEMP test (19.8 ± 23.7 ; $p = 0.033$), see figure 4. However, the percentage of patients with a RoVEMP decrement $\geq 11.3\%$ was similar in both groups (73% and 67%, respectively; $p = 0.530$). With a cut-off value of $\geq 14.3\%$, a non-significant trend towards a higher occurrence of an abnormal RoVEMP test was found in the former (73%) compared to the latter group (58%, $p = 0.133$). Magnitude of decrement was correlated with the time since the last intake of pyridostigmine ($B = 5.40$, $p = 0.019$; figure 5). In the two patients with a follow-up visit, RoVEMP decrements were higher on the day in which pyridostigmine was taken an hour before the test (66% and 45%) than on the day in which the patients did not take pyridostigmine prior to the test (29% and 24%).

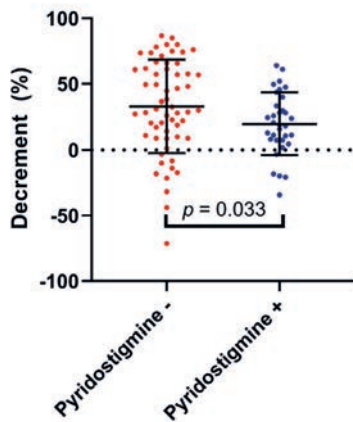


Figure 4. Scatter dot plots showing RoVEMP decrements in MG patients that did not use pyridostigmine before the RoVEMP test (pyridostigmine -) and MG patients that did use pyridostigmine ≤ 4.5 hours before the RoVEMP test. Mean and 95% CI are shown by lines and error bars.

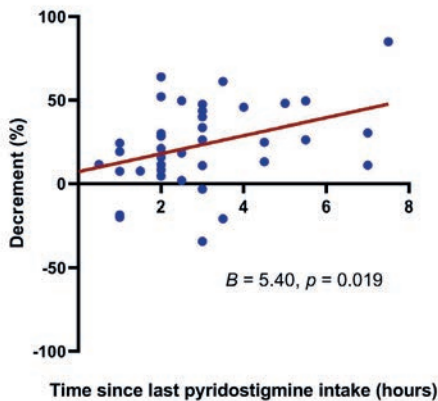


Figure 5. Analysis of the relation between decrement and time since last pyridostigmine intake in 39 MG patients. The correlation between these two variables is shown by the linear regression line (red). The slope indicates the B coefficient (shown in the figure).

DISCUSSION

In this study, we showed that the RoVEMP test accurately differentiates between MG and patients with other neuromuscular diseases as well as healthy controls. Interestingly, the RoVEMP test had a high sensitivity in the diagnostically most challenging myasthenia subgroup: ocular SNMG patients. Six out of 7 of these patients had a positive RoVEMP test.

Valko et al. reported an optimal decrement cut-off of $\geq 15.2\%$ in a cohort of 13 ocular and 14 generalized MG patients compared to a healthy control group ($n = 28$) and found a sensitivity of 89% and a specificity of 64%. The overall sensitivity of the RoVEMP test compared to healthy controls found in our study was somewhat lower (71%). This is probably due to the fact that our cohort contained a lower percentage of ocular MG patients (27% (25/92)) than the cohort of Valko et al. (48% (13/27)). This is supported by the higher sensitivity found in ocular MG patients of our cohort (84%).

As previously described, RNS is often negative in SNMG or OMG patients.⁴ In our study, RNS was abnormal in only 9% of SNMG patients and in 16% of OMG patients. In SNMG, the RoVEMP test was abnormal in 73% of patients that had a normal RNS. In seropositive MG patients, the RoVEMP test also appeared to be more sensitive than RNS (40% of patients with a negative RNS had an abnormal RoVEMP), however this additional diagnostic value was significantly lower than in SNMG. This difference is probably due to the higher number of ocular MG patients in the SNMG group. This study shows that the RoVEMP test is the only diagnostic test for MG that does not have a lower sensitivity in SNMG or OMG. To the contrary, sensitivity of the RoVEMP test in ocular SNMG patients, usually the most difficult subgroup to diagnose, was relatively high.

We used an ROC analysis to identify optimal cut-off values to distinguish between MG patients and healthy controls, and between MG patients and neuromuscular controls. Sensitivity, specificity and area under the curve were similar in the two ROC analyses. The optimal cut-off values were also similar. As neuromuscular controls form a better reference than healthy controls, we propose to use the more conservative cut-off value found in the ROC analysis with neuromuscular controls ($\geq 14.3\%$), with an overall sensitivity of 67% and a specificity 82%. Although this cut-off value lies 3.0% above that of the ROC analysis with healthy controls, sensitivity in SNMG or OMG patients is not affected. This cut-off value also lies closer to that proposed by Valko et al. ($\geq 15.2\%$).

Pyridostigmine use $\leq 4,5$ hours before the RoVEMP test was associated with a lower magnitude of RoVEMP decrement. However, the sensitivity was not significantly different between patients that did or did not use pyridostigmine before the test. Still, we would recommend asking patients not to use pyridostigmine $\leq 4,5$ hours before the RoVEMP test. We also found a significant correlation between the magnitude of decrement and the time since the last intake of pyridostigmine and observed considerably lower decrements in two patients when taking pyridostigmine compared to not taking pyridostigmine. These findings support that RoVEMP decrement reflects reversible neuromuscular transmission failure, probably analogous to RNS decrement. In addition, we showed that RoVEMP decrement is not a measure for diplopia in general, as the prevalence of diplopia was similar in our MG patients and neuromuscular controls.

As RNS is also used in LEMS and CMS patients, we included a small number of these patients to explore the potential value of the RoVEMP test in LEMS and CMS. 3 of 4 LEMS patients and 2 of 2 CMS patients had an abnormal RoVEMP test in our study. Although promising, these results should be confirmed in larger cohorts.

We found an abnormal RoVEMP test in 2 healthy controls and 6 neuromuscular controls. Some of these controls showed a relatively high variation in the latency of the detected peaks and a low signal to noise ratio. In this study, we decided to exclude RoVEMP results only when there were evident blink artifacts that disrupted the RoVEMP signal (overall exclusion rate 3%). In further research, we will focus on quantifying the signal to noise

ratio and establishing a method to objectively determine the confidence bounds of a single RoVEMP measurement to increase the diagnostic yield.

Limitations of this study include the single center of inclusion and our study population within a tertiary referral center that may not fully reflect the total MG population due to a referral bias. Our control group of neuromuscular patients did not reflect the whole range of patients with diplopia due to a neuromuscular disease other than MG. However, we included control patients with disorders that were previously reported to cause diagnostic confusion and delay due to similarities in presenting symptoms compared to OMG.¹⁸

The RoVEMP test is a new neurophysiological test that, in contrast to RNS and single-fiber EMG, is able to measure neuromuscular transmission of EOMs, which are the most affected muscles in MG. As the test is quick (10-15 minutes), less invasive than RNS and single-fiber EMG and easy to perform, we recommend including the RoVEMP test in the routine diagnostic evaluation of patients with suspected MG. Especially in diagnostically challenging patients with negative antibody tests, negative RNS results and isolated ocular muscle weakness, the RoVEMP test has a clear added value in supporting the diagnosis of MG.

ACKNOWLEDGEMENTS

We would like to thank S.W. Genders, MD, from the Ophthalmology Department of the Leiden University Medical Center for recruiting patients with Graves' ophthalmopathy in our study.

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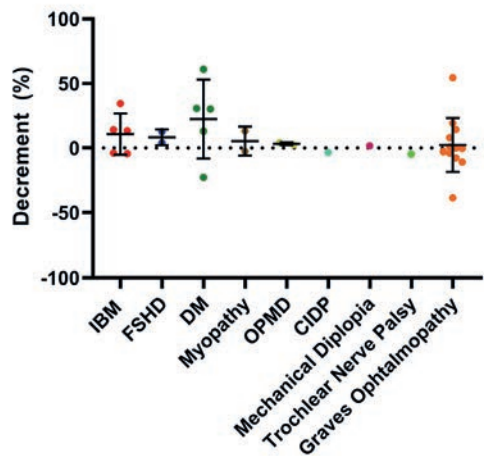
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Supplemental material

SUPPLEMENT 1

RoVEMP decrements in the disease control groups

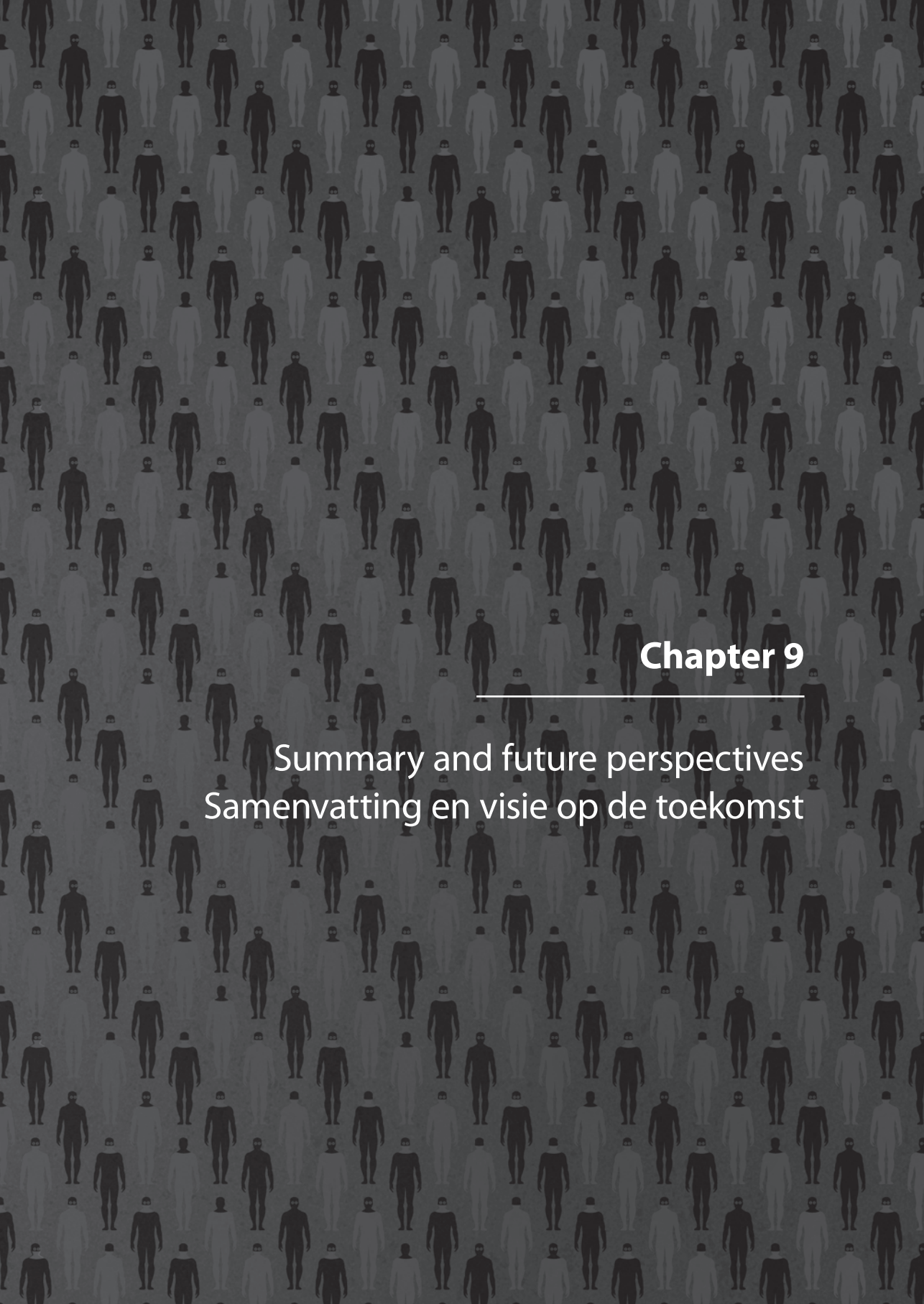


Scatter dot plots showing RoVEMP decrements in all the disease groups that together constituted the neuro-muscular control group. Mean and 95% CI are shown by lines and error bars.



SUMMARY



The background of the entire page is a repeating pattern of stylized human figures. Each figure is composed of a black silhouette and a white outline, creating a high-contrast, grid-like effect. The figures are arranged in a regular, repeating pattern across the entire page.

Chapter 9

Summary and future perspectives Samenvatting en visie op de toekomst

SUMMARY

Over the last years several advancements have been made in MG research. Additional antibodies against for example MuSK, LRP4 or agrin have been discovered, multiple outcome measures have been developed and new therapies have been tested. However, research has focused to a lesser extent on understanding the heterogeneous muscle weakness that we encounter in MG and taking this heterogeneity into account in outcome measures and analyzing therapeutic effects. This thesis aimed to gain more insight in the phenomenology of MG and its reflection in current outcome measures and diagnostic tests.

In a cohort of 225 AChR MG patients, we found a high heterogeneity in the distribution of muscle weakness and a frequent occurrence of shifts between phenotypes in individual patients. In 12 patients (5%) MG was restricted to ocular weakness, whereas 15 other patients (7%) never had any form of ocular weakness throughout their disease course. These phenotypic extremes suggest that other factors aside from the AChR antibody mediated immune response, such as characteristics of individual muscles and their resistance against the antibody mediated attack, are of importance in determining the disease expression in MG. Ocular or bulbar weakness went into remission more frequently than neck/limbs/respiratory (NLR) weakness, suggesting that the latter form of weakness responds less well to therapy or that oculobulbar muscles are more capable of adapting to the autoimmune disorder. Clinical remission occurred more often in patients that did not have any form of NLR weakness in the first 6 months of disease. This suggests that the initial phenotype in AChR MG patients is of prognostic value. **(CHAPTER 2)**

Changes in the side of the ptosis or affected EOMs occur frequently in MG. In 83% of MG patients at least one other EOM was involved at the second visit. EOMs of both eyes were usually affected (75%) and double vision contained both a vertical and horizontal component in most cases (95%). In diagnostically challenging cases, we recommend testing ptosis and diplopia in all gaze directions for 60 seconds during at least two follow-up visits to maximize the chance of observing diplopia or changes in diplopia patterns observed earlier. **(CHAPTER 3)**

Late-onset of symptoms and the presence of additional autoimmune diseases were shown to be prognostic factors for exacerbations and the necessity of emergency treatments in MG. The higher occurrence of exacerbations in these subgroups might reflect distinct underlying pathophysiological mechanisms. Future immunological research on interindividual differences in complement activity and antibody-specificity might provide new clues for understanding the disease fluctuations observed in MG. Besides remission, exacerbations and emergency treatments should also be considered as endpoints in future studies. **(CHAPTER 4)**

Both oculobulbar and generalized MG patients had changes in activity limitations that were most clearly associated with changes in the generalized items of the QMG score. This

finding suggests that we might underestimate the effect of subclinical generalized muscle weakness in patients who are clinically diagnosed with pure oculobulbar MG. This is in line with a previous study that described subclinical generalized weakness in ocular MG patients. Future studies on isolated ocular MG should take into account possible subclinical generalized weakness that might bias the study results. **(CHAPTER 5)**

In the eculizumab study a discrepancy was found between the primary (MG-ADL) and secondary (QMG) outcome measures. The former did not show a significant treatment response whereas the latter did. We suggested that difference in quantitative representation of different muscle groups in QMG and MG-ADL might provide an answer to the mismatch between the outcome measures. It was proposed that discrepancies between QMG and MG-ADL in the eculizumab trial may be explained by a preferential effect of eculizumab on generalized weakness, which would explain the better response on QMG as compared to MG-ADL. To test our hypothesis, we advised reporting the subscores of the oculobulbar and generalized items on both QMG and MG-ADL.

Subsequently, we further investigated MG-ADL and found that it has a lower sensitivity for generalized weakness than for oculobulbar weakness. ACTIVLIM had a significant additional value on top of MG-ADL in the prediction of the generalized items of the QMG, suggesting that adding questions on generalized weakness could improve the sensitivity of the MG-ADL for generalized weakness. This additional value was even higher for changes in generalized weakness over time. **(CHAPTER 6)**

The MGII score was cross-culturally validated in a Dutch cohort of MG patients. Compared to the MG-ADL, the MGII had much lower floor effects. As hypothesized in the previous chapter, increasing the number of generalized items leads to a higher sensitivity generalized weakness: MGII (with 10 generalized items) had a markedly higher sensitivity for generalized weakness than MG-ADL (with 3 generalized items). **(CHAPTER 7)**

The RoVEMP test is a new neurophysiological test that, in contrast to RNS and single-fiber EMG, directly measures neuromuscular transmission of extra-ocular muscles. We were able to show that RoVEMP decrement is not a measure for extra-ocular muscle weakness or diplopia in general as decrement was rarely found in the neuromuscular control group, notwithstanding the similar frequencies of diplopia found in neuromuscular controls and MG patients. In addition, we found a significant correlation between magnitude of decrement and the time since the last intake of pyridostigmine. These findings support that RoVEMP decrement reflects reversible neuromuscular transmission failure, probably analogous to RNS decrement. Especially in diagnostically challenging patients, with isolated ocular muscle weakness, negative antibody tests and negative RNS results, the RoVEMP test might be very helpful for establishing the diagnosis of MG. **(CHAPTER 8)**

FUTURE PERSPECTIVES

Novel assays testing individual differences at the level of epitope specificity, complement function or genes important for immune or muscle function will provide a deeper understanding of the heterogeneity in the distribution of muscle weakness observed in MG. Comparison of the phenotypic extremes described in chapter 2 (isolated ocular versus no ocular weakness at all during the disease course) could be a fruitful approach to explore underlying pathophysiological differences leading to these distinct patterns of muscle weakness.

The great heterogeneity of muscle weakness patterns observed in this thesis and the different response of ocular or generalized weakness to various types of therapies suggest that both inclusion criteria and outcome measures should take into account the different muscle groups that can be affected (e.g. ocular, bulbar and limb-girdle). New outcome measures, such as the MGII, will enable a more detailed analysis of the treatment response in different muscle groups. Moreover, including phenotypic criteria for the inclusion of patients in future trials will increase the success of those trials. If a therapy has a preferential effect on limb-girdle muscles, it may be more adequate to include patients with pronounced limb-girdle weakness than to include patients with a certain total QMG score or MGFA class. The former does not distinguish between ocular, bulbar or limb-girdle weakness and the latter gives only a rough indication whether limb-girdle weakness ('a') or bulbar weakness ('b') is dominant.

The RoVEMP test may become a routine neurophysiological test in patients with the suspicion of MG. It is quick, non-invasive and has a good diagnostic yield. Especially in the diagnostically challenging subgroups of ocular and seronegative MG patients, the RoVEMP test was found to have a higher diagnostic yield than the current neurophysiological standard (repetitive nerve stimulation). Our study suggested that the RoVEMP test might also be useful in LEMS and CMS patients. Future studies on the RoVEMP test will provide further insights in the diagnostic or prognostic value of this test in MG, LEMS and CMS patients.

In conclusion, future research should analyze heterogeneity of disease expression in more detail as it can be used to better understand the pathophysiology of MG. Limiting categorization of MG patients by antibody status, QMG score, MGFA class or ambiguous terms such as 'generalized disease' could impede uncovering one of the most intriguing questions in the field of MG and many other neuromuscular diseases: the differences within and between neuromuscular disorders in the patterns of muscle weakness. Subgroup-specific therapies, better diagnostic strategies and higher success rates of trials could be the rewards of putting effort in solving the puzzle why systemic (autoimmune) muscle diseases affect some muscles more than others.

SAMENVATTING

De laatste jaren is in myasthenie-onderzoek op verscheidene terreinen vooruitgang geboekt. Nieuwe antistoffen tegen onder andere MuSK, LRP4 en agrin zijn ontdekt, nieuwe uitkomstmaten zijn ontwikkeld en nieuwe therapieën zijn getest. Er is echter minder aandacht geweest voor het verklaren van de heterogeniteit in spierzwakte die we observeren in myasthenie en de manier waarop deze heterogeniteit impact heeft op uitkomstmaten en therapeutische effecten. Dit proefschrift richtte zich op het vergaren van meer inzicht in de fenomenologie van myasthenie en de wijze waarop deze uiting vindt in uitkomstmaten en diagnostische onderzoeken.

In een cohort van 225 AChR MG-patiënten vonden we een hoge heterogeniteit in de distributie van spierzwakte en kwamen veranderingen van fenotype binnen individuele patiënten frequent voor. In 12 patiënten (5%) was de myasthene zwakte beperkt tot de oogspieren, terwijl 15 andere patiënten (7%) nooit enige vorm van oculaire zwakte hadden gedurende hun ziektebeloop. Deze fenotypische extremen suggereren dat andere factoren naast de AChR antistof gemedieerde immuunrespons, zoals eigenschappen van individuele spieren en hun resistentie tegen door antistof gemedieerde schade, van belang zijn in het bepalen van de ziekte-uiting in MG. Oculaire en bulbair zwakte gingen vaker in remissie dan nek/ledemaat/respiratoire (NLR) zwakte, wat suggereert dat de laatste vorm van zwakte slechter reageert op therapie en/of dat oculobulbair spieren beter in staat zijn om zich aan te passen aan de auto-immuunziekte. Klinische remissie werd vaker geconstateerd in patiënten zonder enige vorm van NLR-zwakte in de eerste 6 maanden van ziekte. Dit suggereert dat het initiële fenotype in AChR MG-patiënten van prognostische waarde is. **(HOOFDSTUK 2)**

Veranderingen in de kant van ptosis of aangedane extra-oculaire spieren (EOMs) komen vaak voor in MG. In 83% van de MG-patiënten was ten minste één andere EOM betrokken tijdens de tweede visite. Meestal waren EOMs van beide ogen aangedaan (75%) en dubbelzien had in de meeste gevallen zowel een verticale als een horizontale component (95%). In diagnostisch uitdagende casus raden we aan om ptosis en diplopie in alle blikrichtingen gedurende 60 seconden te testen bij ten minste twee opeenvolgende visites om de kans te vergroten diplopie en veranderingen in het patroon van diplopie te observeren. **(HOOFDSTUK 3)**

Het pas op hoge leeftijd ontwikkelen van symptomen en de aanwezigheid van andere auto-immuunziekten zijn prognostische factoren voor exacerbaties en de noodzaak voor noodbehandelingen in MG. Het vaker voorkomen van exacerbaties in deze subgroepen zou een weerspiegeling kunnen zijn van verschillende onderliggende pathofysiologische mechanismen. Toekomstig immunologisch onderzoek naar individuele verschillen in complement-activiteit en antistof-specificiteit zou nieuwe aanknopingspunten kunnen bieden voor het begrijpen van de ziektefluctuaties die geobserveerd worden in MG. Naast

remissie zouden ook exacerbaties en de noodzaak voor noodbehandelingen overwogen worden als uitkomstmaten in toekomstige studies. **(HOOFDSTUK 4)**

Bij zowel oculobulbaire als gegeneraliseerde MG-patiënten werden veranderingen in activiteit-beperkingen aangetoond die het meest duidelijk geassocieerd waren met veranderingen in de gegeneraliseerde onderdelen van de QMG-score. Deze bevinding suggereert dat we het effect van subklinische gegeneraliseerde spierzwakte zouden kunnen onderschatten in patiënten die gediagnosticeerd zijn met geïsoleerde oculobulbaire MG. Dit is in lijn met een eerdere studie die subklinische gegeneraliseerde zwakte aantoonde in oculaire MG-patiënten. Toekomstige studies naar oculaire myasthenie zouden rekening moeten houden met mogelijke subklinische gegeneraliseerde zwakte die studiebevindingen zou kunnen vertroebelen. **(HOOFDSTUK 5)**

In de eculizumab-studie is een discrepantie gevonden tussen de primaire (MG-ADL) en secundaire (QMG) uitkomstmaten. De eerste liet geen significant therapeutisch effect zien, terwijl de laatste dat wél liet zien. We suggereerden dat het verschil in kwantitatieve representatie van verschillende spiergroepen in de QMG en de MG-ADL een verklaring zou kunnen bieden voor het gebrek aan overeenkomst tussen de uitkomstmaten. De discrepantie tussen de QMG en de MG-ADL in de eculizumab-studie zou veroorzaakt kunnen worden door een sterker effect van eculizumab op gegeneraliseerde zwakte, wat het betere effect op de QMG ten opzichte van de MG-ADL zou verklaren. Om onze hypothese te testen, adviseren we om de subscores van de oculobulbaire en gegeneraliseerde onderdelen van de QMG en de MG-ADL te rapporteren.

Vervolgens hebben we de MG-ADL verder onderzocht en vonden we dat deze een lagere sensitiviteit had voor gegeneraliseerde zwakte dan voor oculobulbaire zwakte. ACTIVLIM had een significant aanvullende waarde bovenop de MG-ADL in het voorspellen van de gegeneraliseerde onderdelen van de QMG, wat suggereert dat het toevoegen van vragen over gegeneraliseerde zwakte de sensitiviteit van de MG-ADL zou verhogen voor gegeneraliseerde zwakte. Deze aanvullende waarde was zelfs hoger voor veranderingen in gegeneraliseerde zwakte gedurende het ziektebeloop. **(HOOFDSTUK 6)**

De MGII-score is gevalideerd in een Nederlands cohort van MG-patiënten. Ten opzichte van de MG-ADL heeft de MGII veel lagere vloereffecten. Conform de hypothese uit het vorige hoofdstuk, leidt het verhogen van het aantal gegeneraliseerde items tot een hogere sensitiviteit voor gegeneraliseerde zwakte: MGII (met 10 gegeneraliseerde onderdelen) had een uitgesproken hogere sensitiviteit voor gegeneraliseerde zwakte dan MG-ADL (met 3 gegeneraliseerde onderdelen). **(HOOFDSTUK 7)**

Het RoVEMP-onderzoek is een nieuw neurofysiologisch onderzoek dat, in tegenstelling tot RNS en single-fiber EMG, direct de neuromusculaire transmissie van extra-oculaire spieren meet. We toonden aan dat RoVEMP decrement geen algemene maat is voor extra-oculaire spierzwakte of ongedifferentieerde diplopie, aangezien decrement zelden gevonden werd in de neuromusculaire controlegroep, ondanks de vergelijkbare aanwezigheid van diplopie in

neuromusculaire controles en MG-patiënten. Daarnaast vonden we een significante correlatie tussen de hoogte van decrement en de verstreken tijd sinds de laatste inname van pyridostigmine. Deze bevindingen onderbouwen dat het RoVEMP-decrement reversibel neuromusculair transmissiefalen reflecteert, waarschijnlijk analoog aan RNS-decrement. In het bijzonder in diagnostisch uitdagende patiënten met geïsoleerde oogspierzwakte, negatieve antistofonderzoeken en negatieve RNS-resultaten, zou het RoVEMP-onderzoek erg nuttig kunnen zijn in het stellen van de diagnose MG. **(HOOFDSTUK 8)**

VISIE OP DE TOEKOMST

Nieuwe assays die individuele verschillen opsporen op het niveau van epitoot-specificiteit, complementfunctie en genen die van belang zijn voor spierfunctie en het immuunsysteem, zullen een dieper begrip kunnen geven van de geobserveerde heterogeniteit in de distributie van spierzwakte in MG. Vergelijking van de fenotypische extremen beschreven in hoofdstuk 2 (geïsoleerde oculaire zwakte versus louter non-oculaire zwakte gedurende het gehele ziektebeloop) zou een vruchtbare methode kunnen zijn om de onderliggende pathofysiologische verschillen te exploreren die leiden tot deze verschillende patronen van spierzwakte.

De grote heterogeniteit van spierzwaktepatronen beschreven in dit proefschrift en de verschillende respons van oculaire en generaliseerde zwakte op verscheidene vormen van therapie suggereren dat zowel inclusiecriteria en uitkomstmaten rekening zouden moeten houden met de verschillende spiergroepen die aangedaan kunnen zijn (bijv. oculair, bulbaire en ledemaat-romp). Nieuwe uitkomstmaten, zoals de MGII, zouden een meer gedetailleerde analyse van therapierespons op verschillende spiergroepen kunnen geven. Daarnaast zou het includeren van fenotypische inclusiecriteria in toekomstige studies de succeskans van deze studies kunnen verhogen. Indien een therapie een sterker effect heeft op ledemaat-rompspieren, zou het adequater zijn om patiënten te includeren met uitgesproken ledemaat-rompspierzwakte dan patiënten met een zekere totale QMG-score of MGFA-klasse. De eerste maakt geen onderscheid tussen oculaire, bulbaire en ledemaat-rompzwakte en de laatste geeft slechts een grove indicatie of ledemaat-rompzwakte ('a') of bulbaire zwakte ('b') dominant is.

Het RoVEMP-onderzoek zou een routinematig neurofysiologisch onderzoek kunnen worden in patiënten met de verdenking van MG. Het is snel, non-invasief en heeft een goede diagnostische opbrengst. Met name in diagnostisch uitdagende subgroepen van geïsoleerd oculaire en seronegatieve MG-patiënten, bleek de RoVEMP een hogere diagnostische waarde te hebben dan de huidige neurofysiologische standaard (repetitieve zenuwstimulatie). Onze studie suggereert dat het RoVEMP-onderzoek ook van waarde zou kunnen zijn in LEMS- en CMS-patiënten. Toekomstige studies naar de RoVEMP-test zullen verder

inzicht geven in de diagnostische en prognostische waarde van deze test in MG-, LEMS- en CMS-patiënten.

Toekomstig onderzoek zou de heterogeniteit van ziekte-expressie in meer detail moeten analyseren, omdat de pathofysiologie van MG zo beter kan worden begrepen. Het beperken van de categorisering van MG-patiënten door antistof status, QMG-score, MGFA-klasse of ambigue termen zoals 'gegeneraliseerde ziekte' leidt de aandacht af van een van de meest intrigerende kwesties in het veld van MG en vele andere neuromusculaire ziekten: de verschillen binnen en tussen neuromusculaire stoornissen in patronen van spierzwakte. Een investering in het oplossen van de puzzel waarom systemische (auto-immuun) spierziekten sommige spieren meer aantasten dan andere, kan leiden tot subgroep-specifieke behandelingen, betere diagnostische strategieën en hogere succesansen in klinische studies.





APPENDICES

List of publications
Curriculum Vitae
Dankwoord

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CURRICULUM VITAE

Robert de Meel was born on 1 February 1992 in Voorburg. In 2010 he graduated cum laude from the Gymnasium at the Sint Maartenscollege in Voorburg. During the last two years of secondary school, he participated in the Pre-university Programme of Leiden University, studying Law & Business Administration. Robert studied Medicine and completed the Business Administration minor at Leiden University. During his Medicine studies, he was selected for the MD/PhD program and followed the Honours program and the Leiden Leadership Program. He participated in an Erasmus Project at the University of Geneva and visited the University Federico II in Naples and the Università Cattolica del Sacro Cuore in Rome for clinical internships.

After his Medicine studies, he worked as a PhD student at the department of Neurology of the LUMC under supervision of prof. dr. Jan Verschuuren and dr. Martijn Tannemaat. During his PhD, he also participated in clinical trials, was actively involved in teaching of students and guiding of students during their scientific internship. He was a member of the Selection Committee for Medicine, contributing both as assessor and developer of the conversational component of the selection procedure in the LUMC. Aside from his PhD, he completed a Master's program in Philosophy at the end of 2019 and worked as a visiting scholar at the University of California, Berkeley under the supervision of prof. Alva Noë.

Currently, he is a postdoc within the research group of prof. Amelia Evoli at the department of Neurology of the Università Cattolica del Sacro Cuore at Rome with the support of the Niels Stensen Fellowship grant. In this new project, he will extend the research he did during his PhD by investigating factors explaining the wide variation of disease expression in MG.

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Velen hebben bewust of onbewust bijgedragen aan de totstandkoming van dit proefschrift. Een terloopse discussie, ook niet gerelateerd aan MG, kan een zaadje planten dat leidt tot het opzetten van een onderzoeksproject. In het bijzonder wil ik een aantal personen bedanken die een duidelijke invloed hebben gehad in mijn PhD-periode. Ten eerste wil ik mijn promotor prof. dr. Jan Verschuuren en copromotor dr. Martijn Tannemaat danken voor hun vertrouwen, inspiratie en enthousiasme. Gesprekken over nieuwe onderzoeksprojecten waren mijn favoriete momenten en het uitvoeren van al onze ideeën zou meerdere PhD-trajecten in beslag hebben genomen. Jan en Martijn hebben me geleerd om kritisch te kijken naar de huidige stand van het myasthenie-onderzoek, om interessante problemen te identificeren en om na te denken over haalbaarheid en mogelijke impact van onderzoeksprojecten.

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