Faces and fatigue in myasthenia gravis

Ruiter, A.M.

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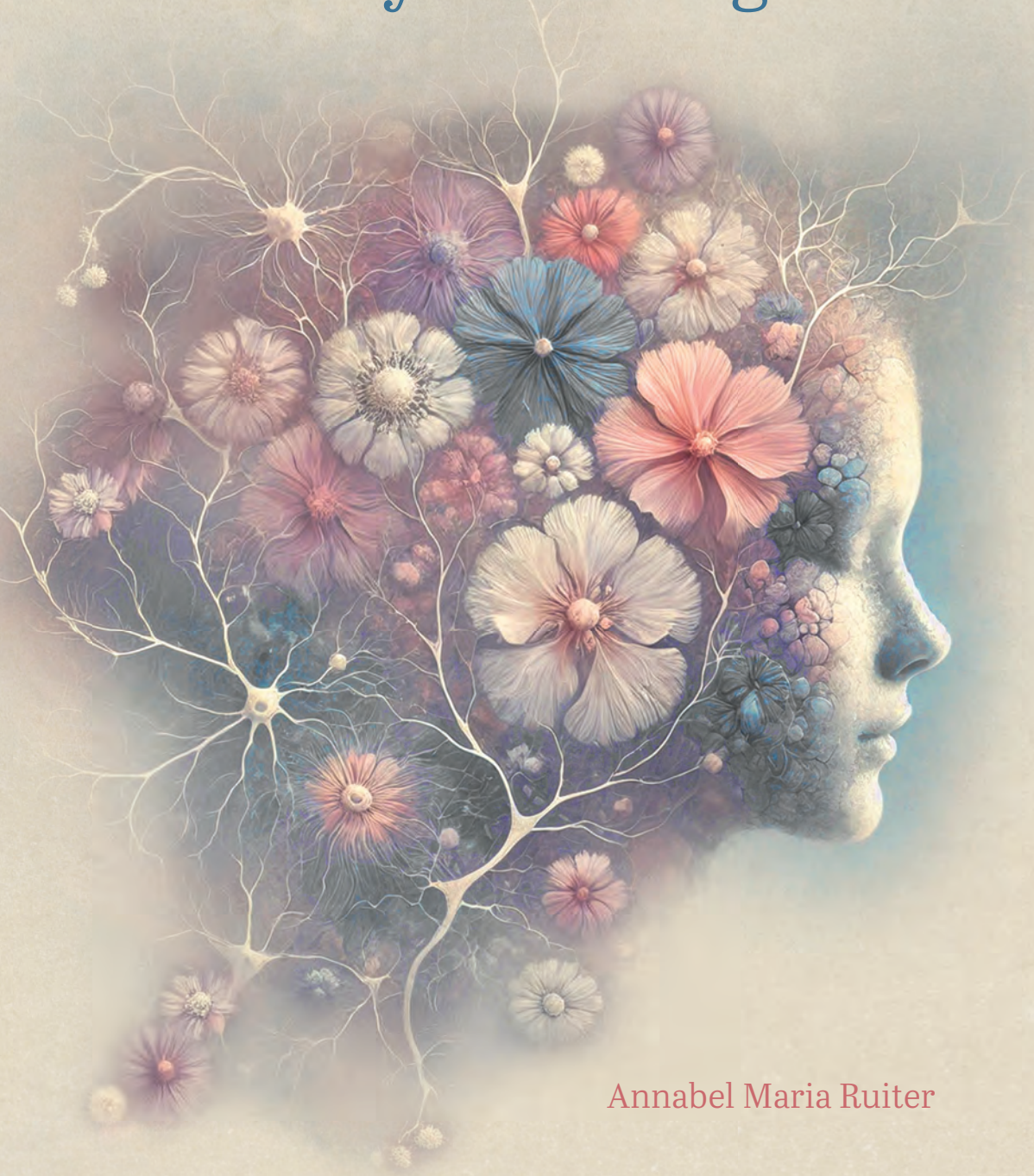

Annabel Maria Ruiter

Stellingen behorende bij het proefschrift getiteld

Faces and fatigue in myasthenia gravis

1. Met het oog op potentiële bijwerkingen en kosten is regelmatige fysieke inspanning voor de hand liggender dan immunoglobulines of plasmaferese als de behandeling van vermoeidheid. (dit proefschrift)

2. Vermoeidheid is voor myastheniepatiënten het meest beperkende symptoom van de ziekte, ongeacht type antistof. (dit proefschrift)

3. MG patiënten worden wel blij van een mobiele app om hun symptomen mee vast te leggen, maar je kunt het aan hun gezicht niet zien. (dit proefschrift)

4. Kunstmatige intelligentie zal het binnenkort mogelijk maken om symptomen van myasthenia gravis op afstand te monitoren, maar het is onwaarschijnlijk dat dit een volledig beeld van de ziekte-ernst oplevert: “there is more than meets the eye”. (dit proefschrift)

5. De prevalentie van cardiovasculaire comorbiditeit is onder patiënten met auto-immuun myasthenia gravis veel hoger dan in de algemene bevolking; derhalve moet er aandacht zijn voor het tijdig inzetten van corticosteroid-sparende behandeling.

6. Vermoeidheid is bij myasthenia gravis sterk gerelateerd aan ziekte-ernst, maar is niet myasthenia gravis specifiek.

7. Spiervermoeidheid en cognitieve vermoeidheid zijn twee verschillende dingen, maar ze zijn onlosmakelijk met elkaar verbonden.

8. Laaggradige ontsteking speelt vermoedelijk een belangrijke rol in het ontstaan of in stand houden van vermoeidheid.

9. Een proefschrift schrijven is als tennis: je grootste tegenstander ben je meestal zelf.

10. Hoewel het doen van onderzoek naar cognitieve vermoeidheid soms vermoeidheid kan opwekken, leidt dit gelukkig voornamelijk tot het ontstaan van juist méér cognitieve energie bij de promovendus.

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Annabel Maria Ruiter

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Faces and fatigue in myasthenia gravis

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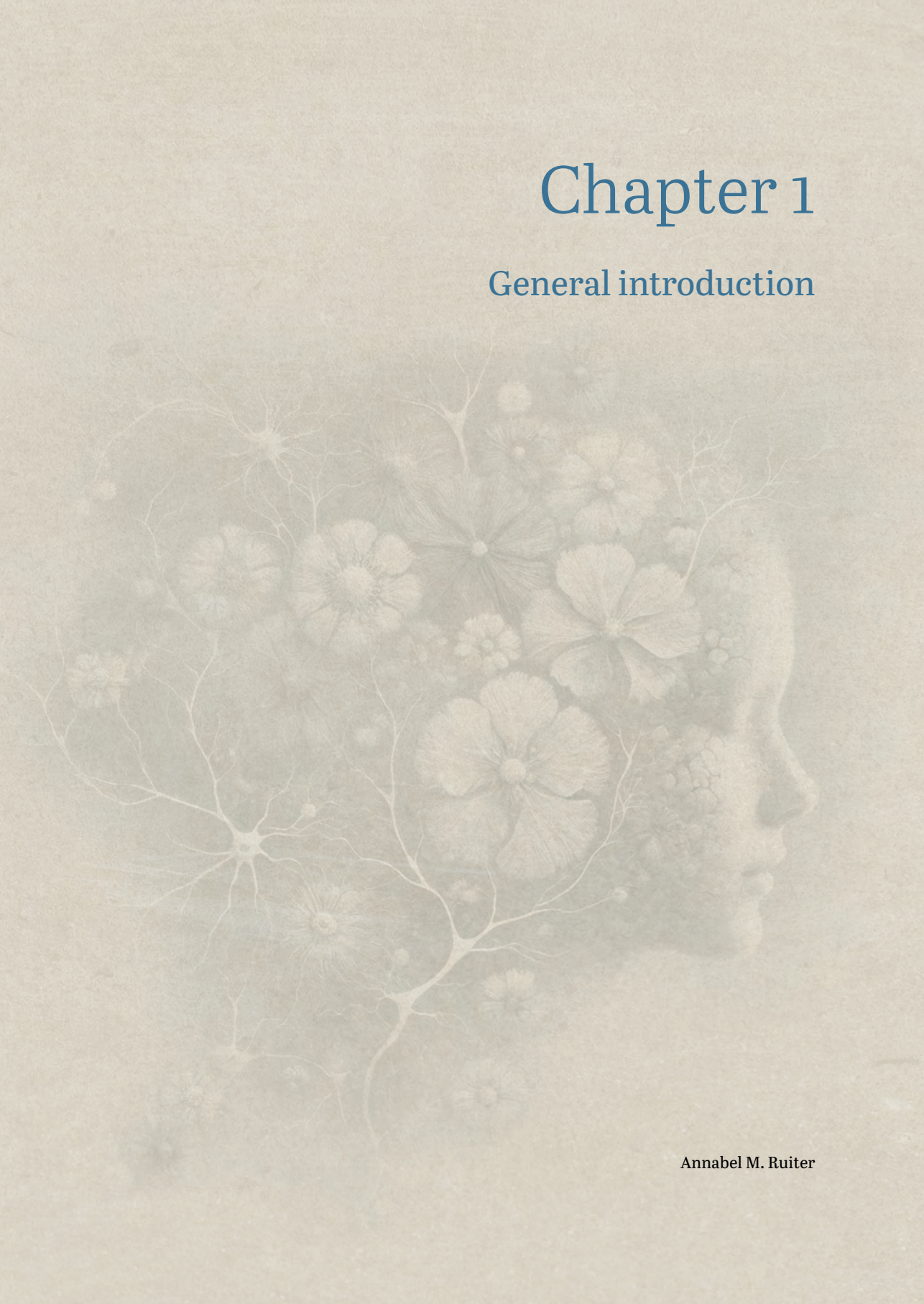
Table of Contents

Chapter 1

General introduction



Annabel M. Ruiter

General introduction and thesis outline

Introduction to myasthenia gravis: a clinical perspective

myasthenia gravis is one of the antibody-mediated autoimmune disorders of the neuromuscular junction (NMJ), the critical synapse where motor neurons communicate with skeletal muscle fibers. Dysfunction of this synaptic communication leads to the disease's hallmark symptoms: fluctuating and fatigable weakness of voluntary muscles. Although, the neuromuscular synapse occupies only 0.01% of the total surface area of a typical muscle fiber, the consequences of the autoimmune attack can be profound, affecting muscles throughout the body and significantly impacting a patient's quality of life.

The term myasthenia is derived from Greek, with *mys* meaning muscle and *astheneia* meaning weakness. *Gravis*, from Latin, means severe, reflecting the potentially life-threatening nature of the condition before the advent of modern therapies. The pathophysiology of myasthenia is rooted in an aberrant immune response. The body produces autoantibodies that target key proteins at the NMJ, disrupting neuromuscular transmission. Based on specific target antigens, the group of myasthenic disorders can be classified into several subtypes, each with distinct clinical and pathophysiological characteristics (table 1).

Table 1. Autoimmune myasthenic disorders.

Location	Name	Antibody target	Antibody frequency*
Presynaptic	LEMS	VGCC	85%
Synaptic	MG	agrin	<1%
Postsynaptic	MG	AChR	85%
Postsynaptic	MG	MuSK	1-10%
Postsynaptic	MG	Lrp4	~1%

Seronegative disorders are not included in table. *Frequencies are relative to either LEMS, or all forms of MG. Abbreviations: AChR = acetylcholine receptor; LEMS = Lambert Eaton myasthenic syndrome; Lrp4 = low-density lipoprotein receptor-related protein 4; MG = myasthenia gravis; MuSK = Muscle specific Kinase; VGCC = voltage-gated calcium channel

This thesis focused predominantly on the most common form of myasthenia gravis (MG), with antibodies against the acetylcholine receptor (AChR), and to a lesser extent on MG with antibodies against muscle-specific kinase (MuSK) and seronegative MG, where conventional antibodies are not detected.

Pathophysiological subtypes and their clinical correlates

approximately 85% of patients with generalized MG have detectable serum antibodies against the postsynaptic AChR[1, 2]. These polyclonal antibodies are predominantly of the IgG1 class. Some have IgG2 or IgG3 AChR antibodies, most often in combination with the presence of IgG1 antibodies[3]. IgM and IgA AChR antibodies have been detected in MG patients, but always in combination with IgG antibodies[3, 4].

These antibodies impair the neuromuscular transmission through three distinct mechanisms:

1. Steric hinderance of AChR functioning. By binding to the AChR, the antibodies directly block the binding of acetylcholine (ACh) or cause conformational changes in the AChR that hinder the activation of the AChR channel function.
2. Crosslinking of AChRs, leading to their internalization and degradation (antigenic modulation) and as a consequence a lower concentration of AChRs at the postsynaptic membrane.
3. Complement activation. The antibodies activate the complement cascade, causing destruction of the postsynaptic membrane and simplification of the junctional folds[5].

All three mechanism lead to a lower availability of well-functioning AChRs at the postsynaptic membrane.

The thymus gland plays a crucial role in the pathogenesis of AChR-MG; about 65% of patients exhibit thymic hyperplasia, and 10-15% have a thymoma (a tumour of the thymus)[5]. Thymectomy is therefore an important therapeutic consideration in AChR MG.

The second most common subtype, found in about 1-10% of MG patients (and up to 40% of those seronegative for AChR antibodies), is MuSK-MG[1, 2, 5]. MuSK is a receptor tyrosine kinase essential for the clustering and anchoring of AChRs at the NMJ during development and maintenance. The autoantibodies in MuSK-MG are typically of the IgG4 subclass[2]. Unlike IgG1, IgG4 does not activate complement but instead disrupts the interaction between MuSK and its co-activator, LRP4 (low-density lipoprotein receptor-related protein 4), which is critical for maintaining the structural integrity of the NMJ[1]. This leads to a reduction in AChR density and impaired synaptic function. Clinically, MuSK-MG often presents with more prominent bulbar (facial, pharyngeal, laryngeal) and respiratory muscle weakness, and muscle atrophy can be more common than in AChR-MG[2].

A small subset of patients has been found to have antibodies against LRP4[1]. These antibodies, similar to MuSK antibodies, interfere with the signaling that maintains AChR clustering. The clinical phenotype of LRP4-MG is generally milder than that of AChR- or MuSK-MG[6]. LRP4-Abs are more effective in inducing cellular FcR-mediated effector mechanisms, than

antibody-dependent complement activation. Their functional signature is likely to be different from AChR-specific antibodies[7].

In a few MG patients agrin antibodies have been found. Agrin antibodies were almost always detected in combination with antibodies against MuSK, LRP4, or AChRs, indicating a high incidence of autoantibodies against several neuromuscular proteins in the agrin-positive MG cases[8, 9].

A significant portion of the remaining patients are classified as 'seronegative' MG (SNMG), as they lack detectable antibodies using the currently available assays[10]. It is likely that other, yet-to-be-identified, antigens account for the remaining seronegative cases[1].

The clinical spectrum of myasthenia gravis

The clinical presentation of MG is highly variable, but its defining characteristic is muscle fatigability and weakness that worsens with repeated or sustained muscle activity and improves with rest[1]. This fluctuation is a direct consequence of the reduced neuromuscular transmission; with fewer functional AChRs, the synapse cannot sustain the high rate of signalling required for continuous muscle contraction[1].

The distribution of muscle weakness determines the clinical subtype. In approximately 15% of patients, weakness remains confined to the extraocular muscles, a condition known as ocular MG[1]. These patients typically present with fluctuating, often asymmetrical, ptosis (eyelid drooping) and diplopia (double vision). Symptoms typically worsen towards the end of the day, after prolonged reading, or in bright sunlight, but some patients experience profound weakness early after rising. In the majority of patients, the weakness becomes generalized within the first two years of onset, a condition known as generalized MG (gMG)[11, 12]. The pattern of weakness is characteristic, with a preference for certain muscle groups[12]. Bulbar muscles are frequently involved, leading to significant morbidity[12]. Facial weakness can result in a 'myasthenic snarl' on attempting to smile and an inability to close the eyes tightly. Weakness of the muscles of mastication causes difficulty in chewing. Pharyngeal and laryngeal muscle involvement leads to dysphagia (difficulty swallowing) and dysarthria, where speech becomes nasal, slurred, and quiet with prolonged talking[12]. Limb weakness is typically proximal and symmetrical, affecting the shoulders and hips more than the hands and feet[12]. Patients may struggle with activities such as combing their hair, climbing stairs, or rising from a chair. Neck extensor and flexor weakness is also common, which can lead to a 'dropped head syndrome', a disabling symptom where the patient is unable to hold their head up. The most feared complication is weakness of the respiratory muscles, primarily the diaphragm and intercostal muscles. This can lead to a myasthenic crisis, a

life-threatening state of respiratory failure requiring mechanical ventilation[1]. Crises are often precipitated by infections, surgery, or certain medications.

MG is a rare disease, with a prevalence of approximately 15-20 per 100,000 and an incidence of about 1-2 per million person-years, which translates to an estimated 3,000 patients in the Netherlands[2, 13, 14].

Clinical assessment and the need for objective measures

The diagnosis of MG relies on a combination of clinical history, physical examination, serological testing for autoantibodies, and electrophysiological studies like repetitive nerve stimulation (RNS) and single-fibre electromyography (SFEMG). Once diagnosed, monitoring disease severity and treatment response is crucial. Clinicians traditionally rely on standardized quantitative scoring systems, such as the Quantitative Myasthenia Gravis (QMG) score and the Myasthenia Gravis Activities of Daily Living (MG-ADL) profile.

The QMG score is a physician-administered scale that measures muscle strength across 13 items, including eye closure, chewing, swallowing, and limb strength[15]. While valuable, it has limitations: it is subject to inter-rater variability, can be time-consuming to perform, and requires a trained examiner. The MG-ADL is a patient-reported outcome measure assessing eight common symptoms, providing a subjective snapshot of the disease's impact[16]. While useful, it lacks the objective refinement to detect subtle changes. The assessment of facial and bulbar weakness, in particular, relies heavily on the subjective clinical judgement of the examiner. This subjectivity presents a challenge for both clinical trials and routine practice, creating a clear need for more objective, sensitive, and automated methods for quantifying weakness. The development of such tools, as explored in the second part of this thesis, could revolutionize how we monitor disease progression and treatment efficacy, particularly for key symptoms like facial weakness.

Disease burden beyond muscle weakness: the enigma of central fatigue

The primary therapeutic goal in MG is to manage muscle weakness and prevent life-threatening crises. This is achieved through a combination of symptomatic treatment with acetylcholinesterase inhibitors (e.g., pyridostigmine) and long-term immunomodulation with corticosteroids, steroid-sparing immunosuppressants (e.g., azathioprine, mycophenolate mofetil), and newer biologic agents targeting specific immune pathways.

Many patients, even those in pharmacological remission with minimal objective muscle weakness, continue to report a debilitating sense of fatigue. This fatigue is conceptually different from the peripheral, activity-induced muscle fatigability that defines MG[17, 18].

Patients describe it as a profound lack of energy, an overwhelming exhaustion, or a 'brain fog' that impairs their ability to sustain both physical and mental activities. This type of fatigue, termed central fatigue, is believed to originate from processes within the central nervous system, but the exact pathophysiology remains unknown[17, 19].

Central fatigue is a major contributor to the overall disease burden in MG, strongly associated with reduced quality of life, unemployment, and depressive symptoms[19]. Its prevalence is significantly higher in MG patients than in the general population, yet its pathophysiology remains poorly understood. Several hypotheses have been proposed, suggesting it may be a protective mechanism to prevent muscular overexertion or as a consequence of chronic systemic inflammation[17, 18].

Understanding the multifactorial nature of central fatigue is a critical frontier in MG research. It requires a comprehensive approach that considers not only disease severity but also psychosocial factors, sleep quality, physical activity levels, and potential biological underpinnings. This necessitates the use of large-scale patient cohorts and registries, such as the Dutch-Belgian myasthenia patient registry described in **Chapter 2**, to capture the full spectrum of the patient experience. Investigating the covariates, potential treatments, and underlying biomarkers of central fatigue, as undertaken in the first part of this thesis, is essential to address this profound unmet need and improve the care of individuals living with myasthenia gravis.

Thesis outlines

In **Chapter two** data is presented from the Dutch-Belgian myasthenia patient registry, 'het Nederlands-Belgisch Myasthenie Register'. It described the results of this patient-reported registry, complemented with physician-reported medical information. It provides an overview on the natural course of MG but also disease burden and comorbidity. Secondly, the accuracy of patient-reported data is discussed. This chapter gives a first glance on the magnitude of the impact of fatigue. **Chapter three** is a systematic review on central fatigue in MG. The aim of this study was to provide a complete overview of fatigue in MG. With a thorough literature search we attempted to summarize all aspects of fatigue. Apart from the strong relationship with disease severity, gender and depressive symptoms, it also provides evidence suggesting that fatigue may be treatable with psychological and/ or physical training programs. In **Chapter four** the aim was to study central fatigue in a large representative cohort of Dutch MG patients. For this study, an extensive online questionnaire was completed by 420 Dutch MG patients. In this cohort study we focused on several covariates with a potential influence on fatigue, including disease severity, gender, depressive symptoms, sleep quality and MG specific medication. Studies in other neuromuscular disorders found a beneficial effect of physical training on central fatigue[20, 21]. Therefore, we explored the relationship between the amount and degree of physical activities and central fatigue, as this has not been studied to this extent before in MG. Secondly, different coping styles and the influence on quality of life were assessed. In the final chapter of the first part of this thesis, **Chapter five**, the serum of 116 anti-AChR positive MG patients was examined in search of a potential circulating biomarker for fatigue. Due to the possible muscular origin of this potential biomarker, we studied 38 candidate serum biomarkers, including several myokines. In the discussion, a model for the pathophysiology of fatigue in myasthenia is proposed.

The second part of this thesis starts with **Chapter six**, in which we show averaged images of 52 patients with MG and 38 healthy controls while portraying several typical facial expressions. With these images we aimed to show that typical hallmarks of MG could be made visible and potentially detectable for automated quantification. **Chapter seven** is a cross-sectional study in which videos of 70 MG patients and 69 healthy controls were assessed by two different advanced computer techniques. For this study, all participants expressed an extensive set of standard facial expressions. The aims of this study were to 1) detect patterns of facial weakness, 2) to differentiate MG patients from controls without facial weakness and 3) to classify disease severity in MG patients. In this study two different techniques were used: facial recognition software and a 3D convolutional neural network (deep learning computer model).

Finally, **Chapter 8** provides a summary, general discussion and proposed future perspectives on central fatigue in MG and the use of advanced computer techniques for assessing facial weakness in MG.

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Part one

Fatigue in myasthenia gravis

Chapter 2

Accuracy of patient-reported data for an online patient registry of autoimmune Myasthenia Gravis and Lambert-Eaton myasthenic syndrome

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Abstract

Disorders of the neuromuscular junction (NMJ) entail a spectrum of rare diseases causing muscle fatigability and weakness, leading to life-long effects on quality of life. We established the Dutch – Belgian registry for NMJ disorders, based on a unique combination of patient- and physician-reported information. Information on natural course, disease burden, prevalence of complications and comorbidity is collected through patient-reported standardized questionnaires and verified using medical documentation. Currently, the registry contains information of 565 Myasthenia Gravis (MG) patients and 38 Lambert-Eaton myasthenic syndrome (LEMS) patients, constituting approximately 25% (MG) and 80% (LEMS) of patients in the Netherlands. This is a very large registry, with the highest participation rate per capita. In addition to confirming many disease characteristics previously described in the literature, this registry provides several novel insights. The reported rate of potentially corticosteroid-related comorbidity, including hypertension, heart disease, osteoporosis and type 2 diabetes was high, emphasizing the need to commence corticosteroid-sparing immune suppressive treatment as soon as possible. The reported rate of other auto-immune diseases is far higher than previously expected: 27% of MG and 38% of LEMS patients, and a surprisingly high number of MG patients (47%) is unaware of their antibody status. In conclusion, this registry provides a valuable collection of information regarding MG and LEMS disease course. Continuous collection of annual follow-up data will provide further longitudinal insights in disease burden, course and treatment effect.

Introduction

National and international patient databases and registries provide large-scale information on various diseases. They are essential for collecting data on the natural course of rare diseases[1-3]. The collection of detailed data on disease course, medication, comorbidity and family history, improves understanding of the disease by both physicians and patients and helps to identify potential novel therapeutic targets. We report the establishment and first results of the Dutch registry for disorders of the neuromuscular junction (NMJ), entailing a spectrum of rare disease entities, which can be due to an acquired autoimmune disease or a congenital genetic defect. The purpose of this registry is to study the epidemiology of patients with Myasthenia Gravis (MG), Lambert-Eaton myasthenic syndrome (LEMS) and congenital myasthenic syndromes (CMS) in the 'Netherlands and Belgium. Second, the registry will collect longitudinal data on the natural disease course and genetic, environmental and immunological factors that may affect disease course.

MG is an autoimmune disorder (AID) with antibodies against the NMJ resulting in various degrees of muscle fatigability and weakness. All striated muscles can be involved, although the extra-ocular muscles are most commonly affected, giving rise to a fluctuating ptosis and diplopia. Antibodies against the acetylcholine receptor (AChR) are present in over 80% of generalized MG patients. In the pure ocular form, AChR antibodies are detectable in nearly 50% of all patients. In approximately 4%, antibodies against the postsynaptic muscle-specific receptor tyrosine kinase (MuSK) are found and in 15% of patients with generalized disease, no serum antibodies are detected[4-6]. Approximately 15% of AChR MG patients has a thymoma, in which case the disease can be classified as a paraneoplastic syndrome[5]. With a prevalence of 1 to 2 per 10.000, MG is considered a rare disease[5].

LEMS is another autoimmune disease affecting the neuromuscular junction, with a prevalence of 2.5 per million[7]. Antibodies against the presynaptic voltage-gated calcium channels (VGCC) are present in 90% of patients[8]. In over 50% of patients, LEMS is a paraneoplastic phenomenon of small-cell lung carcinoma (SCLC)[9]. Clinically, LEMS leads to fluctuating symptoms with proximal muscle weakness, especially in the legs, and autonomic dysfunction[10]. Because of its fluctuating nature, patients are at risk of being misdiagnosed as having MG, although the typical ocular onset of symptoms is rarely seen in LEMS.

The CMS comprehend a spectrum of genetic disorders, usually with an autosomal recessive inheritance pattern. Clinical symptoms commonly develop shortly after birth, but patients with late-onset expression of symptoms have been documented[5]. Symptoms and disease

course depend on the target in the NMJ. There are no exact numbers on prevalence, but most reports only describe a few patients or families per mutation, classifying CMS as an ultrarare disease[11, 12].

Several MG patient registries have been described[1, 13-17]. For some, the data are entered by physicians[1, 14, 15, 17], while other registries are patient-driven[13]. The major advantages of a registry using forms that can be completed online by the patient are that it can quickly reach out to a large number of patients, requires no hospital visit, and is directly available for detailed analysis. However, to obtain high quality data these registries have to be curated and checked for inconsistencies and missing data, preferentially by using the original patient letters from the treating physicians. We describe the results of the Dutch registry for NMJ disorders with special emphasis on the quality of the patient-reported data and process of data curation.

Methods

Design

The Dutch registry for NMJ disorders is a collaborative initiative of 'Spierziektencentrum Nederland' (the Dutch patient support organization for neuromuscular diseases) and Leiden University Medical Center (LUMC). It was approved by the medical ethical committee of the LUMC and initiated in December 2015. It is an active longitudinal database which collects medical information obtained both from patients and treating physicians. The data is stored in a web-based data management system (Project Manager Internet Server; ProMISe) and managed by the LUMC. Privacy-sensitive data is encrypted through a third party (ZorgTTP). In 2017, the registry was renamed to the Dutch-Belgian registry to include patients from Belgium. For the purpose of this paper, we excluded the Belgian patients because of the currently small number of Belgian participants (n=10, 2%).

Data collection

Patients can register voluntarily by filling out a written informed consent form and a short information sheet, which includes a request to include contact details of their current treating physician. Both can be download from the registry website[18]. Information about registration is provided on the website, by treating physicians or the patient support organization.
After completion of the registration, participants receive a digital invitation for an online baseline questionnaire (table 1). A printed version is available upon request. After registration, a request is made to the treating physician for extensive available medical information including information on medical history, antibody status, electromyography (EMG) results and information on thymectomy and thymus pathology. After completion of

the baseline questionnaire, participants receive an invitation for a follow-up questionnaire to assess information concerning disease course and newly diagnosed conditions every year When a patient does not complete all items on the questionnaire, these items are classified as missing data.

Table 1. items of baseline (left) and follow-up (right) questionnaires.

A. Baseline questionnaire	B. Annual follow-up questionnaire
1a. Form of myasthenic syndrome; MG/ LEMS/ CMS	1. Clinical symptoms during the past year (multiple choice)
b. Age at first symptoms	
c. Age at diagnosis	2. Most limiting symptoms during the past year (multiple choice)
2. Symptoms previously misdiagnosed	
3. Auto-antibodies detected? AChR/ MuSK/ VGCC/ other/ none/ unknown	3a. New medical conditions diagnosed in the past year (multiple choice)
	b. Diagnosis of lung cancer‡
4. Clinical symptoms at disease onset (first six months) (multiple choice)	4. Thymectomy in the past year*
5. Clinical symptoms past three months (multiple choice)	5. Degree of independency in daily activities during the past year
6. Most limiting symptoms in the past three months (multiple choice)	6. Hospital admission due to disease in the past year
7. Symptoms when disease was at its worst (multiple choice)	7. Admission to intensive care due to disease in the past year
8a. Other medical conditions (multiple choice) b. Diagnosis of lung cancer‡	8. Intubated due to disease in the past year
9a. Thymectomy*	9. Received immune globulins for disease in the past year
b. Age of thymectomy	
10. Degree of independency in daily activities	10. Received plasma exchange therapy for disease in the past year
11. Hospital admission due to disease	11. Use of medication during the past year
12. Admission to intensive care due to disease	12. Side-effects of medication
13. Ever been intubated due to disease	13. New medical conditions diagnosed in family in the past year (multiple choice)
14. Ever received immune globulins for disease	14. Weight
15. Ever received plasma exchange therapy for disease	15. Smoking
16. Use of medication	
17. Side-effects of medication	
18. Family history (multiple choice)	
19. Ethnicity	
20. Weight & height	
21. Smoking	

*MG questionnaires only; ‡LEMS questionnaires only.

Statistics

All analyses were performed using SPSS Statistics 25. Group data are described by mean and standard deviation (±SD). We used unpaired T-tests or Mann-Whitney U tests for comparisons between two groups. Chi-square tests were applied for nominal data. Significance was accepted at $p<0.05$. Bonferroni correction for multiple testing was applied for subgroup analysis when necessary (3 subgroups $\alpha_{altered}$ 0.017; 5 subgroups $\alpha_{altered}$ 0.01).

Results

Patients characteristics and demographics

On September 20th 2019, the registry contained information on a total of 608 Dutch patients: 335 (55.1%) females and 266 males. Patient characteristics are detailed in table 2. Given the prevalence of 1-2 per 10.000, about 16-33% of all Dutch MG patients have been enrolled in the registry (17.366.356 inhabitants in the Netherlands on September 20th 2019[18]). Approximately 87% of all Dutch LEMS patients are registered in the database, when assuming a prevalence of 2.5 per million[7]. However, there are indications that the actual prevalence in the Netherlands is higher[19].

Two participants declined retrieval of medical information. We retrieved information on antibody status in 78%, medical history in 33% and whether thymectomy was performed from 45% of participants.

All participants received an invitation to complete the baseline questionnaire (table 1a). A total of 509 MG, 36 LEMS and 4 CMS patients completed the questionnaire (90%, 95% and 80% of all participants, respectively). The mean (±SD) disease duration at the time of completing the baseline questionnaire was 12.6 (±13.3) years. Participants who completed the baseline questionnaire received an invitation to complete a follow-up questionnaire (table 1b). A total of 334 MG, 26 LEMS and 1 CMS patient responded (59%, 68%, 20%, respectively). The mean (±SD) time between completing the baseline and follow-up questionnaire was 2.0 (±0.6) years. CMS patients were excluded from further analysis because of their small number (n=5).

Antibodies

Medical documentation on antibody status was available from 78% of all participants with MG (table 2). Of these, 80.4% were AChR positive, 3.6% MuSK and 15% seronegative. Antibody status was also queried in the baseline questionnaire (table 3 and 4). Forty-one percent of MG participants reported having AChR antibodies, 2.8% reported having MuSK antibodies and 8.9% reported being seronegative. Remarkably, almost half of the MG participants did not know their antibody status.

A documented antibody status was available from 87% of participants with LEMS (table 2). VGCC antibodies were present in 82%, 18% was seronegative. In the baseline questionnaire, 53% reported VGCC antibodies, 11% to be seronegative and 36% didn't know (table 4).

Table 2. Patients characteristics and demographics

Characteristic	**Statistic**	
Registered patients, n	608	
MG, n (%)	565 (92.9%)	
LEMS, n (%)	38 (6.3%)	
CMS, n (%)	5 (0.8%)	
Mean age in years		
MG, mean ± SD	63.2 ± 15.0	
LEMS, mean ± SD	62.7 ± 10.2	
CMS, median [IQR]	64.4 [44.5-67.1]	
Female gender, %	55.1%	
MG antibody status (medical documentation), n (%)	443 (78.4%)	
AChR	80.4%	
MuSK	3.6%	
LRP4	0.7%	
Seronegative	15.3%	
Missing data, n (%)	122 (21.6%)	
LEMS antibody status (medical documentation), n (%)	33 (86.8%)	
VGCC	81.8%	
Seronegative	18.2%	
Missing data, n (%)	5 (13.2%)	
Completed baseline questionnaire, n (% of registered patients)		
MG	509 (90.1%)	
LEMS	36 (94.7%)	
CMS	4 (80.0%)	
Completed follow-up questionnaire 1 year after registration, n (% of registered patients)	334 (59.1%)	
MG	26 (68.4%)	
LEMS	1 (20.0%)	
CMS		
Prevalence of registered patients per 10.000 Dutch inhabitants‡		
MG	0.33	
LEMS	0.02	
CMS	0.003	
Age at disease onset in years	**Female**	**Male**
MG, mean ± SD	41.7 ± 19.6	56.5 ± 13.2**
<50 years	62.1%	24.8%
>50 years	37.9%	75.2%
LEMS, mean ± SD	46.6 ± 13.6	53.2 ± 13.4
<50 years	60.0%	40.0%
>50 years	40.0%	60.0%
Age at diagnosis in years	**Female**	**Male**
MG, mean ± SD	44.3 ± 18.9	57.2 ± 13.2**
LEMS, mean ± SD	48.0 ± 13.6	56.6 ± 10.2*
Time between disease onset and diagnosis in years	**Female**	**Male**
MG, mean ± SD	2.3 ± 5.5	0.7 ± 1.5**
LEMS, mean ± SD	1.4 ± 1.5	2.6 ± 5.1

‡17.366.356 inhabitants on September 20th 2019; *p<0.05; **p<0.001

Age at disease onset and diagnosis

Female MG patients were significantly younger at disease onset compared to male MG patients (p<0.001). This holds true for both AChR MG patients (p<0.001) and patients with missing data on antibody status (p<0.001) (figure 1). The same distribution was found in LEMS, but this did not reach significance (p=0.16, table 2).

Table 3. MG baseline and follow-up questionnaires

MG characteristics	Statistic
Disease duration in years, mean ± SD	
Baseline questionnaire	12.5 ± 13.2
Follow-up questionnaire 1 year after registration	14.5 ± 12.3
Patient reported antibody status, %	
AChR	41.0%
MuSK	2.8%
AChR and MuSK	0.1%
Seronegative	8.9%
Unsure of antibody status	47.3%
Missing data, n (%)	*14 (2.8%)*
Initial symptoms in first 6 months of disease, %	
Pure ocular	14.3%
Oculobulbar	6.8%
Generalized	78.8%
Missing data, n (%)	*24 (4.7%)*
Symptoms when disease at worst, %	
Pure ocular	8.7%
Oculobulbar	5.9%
Generalized	84.9%
Missing data, n (%)	*34 (6.7%)*
Symptoms in 3 months prior to completing baseline questionnaire, %	
Pure ocular	9.9%
Oculobulbar	7.2%
Generalized	67.6%
No symptoms	14.5%
Missing data, n (%)	*9 (1.8%)*
Symptoms at follow-up 1 year after registration, %	
Pure ocular	8.4%
Oculobulbar	5.1%
Generalized	73.2%
No symptoms	13.3%
Missing data, n (%)	*2 (0.6%)*
Pure ocular disease course >2 years, % of total	5.0%
Pure ocular disease course >2 years, % of initial ocular	37.3%
Progression after pure ocular disease course >2 years, % of total	0.9%
Thymectomy, %	52.7%
Time in years between disease onset and thymectomy, mean ± SD	2.6 ± 5.3
Missing data, n (%)	*67 (13.2%)*
Hospitalization neurology ward due to MG ≥1 time, %	45.4%
Hospitalization ICU due to MG ≥1 time, %	39.5%
Ventilation necessary during ≥1 ICU admission, %	50.7%
Missing data, n (%)	*43 (8.4%)*
Concomitant AID in medical history, %	26.7%
Missing data, n (%)	*38 (7.5%)*

Age at time of diagnosis was significantly lower for female MG (p<0.001) and LEMS patients (p=0.042) compared to males. The mean (±SD) time from disease onset to diagnosis was significantly longer for females compared to male MG patients: 2.3 (±5.5) vs. 0.7 (±1.5) years (p<0.001), again the trend was similar in LEMS, but again this was not significant, possibly due to the low number of patients (n=16 and 22, respectively).

Table 4. LEMS baseline and follow-up questionnaires

LEMS characteristics	Statistic
Disease duration in years, mean ± SD	
Baseline questionnaire	10.7 ± 11.4
Follow-up questionnaire 1 year after registration	12.7 ± 12.6
Patient reported antibody status, %	
VGCC	52.8%
Seronegative	11.1%
Unsure of antibody status	36.1%
Missing data, n (%)	0
Initial symptoms in first 6 months of disease, %	
Pure ocular	2.8%
Oculobulbar	0%
Generalized	97.2%
Autonomic symptoms	84.8%
Missing data, n (%)	*3 (8.3%)*
Symptoms when disease at worst, %	
Pure ocular	0%
Oculobulbar	0%
Generalized	100%
Autonomic symptoms	87.5%
Missing data, n (%)	*4 (11.1%)*
Symptoms in 3 months prior to completing baseline questionnaire, %	
Pure ocular	2.8%
Oculobulbar	0%
Generalized	94.3%
Autonomic symptoms	88.2%
No symptoms (excl. autonomic symptoms)	2.8%
No symptoms (incl. autonomic symptoms)	0%
Missing data, n (%)	*2 (5.6%)*
Symptoms at follow-up 1 year after registration, %	
Pure ocular	0%
Oculobulbar	0%
Generalized	80.8%
Autonomic symptoms	72.0%
No symptoms (excl. autonomic symptoms)	19.2%
No symptoms (incl. autonomic symptoms)	8.0%
Missing data, n (%)	*1 (2.8%)*
Diagnosis of lung cancer, %	8.6%
Missing data, n (%)	*1 (2.8%)*
Hospitalization neurology ward due to LEMS ≥1 time, % (	22.2%
Hospitalization ICU due to LEMS ≥1 time, %	18.2%
Ventilation necessary during ≥1 ICU admission, %	33.3%
Missing data, n (%)	*3 (8.3%)*
Concomitant AID in medical history, %	38.2%
Missing data, n (%)	*2 (5.6%)*

Signs and symptoms

The mean (±SD) disease duration for MG patients at the time of completing the baseline questionnaire was 12.5 (±13.2) years; 85% had a disease duration longer than two years. In the baseline questionnaire, most MG patients (79%) reported generalized symptoms within the first six months of the disease course (table 3). Pure ocular symptoms were present in 14% and isolated oculobulbar symptoms in 6.8% within the first six months. When the disease was at its worst, 85% of all patients had generalized symptoms and 8.7% experienced only ocular symptoms. In the three months prior to completing the baseline questionnaire, 15% of the MG patients reported having no symptoms. Of all MG patients with a disease duration of two years or longer, 5.0% reported a pure ocular disease course without any bulbar or generalized weakness. This corresponds with 25 (37%) out of 67 initial ocular patients. At the first follow-up questionnaire, three (0.9%) MG patients reported progression of symptoms to bulbar or generalized weakness after an initial pure ocular disease course and a disease duration of more than years at the baseline questionnaire.

Only one LEMS patient had pure ocular symptoms during the first 6 months of the disease, all others (97%) had generalized symptoms. Autonomic symptoms, including constipation, sicca symptoms and erectile dysfunction are frequent among LEMS patients: 85% reported at least one autonomic symptom in the initial six months and 88% when the disease was at its worst. All patients reported having clinical symptoms in the three months prior to the baseline questionnaire. Only one patient reported autonomic symptoms without muscle weakness. At the first follow-up questionnaire, more than 80% of LEMS patients reported generalized symptoms during the previous year. Surprisingly, 19% (n=5) reported no clinical symptoms or only autonomic symptoms. Of these five LEMS patients without clinical symptoms, only one patient did not use any medication. All other patients used symptomatic medication (3,4-DAP and/or pyridostigmine); one patient used corticosteroids and one patient received plasma-exchange therapy in the previous year.

The most limiting symptoms during the past three months are displayed in figure 2a-c. The top three most limiting symptoms in the AChR MG group were fatigue (35%), leg weakness (15%) and diplopia (13%). For MuSK MG patients the most limiting symptom was fatigue (46%) followed by weakness of the hands (15%). Leg weakness (63%), fatigue (20%) and incontinence (5.7%) were the most limiting symptoms for LEMS patients.

Medical files with documentation of initial symptoms were available for 162 (29%) MG patients and 9 (24%) LEMS patients. According to documentation at the time, 41% of MG patients presented with pure ocular symptoms, 24% oculobulbar and 36% started with generalized symptoms. When comparing the initial symptoms in the medical file with the

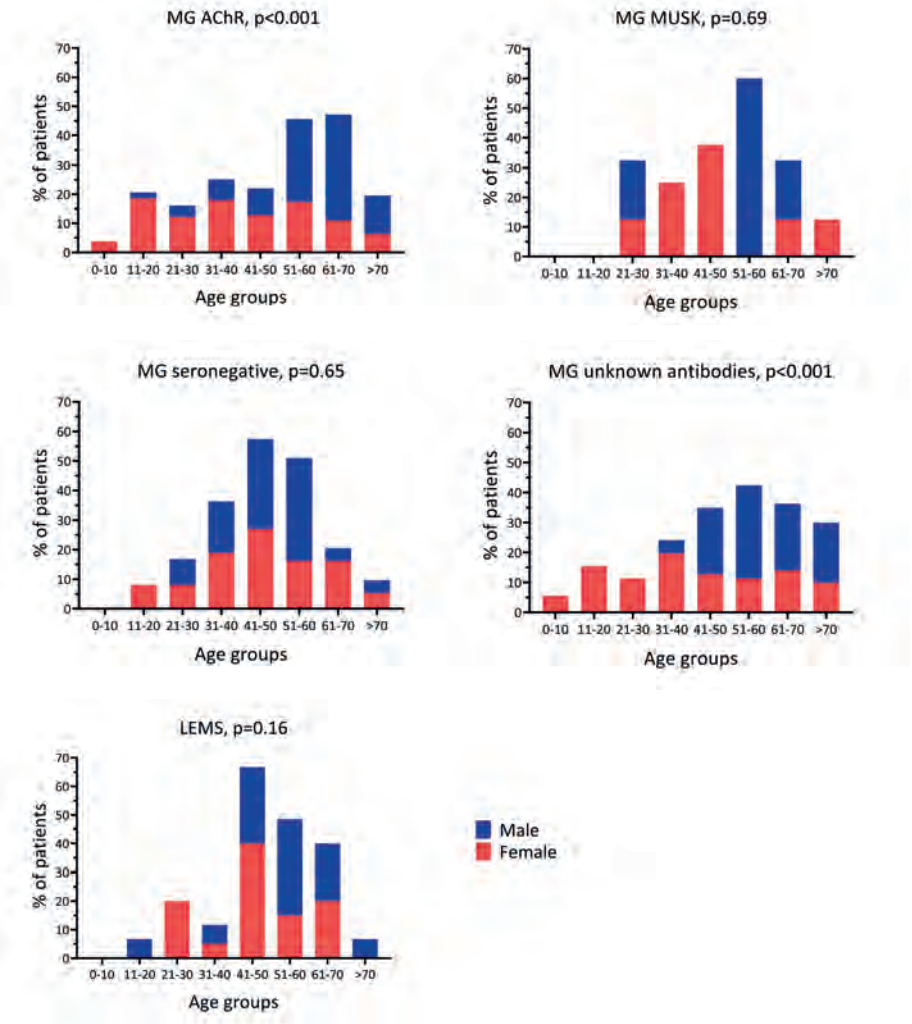

Figure 1. Age at disease onset, MG subgroups and LEMS. P-values represent female-male differences.

patient-reported questionnaire, the medical documentation reported fewer or different symptoms in 52% of MG patients. In LEMS, 100% started with generalized symptoms. This was similar to what the patients reported in the questionnaires.

Medication

The most frequently used medication is pyridostigmine (74%) in MG and 3,4-diaminopyridine (100%) in LEMS. Of MG patients, 8.4% did not use any disease specific medication in the three months prior to completing the baseline questionnaire and 22% used only

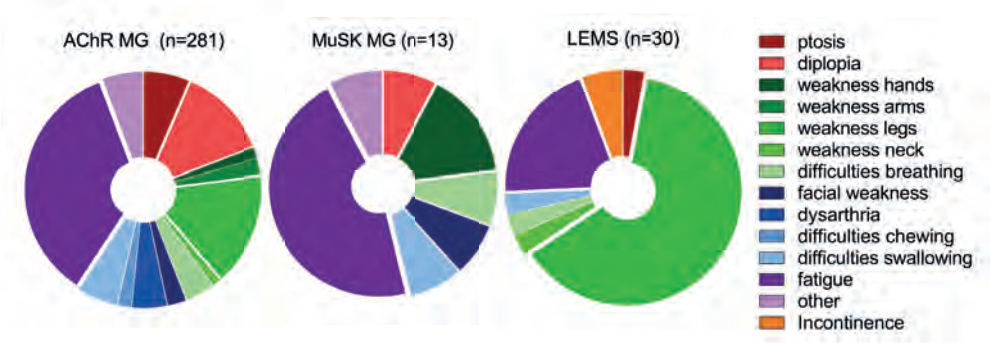

Figure 2. Most limiting symptom in past 3 months.

pyridostigmine (figure 3). A form of immunomodulating therapy was used by 69% of the MG patients; most frequently steroids (46%) and azathioprine (37%). Corticosteroids as a mono-immunosuppressive therapy, with or without pyridostigmine, were used by 13%; azathioprine was used by 11%. A combination of steroids and a second immunomodulating therapy was used by 31%, most commonly corticosteroids and azathioprine. Intravenous immunoglobulins were used by 15% of MG patients, compared to 6.5% of LEMS patients. Of LEMS patients, 71% used a combination of 3,4-diaminopyridine and pyridostigmine. Immunomodulating therapy was used by 49% of LEMS patients. The most frequently used therapies were corticosteroids (36%) and azathioprine (16%).

Comorbidity

Figure 4 shows the patient-reported comorbidities. A documented medical history provided by the treating physician was available for 198 (33%) patients in our cohort. Cardiovascular, potentially corticosteroid-related comorbidities were reported frequently: 47% of both MG and LEMS patients. The highest prevalences were for hypertension (MG 35%; LEMS 31%) and heart diseases (including heart failure and arrhythmias) (MG 18%; LEMS 25%). Type 2 diabetes was reported by 11% of MG patients and 2.8% of LEMS patients. Figure 5 shows the differences in these comorbidities between patients currently on corticosteroids (or during the past three months) compared to patients without corticosteroids for at least three months. Heart diseases were significantly more prevalent in patients currently using corticosteroids: p=0.039. There was a non-significant trend towards a higher prevalence of type 2 diabetes and osteoporosis in those using corticosteroids compared to patients without steroids (p=0.065; p=0.242). No differences were found in the prevalence of hypertension and psychiatric problems (p=0.553; p=0.684). A concomitant AID was reported by 27% of MG patients and 38% of LEMS patients (table 2 and 3, figure 4). The most prevalent second AID was thyroid disease (MG 8.5%; LEMS 14%) The prevalence for a second AID in

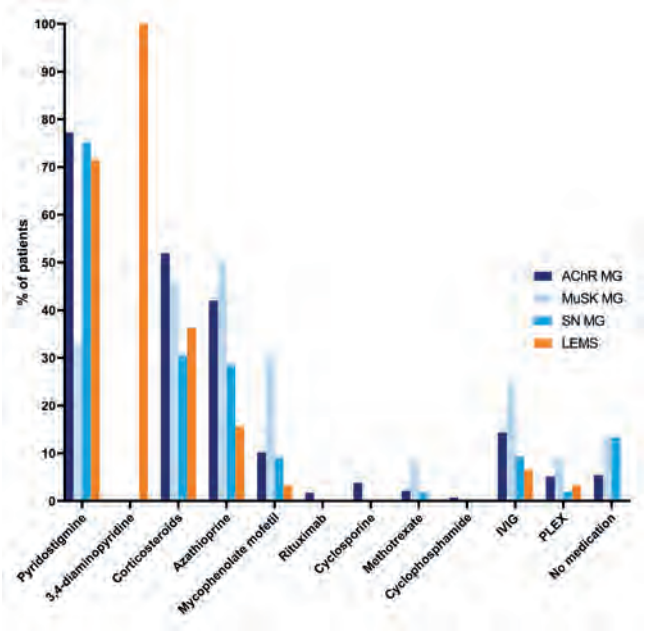

Figure 3. Patient-reported medication use. Abbreviations: PLEX: plasma-exchange therapy; IVIG: intravenous immunoglobulins.

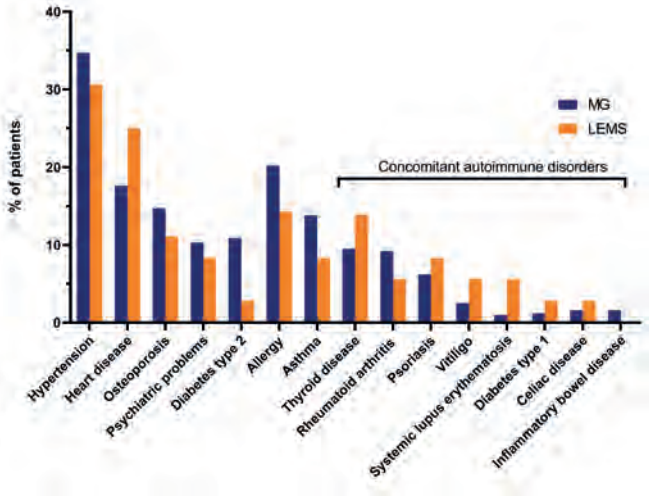

Figure 4. patient-reported comorbidity. Small-cell lung cancer and thymoma not displayed in figure.

the physician-provided medical history was 32% in MG and 47% in LEMS. Thyroid disease was the most prevalent AID in both. Lung cancer was reported by 3 (8.6%) of 35 LEMS patients, in accordance with information from their medical files. Other tumours that were reported in the medical documentation were as following: sigmoid adenoma, adenocarcinoma colon and pulmonary neuro-endocrine tumour. A thymectomy was performed in 233 (53%) out of a total of 442 MG patients, 67 patients (13%) did not complete this item of the questionnaire (table 3). Of the responding AChR MG patients 54% reported a previous thymectomy, 36% of the responding MuSK MG patients and 30% of the responding seronegative MG patients. The mean (±SD) time between disease onset and thymectomy was 2.6 (±5.3) years. Time from disease onset to thymectomy after publication of the large thymectomy trial of the MGTX study group in 2016[20] was 3.4 (±7.6) years, which was not significantly different than before the trial: 2.4 (±4.7) years (p=0.405). Unfortunately, a histological report of the thymus was available in only 26% of all thymectomies. Of all available reports, 60% noted the presence of a thymoma and 20% described normal findings.

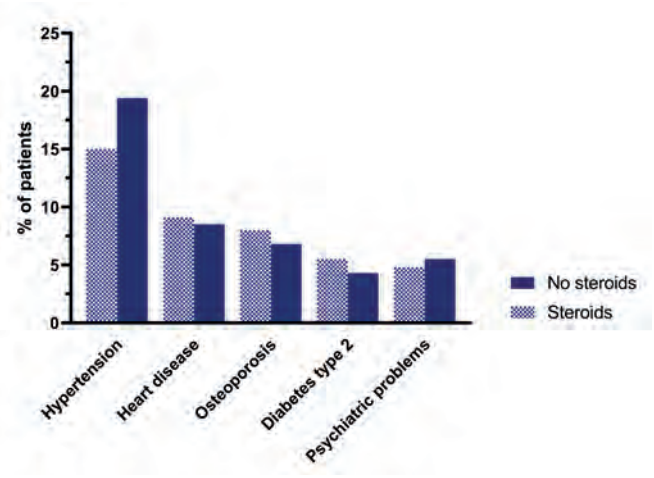

Figure 5. Prevalence of potential side-effects of corticosteroid use, all patients.

Hospitalization

More MG patients reported having been admitted to a Neurology ward at least once due to their disease compared to LEMS patients, 46% vs 22% (p=0.007, table 3 and 4). Within MG subtypes, seronegative patients reported significant less admissions to a Neurology ward (16%) compared to both AChR MG (52%, p<0.001) and MuSK MG (47%, p=0.012). There was no difference in admissions between AChR MG and MuSK MG (p=0.674).

Intensive Care Unit (ICU) admissions were also reported more frequently by MG patients, 40% compared to 18% in LEMS (p=0.015). There was no difference between the percentage of MG and LEMS patients who reported to have been ventilated during an ICU admission (51% vs. 33%, p=0.405). When focusing on MG subtypes, there was no differences between AChR MG and MuSK MG in ICU admissions (40% vs 62%, p=0.121) nor ventilation (22% vs 40%, p=0.184). Seronegative patients were significantly less frequently admitted to an ICU (26%) compared to MuSK MG (p=0.015) but not to AChR MG (p=0.052). Ventilation during an ICU admission was also less frequently reported by seronegative patients (8%) compared to AChR MG (p=0.038) and MuSK MG (p=0.009).

Discussion

Our database includes up to one third of the Dutch MG patients and the majority of the Dutch LEMS patients, which makes it a large and valuable dataset. The complementary combination of patient-reported and physician-reported information helps to study the quality of the patient-reported data and the process of data curation. This is important, especially since patient-reported data is prone to subjectivity and (recall) bias is inevitable[13].

Validity of data

Despite the fact that medical information obtained from treating physicians was often incomplete, we managed to gather a documented antibody status of 476 (79%) MG and LEMS patients. Interestingly, a large number of patients was unable to report their own antibody status, suggesting a need for patient education. The age-distribution in the subgroup with missing data on antibody status resembles the pattern of the AChR MG subgroup (figure 1), suggesting that the majority of these patients are AChR positive. We found no large discrepancies between the patient-reported and the physician-reported antibody status. Only 7 (2.3%) AChR MG patients reported being seronegative, 5 (1.6%) AChR MG patients reported having MuSK antibodies and 3 (5%) seronegative patients reported that AChR antibodies had been detected. In the group of LEMS patients, 2 (8%) of the VGCC positive patients reported being seronegative. In comparison, a recent publication on a large MG patient registry found that patients were unsure or gave no response on AChR or MuSK antibody status in 67% vs. 86%[13]. This percentage is higher than the patient-reported numbers in our cohort, but both studies suggest that patient education can be improved.

Medical documentation on the nature of first symptoms was available for 171 (28%) patients. In more than half of MG patients there was a discrepancy between the reported symptoms in the questionnaires and the symptoms recorded in the medical file at the time of diagnosis.

This may be explained in part by the fact that the questionnaire queried symptoms within the first six months of the disease, whereas medical reports at the time of diagnosis usually describe only the very first symptoms, covering a shorter time period. Alternatively, the results from the questionnaire may have been influenced by recall bias, or the description of symptoms in the initial report may have been incomplete. Ocular symptoms are the presenting symptom in 85% of all MG patients, but 20% will progress to generalized symptoms within one month and 48% within six months[21]. If pure ocular symptoms are present after two years of onset, the disease will most likely remain restricted to the eyelids and extra-ocular muscles. Indeed, we found only three MG patients (0.9%) who reported generalized symptoms in the follow-up questionnaire after having reported a pure ocular course and a disease duration of more than two years at the baseline questionnaire. However, this number is likely an underestimation as it does not include patients who developed generalized symptoms at least two years after disease onset, but before the first questionnaire.

We found a pure ocular MG (OMG) disease course in 5.0% of our entire MG cohort, which is lower than what could be expected based on previous research[21, 22]. One explanation is that our cohort may have an overrepresentation of more severe MG patients because of the voluntary nature of the registry. Secondly, the origin of some generalized symptoms is multi-interpretable, especially weakness in hands, arms or legs, and some patients might have attributed these symptoms to their MG. The distribution of weakness in our LEMS patients follows previously reported patterns[10]. Almost all patients started with limb weakness, predominantly legs, and all patients had generalized symptoms when the disease was at its worst.

The patient-reported prevalence of a second AID was comparable to the physician-reported prevalence. We found that in 22 (5.9%) patients, a second AID was reported by patient, but was not listed in their medical information provided by their physician. In the general population the prevalence of AIDs is estimated between 7.6-9.4% and many autoimmune diseases are associated with AID co-occurrence[23]. Surprisingly, both the patient- and physician-reported rate of a second AID in our cohort is much higher than previously stated in literature (approximately 15% for MG and 20% for LEMS)[24-26]. We did not collect original data on laboratory results or ancillary tests concerning this AID for verification. An unknown number of cases of reported thyroid disease might in fact not be autoimmune mediated but have another etiology. Importantly, a concomitant AID is a prognostic factor for exacerbations and emergency treatments, especially in combination with late-onset MG (>50 years of age)[27].

The reported prevalence of cardiovascular comorbidities (e.g., hypertension, type 2 diabetes) was very high. Additionally, a recent publication showed that 41% of MG patients was overweight and 21% had a body mass index (BMI) corresponding with obesity (BMI >30)[28]. Corticosteroids are likely to contribute to gain weight. Second, a decreased level of physical activity caused by their disease[28, 29] could also be a contributing factor in the development of cardiovascular diseases. In combination with reduced physical activity, these comorbidities pose a serious health risk for patients. Corticosteroid use should therefore be limited and steroid-sparing treatment should be commenced as soon as possible. In addition adequate patient education on the risk of long term corticosteroid use, prevention and treatment is important[30].

The prevalence of SCLC in LEMS has previously been estimated to be around 50%[9]. Given the short life expectancy of tumour-associated LEMS it is not surprising that the prevalence of LEMS patients with lung cancer was only 8.6% in our study.

Data on pathology after thymectomy is inconclusive. A pathology report was received in only 26% of patients who underwent a thymectomy. The major overrepresentation of thymomas (60%) is probably due to the fact that normal findings or hyperplasia are less likely to be reported in the medical file.

Treatment and disease impact

Thymectomy with prednisone compared to prednisone alone has been proven beneficial in non-thymomatous AChR MG patients with a disease duration up to five years[20, 31]. Thymectomy may also be considered in seronegative MG patients with generalized symptoms if immunosuppressive treatment fails. There is no indication for thymectomy in patients with MuSK, LRP4 or agrin antibodies[32, 33]. In our cohort, 53% of 443 MG patients reported a previous thymectomy with a mean (±SD) disease duration of 2.5 (±5.3) years. The majority of these patients were AChR positive or their antibody status was unknown. Four out of 11 MuSK positive patients (36%) reported a previous thymectomy, which were performed in 1983 and 1993, before MuSK antibodies were identified[34].

Almost 74% of MG patients used pyridostigmine in the three months prior to completing the baseline questionnaire, which should be part of the initial treatment of MG, according to the international consensus guideline[32].

Only 22% MG patients were on monotherapy pyridostigmine. Interestingly, 72% of these experienced generalized symptoms during the past three months, although international guideline advises to start immunomodulating therapy (steroids or non-steroids) in all

patients who have not met treatment goals after an adequate trial of pyridostigmine[32]. This finding underlines the importance of an advising role for specialized neuromuscular centers. When immunomodulating therapy is started, steroids and azathioprine are the most commonly prescribed medications, consistent with national and international treatment recommendations.

Half of MG patients and more than 20% of LEMS patients have been admitted to a Neurology ward at least once during their disease course. Publications on admissions related to MG are scarce. Recently, a Finnish study reported on hospital admissions over a 10 year time-period with MG as primary diagnosis or MG as additional diagnosis with a primary infection[35] and found that approximately 54% of the Finnish MG population has been hospitalized at least once because of their MG. This is comparable with the results reported here. Although MuSK patients would seem to be more at risk of an MG crisis because cranial and bulbar muscles are often more severely affected[5], we did not find a difference between MuSK and AChR MG in admissions to a Neurology ward, intensive care unit or the need for ventilation. The rate of ventilatory support in MuSK MG is similar to previous results: one third to half of the patients[36].

A remarkable finding is that the rate of admissions due to MG have not diminished, but increased over time, whereas hospital admission for other autoimmune diseases, such as multiple sclerosis (MS) has decreased[35, 37, 38], despite an increasing prevalence of MS. The increasing available treatment options for MS compared to MG have been suggested as a possible explanation for this difference[35]. Second, intravenous treatment for MG with immune globulins or plasmapheresis often require hospitalization.

Currently, the registry does not provide longitudinal data on admission rates in the Netherlands, but annual follow-up questionnaires should provide future insights.

Limitations

Limitations of our study include different forms of potential bias: first, selection bias is likely, due to the voluntary nature of the registry. This could lead to an overrepresentation of more severe patients. Second, patient-reported questionnaires with retrospective questions are susceptible to recall bias. For example, patients are asked about their symptoms within the first six months of disease, which was on average 12.6 (±13.3) years ago at the time of the questionnaire. Another limitation is that medical records received from treating physicians were often incomplete. We managed to gather medical information from treating physicians on antibody status in 79%, but medical history in only 33% and information on a previously performed thymectomy in 45% of patients. During their disease course, patients are often

treated in more than one hospital. This could be an explanation for the incomplete medical file provided by the treating physician.

Conclusion

This registry provides a valuable collection of information regarding the disease course of Dutch MG and LEMS patients. The large number of participants demonstrates the willingness of patients to be actively involved in scientific efforts to better understand their disease. The unique combination of patient- and physician-reported information enables validation and confirms the reliability of patient-reported data. The reported rate of other auto-immune diseases is far higher than previously expected: 27% of MG and 38% of LEMS patients. Second, the reported rate of other comorbidities, like hypertension and type 2 diabetes, is higher than in the general population. Current clinical practice could be improved by informing patients not only of their own antibody status but also to provide adequate information on the risk of long term steroid use, and prevention and treatment of cardiovascular comorbidities. The continuous efforts to obtain annual follow-up data through questionnaires will provide further longitudinal insights in disease burden, course and treatment effect.

Acknowledgements
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Chapter 3

Fatigue in patients with myasthenia gravis
A systematic review of the literature

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Abstract

Myasthenia Gravis (MG) is a chronic autoimmune disease affecting the neuromuscular junction. Although a hallmark of MG is muscle fatigability due to dysfunction of the neuromuscular junction (peripheral fatigue), a large number of MG patients also report symptoms of central fatigue, defined as an experienced lack of energy, physically and/or mentally. We systematically reviewed the literature on all aspects of central fatigue in MG. Results were categorized in 5 domains: prevalence, diagnosis, pathophysiology, treatment or impact.

The prevalence of patient-reported fatigue varies between 44 and 82%, which is significantly higher than in control subjects. Fatigue severity is usually assessed with standardized questionnaires, but the choice of questionnaire varies widely between studies. The pathophysiology of fatigue is unknown, but it is strongly associated with depressive symptoms, female gender and disease severity. Fatigue is also highly prevalent in ocular MG and patients in remission, suggesting a multifactorial origin. Fatigued MG patients have a lower quality of life. Pharmacological treatment of MG is associated with improvement of fatigue and promising results have been found with physical and psychological training programs.

Fatigue is a highly prevalent symptom of MG with a severe negative impact on quality of life. Physicians treating patients with MG should be aware of this symptom, as it may be treatable with physical or psychological training programs.

Introduction

Myasthenia Gravis (MG) is a chronic autoimmune disease (AID) with a prevalence of 1-2 per 10.000[1]. The clinical hallmark is fluctuating weakness of striated muscles with antibodies directly affecting the neuromuscular junction. In approximately 85% of patients, the initial presenting symptoms are asymmetric ptosis and/or diplopia. Approximately 80% of initially ocular MG patients will develop generalized MG within two years of disease onset[2].

Fatigue is a symptom of growing interest in many neuromuscular disorders[3-5]. A classification using two types of fatigue has been proposed in neuromuscular disorders: peripheral and central fatigue[5, 6]. Peripheral fatigue is a direct result of muscle fatigability due to disorders of the muscle or neuromuscular junction. This type of fatigue will not be discussed in this review. Central fatigue is an experienced lack of energy and feeling of tiredness not related to muscle weakness or pain, and interferes with mental or physical activities. Central fatigue is believed to be important in protecting muscles from further damage by down-regulating physical activities[5, 6]. In this review, we will use the word "fatigue" to describe central fatigue as defined above, unless specified otherwise.

In patients with MG, fatigue has been described as a common symptom[7-14], although its etiology is currently unknown. Due to its fluctuating and effort dependent nature, it may be difficult to distinguish from peripheral fatigue. However, even patients in remission frequently report feeling fatigued[9].

This review summarizes the literature on all aspects of fatigue in MG, with an emphasis on prevalence, diagnosis, pathophysiology, treatment and impact.

Methods

In this systematic review we adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines and a pre-set review protocol (appendix A; prisma-statement.org).

Data sources and search strategy

PubMed, Embase, Web of Science, Cochrane Library, Emcare were systematically searched for potentially relevant studies up to June 1th, 2019 (date of search), using appropriate keywords (appendix B). We reviewed the bibliographies of the studies identified for additional data.

Study selection and data extraction

Eligibility was initially assessed through screening of titles and abstracts by two independent reviewers (A.M.R. and M.R.T.), based on the following inclusion criteria: (1) the subject of the article was autoimmune Myasthenia Gravis with or without antibodies against AChR or muscle specific kinase (MuSK); (2) the article described fatigue, and not only peripheral fatigue; (3) original research; (4) use of a quantitative fatigue questionnaire; (5) article in English, Dutch or German. Articles that did not meet all 5 inclusion criteria were excluded. Missing abstract was no reason for exclusion. Both reviewers (A.M.R and M.R.T.) decided independently on inclusion or exclusion after reading the full text of the paper, and Cohen's kappa coefficient for interrater reliability (κ) was calculated. Discrepancies were discussed until mutual agreement was reached. Articles identified for inclusion in this systematic review were broadly categorized in five domains based on their main subject: prevalence, diagnosis, potential modifiers in pathophysiology, treatment or impact.

Risk of bias assessment

Risk of bias was assessed using the Joanna Briggs Institute (JBI) checklist for case series or case control studies (appendix C). Minimum requirements for inclusion were set as follows: the quality threshold was set at five times "yes" or more in total. At least one positive response was obtained for items 1-3, at least two positive responses for items 4-8. Studies were classified as medium quality studies (JBI 6), high quality (JBI 7-8) or very high quality (JBI 9-10). In case of a randomized controlled trial (RCT) the JBI checklist for RCTs was used (appendix C). Requirements for inclusion of RCTs were as follows: the quality threshold was set at eight times "yes" or more, at least 4x "yes" for items 1-6, 2x "yes" for items 7-10. RCTs were classified as medium quality studies (JBI 8-9), as high quality (JBI 10-11) or as very high quality (JBI 12-13).

Results

Search results

Our initial search produced 445 studies of which 45 were examined in further detail after screening of title and abstract. Twenty-one publications remained for final inclusion (Figure 1). Cohen's kappa coefficient for interrater reliability (κ) was 0.952.

The selected studies are detailed in table 1. A brief description of used questionnaires is provided in table 2.

Prevalence

In MG patients the prevalence of fatigue varies from 44 to 82% compared to 18-40% in control groups[7-9, 11, 13, 15, 16]. The prevalence increases with disease severity from 32% in patients in pharmacological remission to 72% in generalized MG (GMG)[9]. Chronic fatigue, defined as fatigue ≥6 months, occurred in 21 to 72%[8, 9].

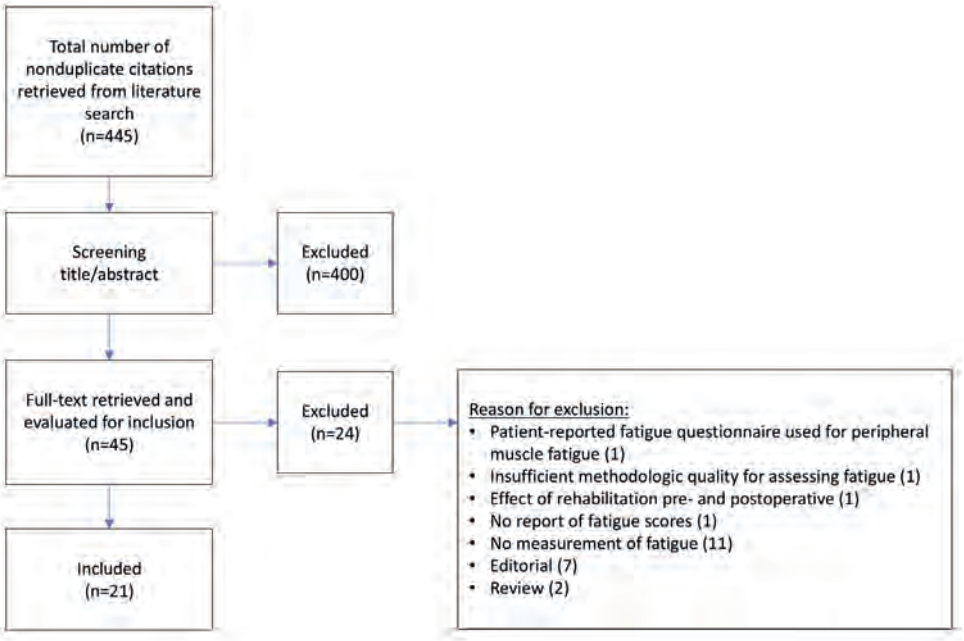

Figure 1. Flow diagram of selected studies

Table 1. Selected studies

Reference	MG, n	Fatigue intervention	Fatigue Questionnaire
Randomized controlled trials			
Andersen et al.[20]	125	Eculizumab	Neuro-QoL-F-SF
Rahbek et al.[28]	15	Physical training program (PRT vs AT)	MFIS
Case control studies			
Alekseeva et al.[7]	73	NA	FSS; FIS; VAS
Alekseeva et al.[15]	69	NA	FIS; FSS
Elsais et al.[8]	82	NA	CFQ
Elsais et al.[26]	10	NA	CFQ
Symonette et al.[19]	20	NA	MGFS; VAS
Jordan et al.[10]	32	NA	MGFS;
Westerberg et al.[16]	40	NA	FSS
Jordan et al.[11]	33	NA	FSMC; MGFS
Paul et al.[13]	28	Cognitive battery	MFI; FIS
Paul et al.[21]	28	Cognitive battery	MFI
Tascilar et al.[22]	19	NA	FSS
Case series			
Chen et al.[29]	29	plasmapheresis	ISS
Grohar-Murray et al.[23]	250	NA	FS; MGFS
Hoffman et al.[9]	200	NA	CFQ
Kittiwatanapaisan et al.[12]	67	NA	MGFS; CFQ
Tran et al.[14]	257	NA	Neuro-QoL-F-SF
Farrugia et al.[27]	10	Physical & psychological training program	FSS; MFIS; VAS
Kassardjian et al.[24]	8	NA	MFIS; VAS
Sabre et al.[25]	76	NA	FSS

Abbreviations: NA = not applicable; D= diagnosis; I = impact; Pa = pathophysiology; Pr = prevalence; T = treatment; a = JBI 6; b JBI 7–8; c JBI 9-10; b*JBI 10-11; c* JBI 12-13
6MWT = 6 minute walking test; AMT = arm movement test; ASP = autonomic symptom questionnaire; AT = aerobic training; B&B = box & block test; BDI = Beck depression inventory; CES-D = center for epidemiological studies depression scale short form; CFQ = Chalder fatigue questionnaire; CMDI = Chicago multiscale depression inventory; CPE=cardiopulmonary exercise; d2-R = attention and concentration test; EQ-5D = euroQoL 5 dimensions; ESS = Epworth sleepiness scale; FIS = fatigue impact scale; FS = fatigue survey; FSMC = fatigue scale for motor and cognitive function; FSS = fatigue severity scale; HADS = hospital anxiety and depression scale; HAM-A/D = Hamilton anxiety/ depression scales; IPQ = illness perception questionnaire; ISI = insomnia severity index; ISS = incapacity status scale; MDI = multiscale depression inventory; MFI = multicomponent fatigue index; MFIS = modified fatigue impact scale; MG-ADL = MG activities of daily living; MGC = MG composite score; MGFS = MG fatigue survey; MGII = MG impairment index; MGQ = MG questionnaire; MG-QoL15(r) = MG-quality of live 15 items (revised); MSLT = multiple sleep latency test; MWT = maintenance of wakefulness test; Neuro-QoL-F-SF = neuro-QoL-fatigue-short form; PASAT = paces auditory serial addition test; PRT = progressive resistance training; PSG = polysomnography; PSQI = Pittsburgh sleep quality index; QMG = quantitative MG; RNS = repetitive nerve stimulation; RWT = Regensburger verbal fluency test; SF-36 = 36-item short form health survey; SCT = stair climb test; STAI = State-Trait Anxiety Inventory; STS = 30-s sit to stand test, VAS visual analog scale.

Fatigue prevalence	Other assessments	Important exclusion criteria	Domain of subject	Quality
	QMG; MG-ADL; MG-QoL15		Pa; T	c*
	6MWT; B&B; MDI; MG-QoL15; SCT; STS		T	b*
69.9%	Data on sleep; BDI; ESS	Sleep-wake disorders	D; I; Pa; Pr; T	c
68.1%	BDI; ESS; STAI		Pa; Pr	b
44%	ASP; MGQ		Pa, Pr;	b
	CPE; MGQ; spirometry	Low CFQ score	Pa	b
	Dynamo; MG-ADL; RNS; QMG		Pa	c
	6MWT; AMT; CES-D; Dynamo; MG-ADL; MG-QoL15; PSQI; QMG; VAS	History of psychiatric illness	Pa; I	c
62-100%	MGC; MG-QoL15; RNS		Pa; Pr	b
57.5%	CES-D; D2-R; MG-ADL; MG-QoL15; QMG; PASAT; PSQI; RWT; timetap-task; VAS	History of psychiatric illness	Pa; Pr; I	b
82%	MDI	History of major psychiatric illness	Pr; I	b
	CMDI	History of psychiatric illness	I; Pa	b
	ESS; HAM-A/D; MG-QoL15; PSG; PSQI	History of psychiatric illness	I; Pa	c
	HADS; IPQ; SF-36		Pa; T	b
	NA		Pa; T	a
56.1%	HADS; ISI; MG-ADL; MG-QoL15; QMG		Pa; Pr; I	b
	CES-D; QMG		D; I; Pa	a
	EQ-5D; MGC; MG-ADL; MGII; MG-QoL15; QMG; SF-36		D; I; Pa; T	b
	HADS; MGC; MG-ADL; MG-QoL15r		T	b
	ESS; MSLT; MWT; PSG; MG-QoL15; QMG		I; Pa	b
NA	QMG; RNS		I; Pa	b

Diagnosis

All included studies used patient-reported questionnaires to assess fatigue; the choice of questionnaire varied widely (table 1). Three studies cross-validated fatigue questionnaires[7, 12, 14]. Fatigue Severity Scale (FSS) scores correlated significantly with all three subscales of the Fatigue Impact Scale (FIS) in both 73 MG patients and 230 controls[7]. Both scales had excellent internal consistencies. The Neuro-QoL-Fatigue short form (Neuro-QoL-Fatigue-SF) was validated by assessing responsiveness to treatment and by estimating the minimal important difference at group level and individual level[14]. At an individual level, sensitivity was 71% and specificity was 76%. The Myasthenia Gravis Fatigue Scale (MGFS) is a questionnaire developed especially for MG patients. Validation of its psychometric properties shows an excellent coefficient alpha scores for internal consistency (between .850 and .934). Its test-retest reliability is high (r = .872, p <.001)[12].

Potential modifiers in pathophysiology

Literature on the exact pathophysiology of fatigue is scarce and most publications report associations without providing evidence of causality. Publications in other neuromuscular disorders point towards a multidimensional cause[4, 17, 18]. In this review, we therefore classified studies reporting on factors associated with fatigue as potential modifiers in its pathophysiology.

The most frequently studied potential pathophysiological factor is MG disease severity[7-9, 12, 14, 16, 19, 20]. Fatigue scores showed a positive correlation with disease severity and in logistic regression analyses, higher disease severity is an independent associated factor for higher fatigue scores and more frequent fatigue[7-9, 14, 16]. There was no difference in fatigue scores between patients with bulbar or limb predominant MG[14]. In contrast, two smaller studies found no association between higher fatigue rates and increasing disease severity[12, 19]. One study (n=20) found no significant difference in fatigue scores between MG patients with and without a decrement upon repetitive nerve stimulation while patients with a typical decrementing response had significantly higher quantitative myasthenia gravis (QMG) scores[19]. Another study on 67 MG patients did not find a significant correlation between QMG scores and fatigue[12].

Higher scores on patient-reported depression scales significantly correlated with higher patient-reported fatigue rates[7, 10-12, 21]. Using the Hospital Anxiety and Depression Scale (HADS), the overall rate for depression among 200 MG patients was 20%[9]. Higher depression rates are found in GMG compared to OMG or patients in pharmacological remission: 25%, 15% and 10% respectively. In both univariate and multivariable logistic regression analyses, higher scores on the HADS were significantly associated with more fatigue. In one study

Table 2. Short overview of commonly used fatigue questionnaires and the domains queried

Questionnaire	Domains	No. items	Assessed by	Period
Chalder Fatigue Questionnaire (CFQ)[53]	Cognitive and physical.	11	Patient	Past week
Fatigue Impact Scale (FIS)[54]	Cognitive, physical and social.	40	Patient	Past week
Fatigue Scale for Motor and Cognitive function (FSMC)[55]	Cognitive and physical.	20	Patient	Unknown
Fatigue Severity Scale (FSS)[56]	Fatigue severity in different situations.	9	Patient	Past week
Fatigue Survey (FS)[23]	Demographic and medical information, fatigue characteristics, precipitating factors, interventions to reduce fatigue and rating of current functional state.	Unknown	Patient	Unknown
Incapacity Status Scale (ISS)[57]	Climbing, ambulation, transfers, bowel function, bladder function, bathing, dressing, grooming, feeding, vision, speech and hearing, medical problems, mood, thought disturbances, mentation, physical fatigue, and sexual function.	16	Patient	Unknown
Modified Fatigue Impact Scale (MFIS)	See Fatigue Impact Scale.			
Multicomponent Fatigue Index (MFI)[58]	Cognitive and physical. Developed to examine current levels and acute changes in fatigue.	15	Patient	Past 4 weeks
Myasthenia Gravis Fatigue Scale (MGFS)[23]	Perception, task avoidance, motor symptoms.	26	Patient	Unknown
Neuro-QoL-Fatigue short form (Neuro-QoL-F-SF)[44]	Physical, functional, social and mental activities.	8	Patient	Past week

(n=19), the difference in fatigue between patients and healthy controls disappeared when adjusting for depression and anxiety scores suggesting an association between depression and fatigue[22].

Most studies found that the female gender was independently associated with higher fatigue rates or higher fatigue-impact rates[7, 14, 23]. Only one study found no difference between genders[8].

Varying results have been published on sleep and fatigue[7-11, 24]. When comparing MG patients with and without fatigue, patients with fatigue scored significantly higher on the Epworth Sleepiness Scale (ESS)[7]. A significant correlation is found between the Pittsburgh Sleep Quality Index and fatigue and between sleep disturbances on the Autonomic Symptom

Profile and fatigue[8, 10, 11]. However, no significant association was found between fatigue and the Insomnia Severity Index[9]. In addition, a small study of eight MG patients with mild-to-moderate disease (QMG <20) found no correlation between patient-reported fatigue or results of the ESS and objective measurements of sleepiness using the Maintenance of Wakefulness Test and Multiple Sleep Latency Test[24].

Restriction of physical activity was the best predictor of fatigue severity after controlling for the effect of depression[12]. A cohort comparing 36 Estonian and 40 Swedish MG patients showed lower levels of physical activity and higher fatigue scores in Estonian patients, although QMG scores between the two groups did not differ[25].

The presence of both AChR and muscle-specific receptor tyrosine kinase (MuSK) antibodies was significantly associated with fatigue in a univariate analysis, but in a multivariate regression analysis only a strong association was found with the presence of MuSK antibodies[9]. In another study, the presence of AChR antibodies was significantly correlated with a decline in performance on one specific test of a cognitive assessment battery. However, this decline did not show a relationship with baseline fatigue scores[11].

The presence of a thymoma did not affect fatigue according to two small studies[10, 16]. One study found that 100% of patients in the thymoma subgroup (n=5) were fatigued, however fatigue was comparable among subgroups (early onset vs late onset vs thymoma)[16]. This study did not report on the current thymoma state. Another study concluded that a prior, successfully treated thymoma was not associated with fatigue[10] .

There was no difference in fatigue severity between 69 MG patients with and without autoimmune comorbidities[15]. In this study, 20% of the population had concomitant autoimmune thyroid disease and one patient had rheumatoid arthritis as a third AID.

In a small study of 10 MG patients assessing pulmonary function, stable MG patients with only mild ocular symptoms who scored high on fatigue appeared to have a minor degree of airway obstruction compared to healthy controls[26]. However, this study did not include a non-fatigued MG group.

One study reported on autonomic symptoms in 82 MG patients[8]. Higher fatigue scores were independently associated with higher scores on the Autonomic Symptom Profile (ASP), especially with the items sleep disturbance and sudomotor/ thermoregulation. A significant correlation between ASP and QMG scores indicated more autonomic symptoms with increasing disease severity.

Treatment

A ten-week physical and psychological training program in nine MG patients showed a small, temporary improvement mid-program and a non-significant trend towards improvement of fatigue at the end of the program, although scores had deteriorated again after three months[27]. Four out of nine patients did report that they had learned how to manage their MG symptoms and fatigue better, despite lacking a persistent improvement in fatigue. An eight-week exercise program comparing aerobic training (AT) versus progressive resistance training (PRT) showed a non-significant trend towards lower fatigue scores after the PRT program[28]. Remarkably, the AT group showed a non-significant trend towards higher fatigue scores. The QMG score in the latter study improved 1-2 points on average, not reaching statistical significance.

Reports on the effect of MG specific medication on fatigue are limited. Oral medication was assessed in only one study: fatigue scores did not differ between MG patients with and without oral corticosteroids[7]. Fatigue scores of a group of 95 MG patients who received a course of intravenous immunoglobulins (IVIG), plasma-exchange (PLEX) or prednisone improved significantly at their second visit 3-4 weeks later[14]. The improvement was similar for all three types of treatment. The effect of PLEX on fatigue was also confirmed in a different study[29], in which fatigue was the most reported symptom prior to treatment (n=29). One month after treatment, mean fatigue scores decreased and fatigue was no longer the most reported symptom, although this difference was not statistically significant.
In the phase 3 REGAIN study, the monoclonal antibody eculizumab was tested as a novel treatment for AChR positive generalized MG (GMG)[20]. Eculizumab was significantly correlated with greater improvement of fatigue than the placebo group; patients in the treatment group had a mean improvement from baseline of -16.3 points compared to -7.7 in the placebo group at week 26.

A majority of MG patients use self-care actions to manage fatigue (both physical and cognitive). In a large survey among 250 MG patients, mental interventions are reported by 76% of the respondents, 78% report applying physical interventions and 80% report resting or sleeping to manage fatigue[23]. However, no relationship or pattern was found between reported fatigue scores and self-care actions.

Impact

Fatigue is associated with more depressive symptoms (see pathophysiology)[7, 9-12, 21, 22]. None of the studies differentiated between depression as a cause or result of fatigue.

A patient-reported lower quality of life correlated with a higher prevalence and severity of fatigue[9, 11, 14, 24]. Improvement in fatigue scores correlated significantly with improvement in quality of life in both the treatment and placebo group in the REGAIN study[20]. In a study comparing 2 groups of MG patients from Estonia and Sweden, fatigue correlated strongly with self-perceived health status[25].

No relationship was found between cognitive performance and baseline fatigue[11, 21], but increased fatigue after completing a cognitive assessment is related with a poorer performance in MG patients[13, 21]. In contrast, controls did not have higher fatigue scores after completing the cognitive battery. In these studies there was no difference on the mood scale of the Multiscale Depression Inventory between MG patients and controls or depression was ruled out as an influencer by linear regression analysis. Outcomes of the Pittsburgh Sleep Quality Index were not of influence.

Discussion

The recent surge in studies on fatigue in MG indicates a growing awareness of the importance of the subject. The prevalence of fatigue in MG is comparable with fatigue in other neuromuscular diseases: a large study found fatigue rates between 61%-74% in facioscapulohumeral muscular dystrophy (FSHD), myotonic dystrophy and hereditary motor and sensory neuropathy type 1[3]. It has been hypothesized that muscle damage induces fatigue through a central nervous system mediated mechanism in order to temporarily down-regulate physical activities and protect muscles from further damage[5, 6, 30]. In patients with an ongoing neuromuscular disease, chronification of this feedback mechanism could induce continuous minimization of physical activity, although this remains speculative. This mechanism might also explain why patients with a neuromuscular disease appear to suffer from “central activation failure” during maximal voluntary muscle contraction[30]. Secondly, chronically decreased levels of physical activity have a negative effect on muscle mass and strength[31, 32], likely causing a vicious circle in patients with neuromuscular disorders. Indeed, restriction of physical activity is a good predictor for fatigue severity[12, 25]. Physical inactivity may lead to obesity. Due to a combination of inactivity and chronic steroid use, a large proportion of MG patients are overweight (BMI ≥25)[12]. In turn, obesity could contribute to fatigue, although a direct link between BMI and fatigue has never been established.

Patients with an AID are more likely to develop a second AID[34]. Having a second, possibly active, AID could contribute to the development of fatigue, but no difference was found between MG patients with and without concomitant autoimmune comorbidity[15]. Noteworthy

is the fact that autoimmune thyroid disease was the second most prevalent AID in all MG patients in this study, and fatigue is a main clinical feature of thyroid disease[35]. Furthermore, non-autoimmune comorbidity, including type 2 diabetes or non-allergic pulmonary disease, may also contribute to the development of fatigue[36, 37]. Active malignancy and subsequent treatment is likely to affect fatigue scores, as fatigue is a common symptom in patients with cancer and up to 80% experience fatigue during treatment[38].

Findings on the relation between fatigue and sleep disorders in MG vary widely. More objective sleep data is necessary to understand the role of sleep disorders in the context of fatigue.

People with a chronic disease have an increased risk for developing a depression[39]. The observed association between fatigue and depression in MG patients provides no evidence of causality, as depressive symptoms may be the cause or the result of fatigue[7, 9-12, 21, 22]. However, the observed correlation could also be caused by similarities between fatigue and depression questionnaires, e.g. the items 'Do you have problems starting things?' on the Chalder Fatigue Questionnaire and 'I could not get going' on the Center for Epidemiological Studies Depression Scale is likely to result in the same answer.

The reason for the observed higher prevalence of fatigue in women is not well understood. One hypothesis is that differences in hormonal and immunological homeostasis play a role[40].

For fatigue in general, evidence-based pharmacological treatment options are currently very sparse, but fatigue-specific programs with physical and psychological training have been proven to be effective in a range of neuromuscular diseases, including FSHD, Guillain-Barre syndrome, chronic inflammatory demyelinating polyneuropathy and myotonic dystrophy type 1[41-43]. So far, similar studies in MG have been far more limited in scope and size[27, 28]. This systematic review does not provide evidence that immunosuppressive treatment improves fatigue independently of improvement of neuromuscular weakness. Therefore, there is no evidence to support the initiation or intensification of immunosuppressive therapy to treat fatigue when there is no clear neuromuscular weakness.

All studies included used self-report questionnaires to assess fatigue with a known cut-off point for abnormal or clinically relevant fatigue. There are no other diagnostic tools for evaluating fatigue. Currently, there is no consensus on which questionnaire is most appropriate for assessing fatigue in MG.

Limitations of available studies

The most important limitation of included studies is the use of patient-reported questionnaires, not only for fatigue but also depression, sleep and autonomic symptoms. Self-assessments are inherently subject to external influences, mood and probably time of day, as daily fluctuations are a hallmark of MG. Secondly, the use of different questionnaires to measure fatigue and the absence of consensus on the most appropriate scale complicates comparing results of different studies. In a number of studies fatigue was a secondary outcome rather than its main subject, and conclusions on fatigue are therefore not always reported comprehensively. Another limitation is the absence of data on spontaneously reported fatigue rather than in response to questionnaires. Information on how often patients spontaneously report fatigue would provide some indication on its relevance and impact on quality of life.

Recommendations for future research

Future research on fatigue in MG would benefit from the use of one multidimensional assessment tool[45]. Despite its ability to detect change over time, the FSS provides little information on different aspects of fatigue. The multidimensional Checklist Individual Strength appears to be highly promising, as it detects change over time and is sensitive to treatment[45-47]. It consists of four subscales: subjective experience of fatigue, concentration, motivation and physical activity. The scale has been developed for chronic fatigue syndrome and although it has not been previously used in MG, it has been used in several other neuromuscular disorders[41, 48, 49], allowing a comparison between different diseases.

There is a surprising paucity of potential biomarkers for fatigue in MG. Previous studies in other diseases point towards inflammatory circulating proteins as potential causative factors, in particular cytokines such as IL-6 and TNFα[50]. In addition, thyroid function, antibody levels and complement activation could be of interest. Recently, circulating inflammatory proteins and micro-RNAs, in particular MMP-10, miR-150-5p and miR-21-5p, have emerged as a novel potential biomarkers for MG[51, 52].

Pilot data on an aerobic exercise program and cognitive behavioural therapy (CBT) in MG and their substantial benefits in other neuromuscular diseases warrant a large randomized clinical trial[41-43]. Exercise should be of sufficient duration and intensity and be performed under the supervision of an experienced physiotherapist. CBT modules for fatigue in other neuromuscular disorders are readily usable in MG patients[41, 43].

Conclusion

In this systematic review of the literature, we show that fatigue is a prevalent symptom in patients with MG. It is associated with higher disease severity, higher rates of depression and lower quality of life. Its pathophysiology is likely multifactorial in nature but fatigue may lead to a vicious cycle by reducing physical activity which in turn has negative effects on muscle strength and fatigue. Figure 2 represents a model summarizing the multifactorial nature of fatigue and the possible targets for therapy. There is no consensus on the most appropriate method to diagnose fatigue in MG. Encouraging results from physical and psychological training programs in MG and other neuromuscular diseases suggest that this vicious cycle can be reversed.

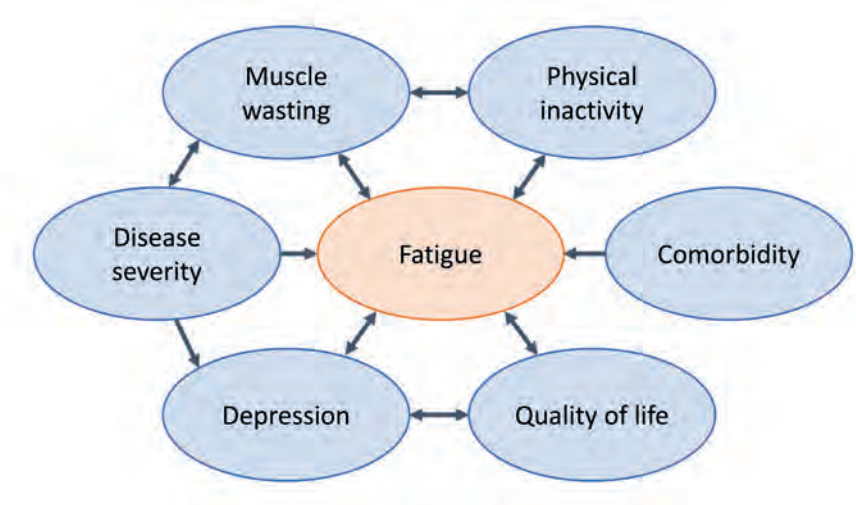

Figure 2. Model summarizing the multifactorial nature of fatigue and the possible targets for therapy

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Appendix A. PRISMA checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	NA
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Appendix 2
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6 Appendix 3
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	NA

Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	NA
Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	NA
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	NA
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	NA
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	8 Figure 3
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Table 1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Table 1
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	NA
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	NA
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	NA
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	NA
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	16-20
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	20
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	20-22
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	2

Appendix B. Full search strategy

Pubmed

((”Myasthenia Gravis”[Mesh] OR ”Myasthenia Gravis”[tw]) AND (”subjective fatigue”[tw] OR ”Mental Fatigue”[Mesh] OR ”Mental Fatigue”[tw] OR ”cognitive fatigue”[tw] OR ”central fatigue”[tw] OR ”perceived fatigue”[tw] OR (”Fatigue”[mesh] AND (”Cognition”[mesh] OR ”Perception”[mesh])) OR ”Fatigue”[Mesh] OR ((”Fatigue”[tw] OR Fatigue*[tw]) NOT (”muscle fatigue”[tw])))) NOT ((”Case Reports”[ptyp] OR ”case report”[ti]) NOT (”Review”[ptyp] OR ”review”[ti] OR ”Clinical Study”[ptyp])) NOT (”Animals”[mesh] NOT ”Humans”[mesh]))

Embase

((exp *”Myasthenia Gravis”/ OR ”Myasthenia Gravis”.ti,ab) AND (”subjective fatigue”.mp OR ”Mental Fatigue”.mp OR ”cognitive fatigue”.mp OR ”central fatigue”.mp OR ”perceived fatigue”.mp OR (”Fatigue”/ AND (exp ”Cognition”/ OR exp ”Perception”/)) OR *”Fatigue”/ OR ((”Fatigue”.mp OR Fatigue*.mp) NOT (”muscle fatigue”.mp))) NOT ((”Case Report”/ OR ”case report”.ti) NOT (exp ”Review”/ OR ”review”.ti OR ”Clinical Study”/ OR exp ”Clinical Trial”/)) AND exp ”Humans”/)

Web of science

(ts=(”Myasthenia Gravis” OR ”Myasthenia Gravis”) AND ts=(”subjective fatigue” OR ”Mental Fatigue” OR ”cognitive fatigue” OR ”central fatigue” OR ”perceived fatigue” OR (”Fatigue” AND (”Cognition” OR ”Perception”)) OR ((”Fatigue” OR Fatigue*) NOT (”muscle fatigue”))) NOT ti=(”case report” NOT (”Review” OR ”review” OR ”Clinical Study” OR ”Trial”)) NOT ti=(”veterinary” OR ”rabbit” OR ”rabbits” OR ”animal” OR ”animals” OR ”mouse” OR ”mice” OR ”rodent” OR ”rodents” OR ”rat” OR ”rats” OR ”pig” OR ”pigs” OR ”porcine” OR ”horse” OR ”horses” OR ”equine” OR ”cow” OR ”cows” OR ”bovine” OR ”goat” OR ”goats” OR ”sheep” OR ”ovine” OR ”canine” OR ”dog” OR ”dogs” OR ”feline” OR ”cat” OR ”cats”))

Cochrane

((”Myasthenia Gravis” OR ”Myasthenia Gravis”) AND (”subjective fatigue” OR ”Mental Fatigue” OR ”cognitive fatigue” OR ”central fatigue” OR ”perceived fatigue” OR (”Fatigue” AND (”Cognition” OR ”Perception”)) OR ((”Fatigue” OR Fatigue*) NOT (”muscle fatigue”))):ti,ab,kw

Emcare

((exp *”Myasthenia Gravis”/ OR ”Myasthenia Gravis”.ti,ab) AND (”subjective fatigue”.mp OR ”Mental Fatigue”.mp OR ”cognitive fatigue”.mp OR ”central fatigue”.mp OR ”perceived fatigue”.mp OR (”Fatigue”/ AND (exp ”Cognition”/ OR exp ”Perception”/)) OR *”Fatigue”/ OR ((”Fatigue”.mp OR Fatigue*.mp) NOT (”muscle fatigue”.mp))) NOT ((”Case Report”/ OR ”case report”.ti) NOT (exp ”Review”/ OR ”review”.ti OR ”Clinical Study”/ OR exp ”Clinical Trial”/)) AND exp ”Humans”/

Appendix C. Risk of bias assessment

Risk of bias was assessed using the Joanna Briggs Institute (JBI) checklist for case series or case control studies. Minimum requirements for inclusion were set as follows: the quality threshold was set at five times “yes” or more in total. At least one positive response was obtained for items 1-3, at least two positive responses for items 4-8. Studies were classified as medium quality studies (JBI 6), high quality (JBI 7-8) or very high quality (JBI 9-10). In case of a randomized controlled trial (RCT) the JBI checklist for RCTs was used (online appendix 3). Requirements for inclusion of RCTs were as follows: the quality threshold was set at eight times “yes” or more, at least 4x “yes” for items 1-6, 2x “yes” for items 7-10. RCTs were classified as medium quality studies (JBI 8-9), as high quality (JBI 10-11) or as very high quality (JBI 12-13).

JBI Critical Appraisal Checklist for case control studies

Reviewer _____ Date_____

Author _____ Year _____Record Number_____

	Yes	No	Unclear	Not applicable
1. Were the groups comparable other than the presence of disease in cases or the absence of disease in controls?	☐	☐	☐	☐
2. Were cases and controls matched appropriately?	☐	☐	☐	☐
3. Were the same criteria used for identification of cases and controls?	☐	☐	☐	☐
4. Was exposure measured in a standard, valid and reliable way?	☐	☐	☐	☐
5. Was exposure measured in the same way for cases and controls?	☐	☐	☐	☐
6. Were confounding factors identified?	☐	☐	☐	☐
7. Were strategies to deal with confounding factors stated?	☐	☐	☐	☐
8. Were outcomes assessed in a standard, valid and reliable way for cases and controls?	☐	☐	☐	☐
9. Was the exposure period of interest long enough to be meaningful?	☐	☐	☐	☐
10. Was appropriate statistical analysis used?	☐	☐	☐	☐

Overall appraisal: Include ☐ Exclude ☐ Seek further info ☐

Comments (Including reason for exclusion)

JBI Critical Appraisal Checklist for Case Series

Reviewer _____ Date_____

Author _____ Year _____Record Number_____

		Yes	No	Unclear	Not applicable
1	Were there clear criteria for inclusion in the case series?	☐	☐	☐	☐
2	Was the condition measured in a standard, reliable way for all participants included in the case series?	☐	☐	☐	☐
3	Were valid methods used for identification of the condition for all participants included in the case series?	☐	☐	☐	☐
4	Did the case series have consecutive inclusion of participants?	☐	☐	☐	☐
5	Did the case series have complete inclusion of participants?	☐	☐	☐	☐
6	Was there clear reporting of the demographics of the participants in the study?	☐	☐	☐	☐
7	Was there clear reporting of clinical information of the participants?	☐	☐	☐	☐
8	Were the outcomes or follow up results of cases clearly reported?	☐	☐	☐	☐
9	Was there clear reporting of the presenting site(s)/clinic(s) demographic information?	☐	☐	☐	☐
10	Was statistical analysis appropriate?	☐	☐	☐	☐

Overall appraisal: Include ☐ Exclude ☐ Seek further info ☐

Comments (Including reason for exclusion)

JBI Critical Appraisal Checklist for randomized Controlled trials

Reviewer _____ Date_____

Author _____ Year _____Record Number____

	Yes	No	Unclear	NA
1. Was true randomization used for assignment of participants to treatment groups?	☐	☐	☐	☐
2. Was allocation to treatment groups concealed?	☐	☐	☐	☐
3. Were treatment groups similar at the baseline?	☐	☐	☐	☐
4. Were participants blind to treatment assignment?	☐	☐	☐	☐
5. Were those delivering treatment blind to treatment assignment?	☐	☐	☐	☐
6. Were outcomes assessors blind to treatment assignment?	☐	☐	☐	☐
7. Were treatment groups treated identically other than the intervention of interest?	☐	☐	☐	☐
8. Was follow up complete and if not, were differences between groups in terms of their follow up adequately described and analyzed?	☐	☐	☐	☐
9. Were participants analyzed in the groups to which they were randomized?	☐	☐	☐	☐
10. Were outcomes measured in the same way for treatment groups?	☐	☐	☐	☐
11. Were outcomes measured in a reliable way?	☐	☐	☐	☐
12. Was appropriate statistical analysis used?	☐	☐	☐	☐
13. Was the trial design appropriate, and any deviations from the standard RCT design (individual randomization, parallel groups) accounted for in the conduct and analysis of the trial?	☐	☐	☐	☐

Overall appraisal: Include ☐ Exclude ☐ Seek further info ☐

Comments (Including reason for exclusion)

Chapter 4

Prevalence and associated factors of fatigue in autoimmune myasthenia gravis

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Abstract

Fatigue is usually defined as a subjective perception of lacking energy, mentally or physically, with a difficulty sustaining voluntary activities. It is a common symptom of many diseases and most likely has a multifactorial cause. In myasthenia gravis (MG), fatigue has a high prevalence and is correlated with female sex and disease severity. However, no large scale studies have been performed. Therefore, we aimed to evaluate fatigue in the Dutch participants (n=420) of the Dutch-Belgian Myasthenia Patient Registry using an online survey. Additional information was obtained on mood, sleep, coping, quality of life, disease severity, physical activities and medication. Severe fatigue was present in 62% with a mean score of 37.1 ± 13.2 points. Fatigue severity and prevalence increased significantly with disease severity. A positive correlation was found for female gender, BMI, disease severity and depressive symptoms. A negative correlation was found for strenuous physical activities and older age. The strong association with disease severity suggests that fatigue should be recognized as an element of the symptomatology of MG. The observed association between strenuous activity and fatigue and differences in coping style between fatigued and non-fatigued patients warrant future clinical trials on exercise and cognitive behavioral therapy.

Introduction

Abnormal or chronic fatigue is a symptom of many diseases, both somatic and psychiatric[1]. Without an underlying disease, it can be diagnosed as an entity on its own: chronic fatigue syndrome[2]. No exact definition of fatigue exists, but it is usually referred to as a subjective perception of lacking energy, mentally or physically, with a difficulty sustaining voluntary activities[3]. It is conceptually different from the muscle fatigability that is commonly observed in many neuromuscular disorders (NMDs). To approach these different types of fatigue, it has been proposed to distinguish peripheral and central fatigue[3, 4]. Peripheral fatigue is related to muscle fatigue or fatigability and is the direct result of disorders of muscle, neuromuscular junction or nerve. Central fatigue is a perceived subjective lack of energy which can be both physical or mental but is not directly related to muscle weakness or pain. This type of fatigue is also frequently referred to as cognitive or mental fatigue. It has been hypothesized that central fatigue is a physiological mechanism arising in the central nervous system with the aim of downregulating physical activities to protect the body from (further) damage[3, 5]. In this paper, we will use the word 'fatigue' to describe central fatigue as defined above, unless specified otherwise.

Myasthenia Gravis (MG) is a chronic autoimmune disease with antibodies directed against the neuromuscular junction. The clinical hallmark of MG is fluctuating muscle weakness with an ocular, oculobulbar or generalized distribution[6]. Although MG is characterized by fluctuating skeletal muscle fatigue, central fatigue is a prevalent patient-reported symptom in Myasthenia Gravis (MG)[7-11]. Concomitant depressive disorders are frequent and it has a negative impact on quality of life in several studies[7-10, 12-16]. Recently, a number of studies have addressed the problem of fatigue in MG, stressing the need for a better understanding of the underlying pathophysiology[17]. The prevalence of fatigue ranges between 44 and 82%[7-11], with the lower limit containing only MG patients with mild to moderate disease (Myasthenia Gravis Foundation of America (MGFA) scale 0-II)[8]. Disease severity is strongly correlated with the prevalence and degree of fatigue. However, the high prevalence of fatigue in ocular MG or patients in pharmacological remission suggests that disease activity is not the only relevant factor[7-9, 16, 18, 19].

In this study, we aimed to address fatigue in a representative cohort of the Dutch MG population, by studying fatigue and its relationship with disease severity, quality of life, sleep disturbances and mood. Additionally, we aimed to assess applied coping strategies, which have not been previously studied in fatigued MG patients. At last, we aimed to explore the influence of frequently used medication, comorbidity, body mass index (BMI) and physical activity.

Material and Methods

Subjects

All MG patients from the Dutch – Belgian Myasthenia Patient Registry[20] were invited to participate in this study. Participation in the patient registry is voluntary, and all patients with a myasthenic syndrome can sign up. At the start of the current study, approximately 18-30% of Dutch MG patients were enrolled in the registry. Only Dutch MG patients were included because of the underrepresentation of Belgian patients in the registry at the time of this study (n=8). The study protocol was reviewed by the medical ethical committee of the Leiden University Medical Center.

Procedures

A personal link with a study invitation and an information letter were sent out by email. Digital informed consent was obtained before entering the study. Participants were asked to fill out a set of digital questionnaires, which they could complete at home. Printed questionnaires were available upon request. A reminder was sent in case of no response within 2 weeks; a second reminder was send 4 weeks after the initial invitation. Participants completed self-report questionnaires on fatigue, depression, sleep, coping, quality of life and disease severity. In addition, participants were asked which medication they used now or during the past three months and to provide information on weight, height and level of physical activity. Physical activities were divided in moderate (walking, cycling of swimming at a regular pace, etc.) and strenuous activities (running, tennis, cycling at an increased pace, etc.). Data provided by the patient registry contained information on antibody status, disease duration and autoimmune comorbidity. Data on non-autoimmune comorbidity in the registry was not collected systematically.

Questionnaires

Checklist Individual Strength (CIS-f)[21]. Fatigue severity was measured with the fatigue subscale of the CIS (appendix A). This is an 8-item self-assessment on experienced fatigue during the past two weeks and is scored on a 7-point Likert scale. The range is between 8-56 points with ≥35 indicating severe or clinically relevant fatigue. After a review of different fatigue questionnaires[22], this fatigue questionnaire was selected based on its use in previous research in other NMD's, including clinical trials[23-25] and its focus on central fatigue. The CIS has been validated in the general population, chronic fatigue syndrome, rheumatoid arthritis, cancer survivors and multiple sclerosis[26-28]. In contrast, several other scales include items that are likely to be influenced by peripheral fatigue. E.g. 'Do you make slips of the tongue when speaking?' (Chalder Fatigue Questionnaire), 'My muscles have felt weak' ((Modified) Fatigue Impact Scale).

Hospital Anxiety and Depression Scale (HADS)[29]. The HADS is a self-assessment consisting of an anxiety and depression subscale both containing seven items regarding symptoms over the past week. The answers are scored on a 4-point Likert scale. The maximum score for each subscale is 21 points; a cut-off score of ≥8 indicates a possible depression or anxiety disorder. This questionnaire does not contain items addressing symptoms of mood disorders which are also likely to be present in somatic diseases (e.g. questions on fatigue or insomnia).
Pittsburgh Sleep Quality Index (PSQI)[30]. Quality of sleep during the past month was assessed with the PSQI. The scale consists of 18 items with a maximum score of 21. A score > 5 indicates the presence of sleep disturbances.

The Utrecht Coping List (UCL)[31]. The UCL is a 47-item questionnaire that measures seven strategies of coping. An explanation of different strategies is provided in the UCL manual (appendix table B.1). Items are scored on 4-point Likert scale. Interpretation of results depends on gender and age. Reference ranges of representative samples are available in the UCL manual (for used reference group, see appendix B.2).

MG Quality of Life 15-items (MG-QoL-15)[32]. Health-related quality of life was measured with the MG-QoL15, an MG specific instrument. Questions cover the past couple of weeks. The scale contains 15-items on a 5-points Likert scale with a score ranging from 0 to 60. Higher scores indicate lower quality of life.

MG-Activities of Daily Living (MG-ADL)[33]. The MG-ADL is an eight item survey of symptom severity for estimating disease severity over the past week. Items are scored on a 4-point Likert scale and include questions on ocular, oropharyngeal, respiratory and motor function. The total score ranges from 0 to 24, with higher scores indicating more symptoms. For further analysis, subgroups were defined as following: no symptoms (MG-ADL of 0 points), ocular MG (positive scores on item(s) 7 and/or 8, no points on other items), oculobulbar MG (positive scores on item(s) 1, 2 and/or 3, no scores on item 5 or 6), generalized MG (positive scores on item(s) 5 and/or 6).

Statistical analysis

For the descriptive analyses, mean and standard deviation (SD) of the variables were calculated. T-test, or Chi-square tests were used for comparison between subgroups. A multivariate linear regression analysis is used for independent association of variables with severe fatigue. The unstandardized beta (B) represents the slope of the line between the variable and fatigue: for every one unit increase in the variable, the fatigue score changes with B. Testing was performed with IBM SPSS Statistics software (version 25.0).

Results

Clinical characteristics

Out of 558 invited patients, 420 (75.3%) completed the survey. The mean age was 62.4 ± 13.9 years. Disease duration was 12.2 ± 11.2 years (table 1). Female patients were significantly younger (p<0.001) and had a longer disease duration (p<0.001). Medical information on antibody status was available in 81% of patients. Antibodies against the acetylcholine receptor (AChR) were present in 80% of the study population, muscle specific kinase (MuSK) antibodies in 4% and 16% was seronegative. Most patients (86%) used medication. Of those, 21% was on pyridostigmine monotherapy, 41% used corticosteroids and 25% a combination of corticosteroids with a non-steroid immunosuppressant. A second autoimmune disorder was reported by 24% of patients. Thyroid disease (9%) and rheumatoid arthritis (8%) were most frequently reported.

Table 1. Clinical characteristics

	Overall	Male	Female	Sig.
Total number of MG patients, n (%)	420	197 (46.9)	223 (53.1)	
Age, mean ± SD	62.4 ± 13.9	66.6 ± 10.5	58.7 ± 15.4	<0.001
Disease duration in years, mean ± SD	12.2 ± 11.2	9.8 ± 8.9	14.3 ± 12.5	<0.001
Antibodies (missing 19%)				
· AChR	80.0%			
· MuSK	4.1%			
· LRP4	0.3%			
· Seronegative	15.6%			
Thymectomy, n (%) (missing 4.8%)	149 (37.3)			
Body Mass Index (BMI), mean ± SD	27.1 ± 5.0	27.8 ± 4.5	26.5 ± 5.3	0.007

Questionnaires. Table 2 shows the results of all questionnaires. The mean fatigue score (CIS-f) was 37.1 ± 13.2 points. Severe fatigue (CIS-f ≥35) was present in 62%, with a higher prevalence in women compared to men: 74% vs. 49% (p<0.001). The mean MG-ADL score for disease severity was 4.0 ± 3.5 points. Most patients (66%) reported generalized symptoms during the past week. Only 15% reported no clinical symptoms. The prevalence and mean score of fatigue increased with disease severity (figure 1). Severe fatigue was present in 22% of patients with no clinical symptoms compared to 76% of patients with generalized symptoms. In patients without clinical symptoms, the mean fatigue score was 22.2 ± 13.7, compared to 41.7 ± 10.3 in patients with generalized symptoms (p<0.001). Women had significantly higher fatigue scores compared to men within all MG-ADL subgroups except oculobulbar symptoms: no clinical symptoms 28.8 ± 15.2 vs. 19.3 ± 12.1 (p=0.012); only ocular symptoms 37.6 ± 12.6 vs. 28.7 ± 11.5 (p=0.015); generalized symptoms 43.7 ± 9.3 vs. 38.7 ± 11.0 (p<0.001).

Table 2. Results of questionnaires

Questionnaire (mean ± SD)	Overall	Male	Female	Sig.
· MG-Activities of Daily Life (MG-ADL)	4.0 ± 3.5	3.1 ± 2.9	4.9 ± 3.7	<0.001
· Checklist Individual Strength-fatigue subscale (CIS-f)	37.1 ± 13.2	32.8 ± 13.6	40.9 ± 11.6	<0.001
· MG-Quality of Life (MG-QoL-15)	21.1 ± 12.4	18.2 ± 12.3	23.6 ± 12.0	<0.001
· Hospital Anxiety and Depression Scale-depression subscale (HADS-d)	5.4 ± 3.7	5.2 ± 3.7	5.6 ± 3.7	0.276
· Pittsburgh Sleep Quality Index (PSQI)	7.7 ± 3.9	6.5 ± 3.5	8.8 ± 3.9	<0.001

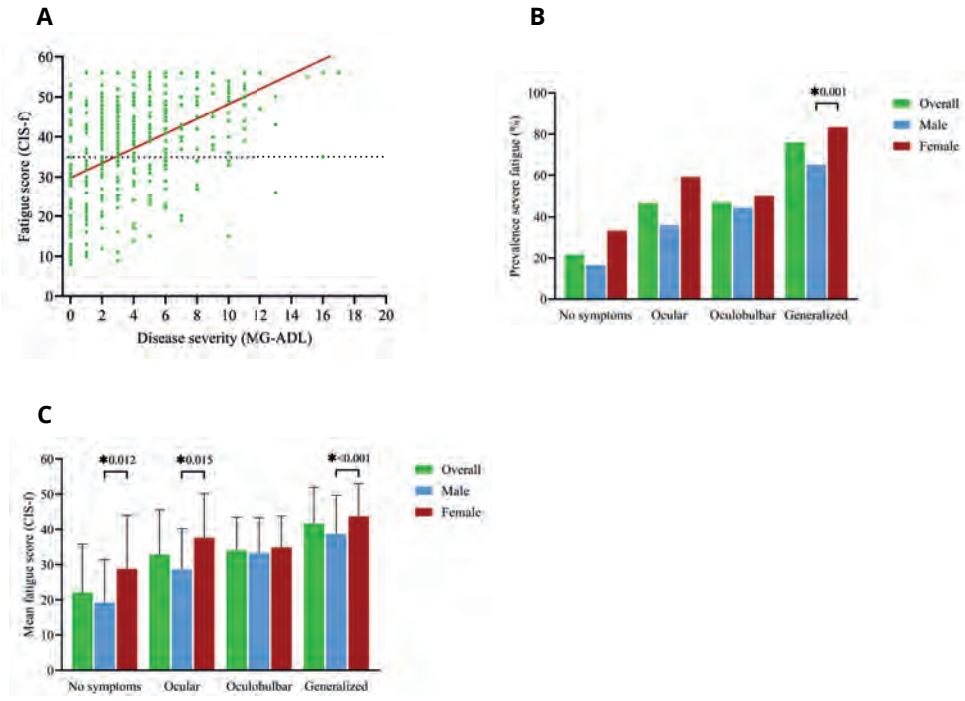

Figure 1. A) Correlation between fatigue (CIS-f) and disease severity (MG-ADL). Horizontal line indicates the cut-off for clinically relevant fatigue (≥35). B) Prevalence of severe fatigue (CIS-f ≥35) per MG-ADL subgroup. C) Mean fatigue (CIS-f) score per MG-ADL subgroup.

The mean score on the HADS-d was 5.4 ± 3.7 points. A possible depressive disorder (score 8-10) was present in 18% and a probable depressive disorder (score ≥11) in 10%. Fatigued patients scored significantly higher than non-fatigued patients, 6.5 ± 3.6 vs. 3.5 ± 3.0 points ($p<0.001$). Quality of life was significantly lower for fatigued patients with 25.9 ± 10.7 points on the MG-QoL-15 compared to 13.0 ± 10.9 for non-fatigued patients ($p<0.001$, figure 2). The mean score for sleep quality, as measured with the PSQI, was 7.7 ± 3.9 points. Fatigued

patients had a lower sleep quality compared to non-fatigued patients: 8.5 ± 3.8 vs. 6.3 ± 3.5 ($p<0.001$).

Figure 3 shows coping strategies according to the UCL in comparison with values for the reference group. Fatigued male patients scored higher on avoiding and passive coping strategies compared to non-fatigued male patients ($p=0.009$; $p<0.001$). Fatigued female patients scored higher on passive coping strategies ($p=0.047$). Avoiding and passive coping strategies are both associated with expressing mental and physical distress (appendix table B.1).

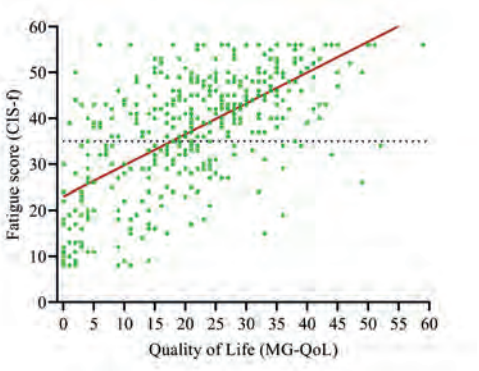

Figure 2. Correlation between fatigue and quality of life. Horizontal line indicates the cut-off for severe fatigue (CIS-f ≥35).

Significantly higher fatigue scores and MG-ADL scores were found for patients treated with pyridostigmine, intravenous immunoglobulins or plasma exchange therapy compared to patients not treated with this medication (table 3). Fatigue was similar in patients with and without a previous thymectomy. The mean duration between thymectomy and participation in this study was 14.8 ± 12.7 years. The mean score and prevalence of fatigue were significantly higher in patients with a concomitant autoimmune disorder compared to patients without: 41.6 ± 11.4 vs. 35.8 ± 13.3, $p<0.001$ with a prevalence of 24% vs. 16%, $p=0.003$.

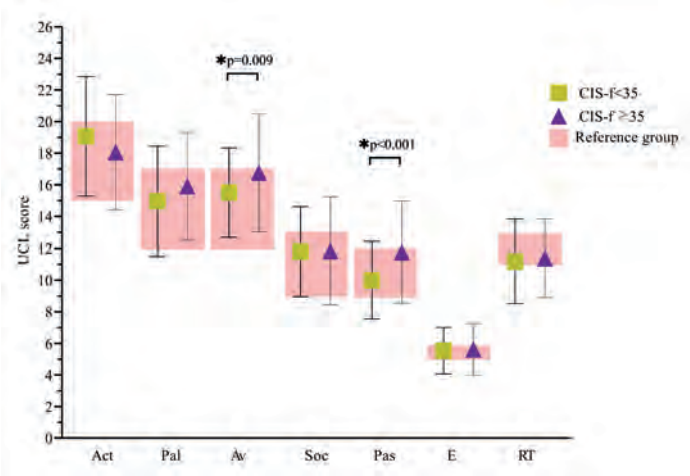

Figure 3A. Results Utrecht Coping List (UCL), males (n=197).

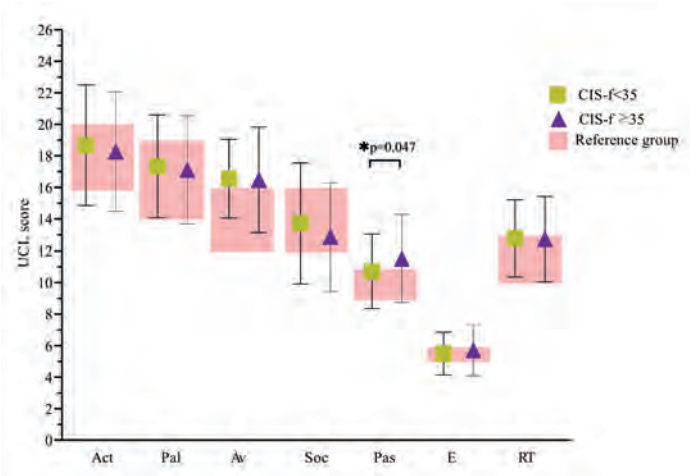

Figure 3B. Results Utrecht Coping List (UCL), females (n=218).

Fatigue and physical condition. According to their BMI score, 1.7% of all participants were underweight (BMI <18.5), 41% were overweight (BMI 25-30) and 21% were obese (BMI >30). The mean BMI was 27.1 ± 1.0 points. There was no difference in BMI between patients with or without corticosteroids (p=0.903). Fatigue scores were significantly higher in obese patients compared to those with a healthy weight (BMI 18.5-25) and those who were overweight (BMI 25-30; p<0.001; p<0.001).

Most patients (78%) engaged in moderate physical activities >50 minutes every day (figure 4a).

Table 3. Fatigue (CIS-f) and disease severity (MG-ADL) within different types of treatment.

	With treatment	Without treatment	Sig.
Pyridostigmine			
CIS-f (mean ± SD)	38.9 ± 11.7	34.4 ± 14.7	0.001
MG-ADL (mean ± SD)	4.7 ± 3.4	3.0 ± 3.3	<0.001
Corticosteroids			
CIS-f (mean ± SD)	37.4 ± 12.3	37.0 ± 13.7	0.732
MG-ADL (mean ± SD)	3.9 ± 3.4	4.2 ± 3.5	0.388
Non-steroid immunosuppressants			
CIS-f (mean ± SD)	38.2 ± 12.5	36.2 ± 13.6	0.110
MG-ADL (mean ± SD)	3.9 ± 3.4	4.1 ± 3.5	0.558
IVIG and/ or PLEX			
CIS-f (mean ± SD)	41.4 ± 12.2	36.4 ± 13.2	0.006
MG-ADL (mean ± SD)	5.4 ± 3.8	3.8 ± 3.3	0.002
Thymectomy			
Time (years) between study and thymectomy (mean ± SD)	14.8 ± 12.7	-	
CIS-f (mean ± SD)	37.2 ± 13.0	37.2 ± 13.2	0.984
MG-ADL (mean ± SD)	3.7 ± 3.1	4.1 ± 3.5	0.181

Abbreviations: CIS-f = Checklist Individual Strength – fatigue; IVIG = intravenous immunoglobulins; MG-ADL = MG – Activities of Daily Living; PLEX = plasma exchange therapy

The mean fatigue score was higher in patients with <50 active minutes every day (41.7 ± 11.3) compared to patients with 100-150 active minutes (36.8 ± 13.5, p=0.012) and >150 active minutes (32.9 ± 12.9, p<0.001). Only a quarter of patients engaged in strenuous physical activities were (figure 4b). Fatigue scores were lower in all patients with one or more periods of strenuous activity per week compared to those without these activities (39.7 ± 12.1 (none) vs. 30.6 ± 13.6 (1 time); 31.8 ± 13.1 (2 times); 26.3 ± 11.7 (>2 times), p<0.001 for all frequencies).

Multivariate linear regression analysis. Table 4 shows the results of the multivariate linear regression analysis with CIS-f as dependent variable. Twenty-three (5.5%) patients were excluded from analysis because of missing data, mostly regarding medical history. An independent positive correlation for higher fatigue scores was found for female gender (P<0.001), BMI (p=0.001), MG-ADL (p<0.001) and HADS-d (p<0.001). Age was negatively correlated with fatigue scores (p=0.001), older patients had lower fatigue scores. All frequencies of strenuous physical activities were negatively correlated with fatigue scores. This effect was strongest for a frequency of >2 times weekly, fatigue scores decreased with 7.9 points compared to those of subjects with no strenuous activities (p<0.001) (total range CIS-f: 8-56 points). There was no correlation with moderate physical activity, sleep quality, concomitant autoimmune disorders, type of antibodies and disease duration.

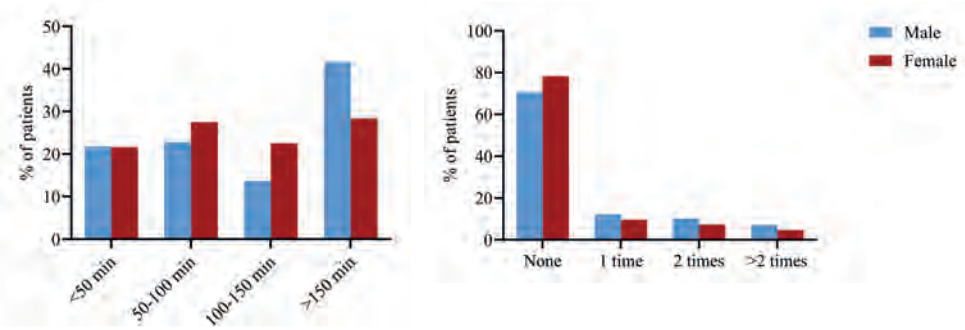

Figure 4. A) Moderate physical activities (e.g. walking or cycling on regular pace), minutes per day. B) Strenuous physical activities (e.g. running, cycling at increased pace), times per week.

Table 4. Multivariate linear regression analysis, n=397

Parameter	B	Std. Error	95% Wald Confidence Interval		
			Lower	Upper	Sig.
Female gender	4,648	1,0945	2,503	6,794	**<0,001**
Age	-0,124	0,0383	-0,199	-0,048	**0,001**
Disease duration	-0,049	0,0437	-0,134	0,037	0,267
Antibodies					
· AChR	-0,716	1,4894	-3,636	2,203	0,631
· MuSK	0,441	2,9151	-5,272	6,154	0,880
· Seronegative	0,716	1,4984	-2,203	3,636	0,631
Other AID	1,713	1,1123	-0,467	3,893	0,124
BMI	0,338	0,0975	0,147	0,529	**0,001**
MG-ADL	0,827	0,1650	0,503	1,150	**<0,001**
HADS-d	1,254	0,1454	0,969	1,539	**<0,001**
PSQI	0,256	0,1409	-0,020	0,533	0,069
Moderate physical activities					
· 50-100 minutes daily	1,310	1,3744	-1,384	4,004	0,340
· 100-150 minutes daily	0,458	1,5237	-2,528	3,444	0,764
· >150 minutes daily	-1,828	1,3476	-4,469	0,814	0,175
Strenuous physical activities					
· 1x/ week	-4,625	1,5584	-7,679	-1,570	**0,003**
· 2x/ week	-3,999	1,7453	-7,419	-0,578	**0,022**
· >2x/ week	-7,951	2,0982	-12,063	-3,838	**<0,001**

Abbreviations: AChR = acetyl choline receptor; AID = autoimmune disorder; B = unstandardized beta; BMI = body mass index; HADS-d = Hospital Anxiety and Depression scale – depression subscale; MG-ADL = Myasthenia Gravis – Activities of Daily Living; MuSK = Muscle specific tyrosine kinase; PSQI = Pittburgh Sleep Quality Index

Discussion

With a prevalence of 1-2 per 10.000[6], approximately a quarter of Dutch MG patients had signed up for the registry at the start of this study. We estimate that we have included 14-23% of all Dutch MG patients in this study. Baseline characteristics of our cohort were similar to the baseline characteristics of a previous cross-sectional study which included all (n=671) MG patients in a predefined region in the Netherlands[34]. Therefore, we believe our cohort is a correct representation of the Dutch MG population. The high response rate of 75% indicates that this topic is highly important to MG patients. The high prevalence of severe fatigue (62%) was comparable with previous studies on fatigue in MG[7-11, 19] but is not unique to MG. Many other medical (chronic) conditions are accompanied by severe fatigue with a similar prevalence[27, 35, 36].

The intensity and prevalence of fatigue in the general population is much lower. Among 1923 healthy Dutch representatives (age 16-88) the mean fatigue score, measured using the CIS-f, was 23[27]. The prevalence of fatigue in the general population of Northern & Western Europe and United States varies between 18-38%[1, 8, 10, 37-39]. Interestingly, the extent of fatigue among MG patients without clinical symptoms in our study (22%) was within this range. Unfortunately, because of the nature of questioning it was not possible to further distinguish between patients in pharmacological remission and patients in remission without medication. Similar findings have been demonstrated in one previous study[7] but two studies found opposite results with a higher prevalence of fatigue in MG patients in (pharmacological) remission compared to the general population[8, 9].

Fatigue increased with disease severity, in accordance with previous findings[7-11, 19]. The current study does not provide longitudinal data to assess effects of MG-specific treatment on fatigue. However, our results suggest that chronic medication does not have a significant effect on fatigue, whereas short-acting medication does. However, the lack of information on dosage and duration precludes a definitive conclusion. Data from the literature on this subject is scarce. Three studies with sample sizes ranging from 29 to 257 participants, all demonstrated a decline in fatigue after successful treatment of clinical MG symptoms[16, 18, 40]. Our study does not provide additional answers to the question whether therapy should be initiated or intensified in fatigued patients.

A novel finding in this study is the difference in coping strategy between fatigued and non-fatigued patients. This finding could be a potential option for treatment of fatigue with cognitive behavior therapy. The identification of coping domains may form the basis of individualized, targeted therapy. Such an approach has been proven effective as treatment

of fatigue in other NMD's[25, 41] and limited pilot data is available in MG[42]. However, these studies did not provide information on differences in coping styles between fatigued and non-fatigued patients.

In addition, we gathered extensive information on physical activity. Strenuous physical activities showed a highly significant and strong negative association with fatigue. In facioscapulohumeral dystrophy, 3 weekly sessions of 30 minutes cycling on an ergometer significantly improved fatigue compared to standard treatment[25]. This effect was still present at 12 weeks follow-up. Similar results were found in post-Guillain Barre syndrome and chronic inflammatory demyelinating polyneuropathy patients[43]. Fatigue severity reduced with 20% after a 12-week training program with 3 weekly cycling sessions on an ergometer. In myotonic dystrophy type 1, no additional improvement of fatigue was found after adding graded exercise to cognitive behavior therapy[41], although the group receiving exercise therapy in this study was relatively small (26% of participants). In MG, pilot data is available which demonstrates non-significant improvements of fatigue in small groups of patients[42, 44]. These results, together with the strong negative association of strenuous activities with fatigue, suggest that a trial that tests the effect of an exercise program in MG, might provide interesting information. If successful, it could provide the basis for a therapy for fatigue that is relatively easy to apply and has few side-effects.

We found a negative correlation between increasing age and fatigue, although this effect was small (B -0.124). Possible explanations could be that younger people have a more demanding lifestyle or that older people applied more effective coping strategies for fatigue. The observed association with female gender and depressive symptoms is consistent with previous research[7-10, 12, 14, 16, 19]. Gender differences in fatigue have been previously demonstrated in other medical conditions as well as in the working population[45, 46]. The exact pathophysiology of these is unclear, but may be related to differences in the immune system and responses[47], or perhaps differences in social roles and activities contribute to the observed gender differences.

The association with depressive symptoms is not surprising, since fatigue is one of the symptoms of a depressive disorder according to the Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-5)[48]. Therefore, this association does not provide any evidence of causality, since depressive symptoms may be the cause or result of fatigue. In addition, the observed association may have been caused by an overlap in fatigue and depression questionnaires, e.g. 'I feel weak' and 'I feel fatigued' (CIS-f) and 'I feel like everything is going more difficult' (HADS-d).

Limitations

This study has a number of limitations. First, the nature of this study, in which an invitation was sent out to all MG patients in the Dutch-Belgian MG registry, may have led to potential selection bias with an over-presentation of more fatigued MG patients. It is likely that fatigued patients are more willing to participate in a fatigue survey compared to non-fatigued patients. However, as 75% of all registry participants completed all questionnaires, this likely reduced the risk of selection bias. Selection bias may also affect participation in the MG registry itself: patients with more severe disease could be more willing to sign up for the registry compared to patients with no or only minimal symptoms. It is also possible that patients treated in university hospitals are more likely be made aware of the existence of the registry compared to patients treated in smaller local hospitals.

Second, this cross-sectional study does not provide information on causality of the observed significant observations. Third, we did not have sufficient information on type and dose of MG-specific medication used by participants to adequately investigate a potential influence of medication on fatigue. Finally, fatigue was assessed with a fatigue-specific questionnaire. We have no data on how frequently fatigue is reported spontaneously. The strong correlation of fatigue with disease severity suggests an overlap between the two types of fatigue. This is further demonstrated by the fact that the number of patients with a high MG-ADL who are not fatigued is extremely low (figure 1a)

Conclusion

Fatigue in Dutch MG patients is highly prevalent, consistent with previous findings in MG, other NMDs and non-neuromuscular chronic diseases. . The strong association with disease severity suggests that fatigue should be recognized as an element of the symptomatology of MG, although it cannot be used to distinguish MG from other NMD's. The high response rate for this study demonstrated the importance of the topic for patients. Its high prevalence and strong negative effect on quality of life, suggest that fatigue should be systematically evaluated during consultations with MG patients. Unfortunately, literature on the effect of MG-specific treatment on fatigue is scarce and this study does not provide additional insights on this topic. This study however, demonstrates a strong negative association of strenuous physical activities with fatigue in a large group of MG patients. Our results suggest that increasing physical fitness could provide a potential treatment of fatigue. In addition, the observed differences in coping styles between fatigued and non-fatigued patients suggest that cognitive behavioral therapy or even targeted coaching may be effective as a treatment for treating fatigue in MG. Naturally, this would require randomized clinical trials to prove their effectiveness.

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Appendix A

English translation of the Checklist Individual Strength – items of the fatigue subscale in bold

Instruction: On the next page you will find 20 statements. With these statements we aim to get an impression of how you have felt during the past two weeks. For example:

I feel relaxed

If you feel that this statement is completely true , place a cross in the left box; like this:

I feel relaxed

yes, that is true ☒☐☐☐☐☐☐ no, that is not true

If you feel that this statement is not true at all, place a cross in the right box; like this:

I feel relaxed

yes, that is true ☐☐☐☐☐☐☒ no, that is not true

If you feel that this statement in not "yes, that is true", but also not "no, that is not true", place a cross in the box that is most in accordance with how you have felt. For example, if you feel relaxed, but not very relaxed, place a cross in one of the boxes close to "yes, that is true": like this:

I feel relaxed

yes, that is true ☐☐☒☐☐☐☐ no, that is not true

Do not skip any statement and place only one cross for each statement.

1. I feel tired

yes, that is true ☐☐☐☐☐☐☐ no, that is not true

2. I feel very active

yes, that is true ☐☐☐☐☐☐☐ no, that is not true

3. Thinking requires effort

yes, that is true ☐☐☐☐☐☐☐ no, that is not true

4. Physically I feel exhausted

yes, that is true ☐☐☐☐☐☐☐ no, that is not true

5. I feel like doing all kinds of nice things

yes, that is true ☐☐☐☐☐☐☐ no, that is not true

6. I feel fit

yes, that is true ☐☐☐☐☐☐☐ no, that is not true

7. I do quite a lot within a day

yes, that is true ☐☐☐☐☐☐☐ no, that is not true

8. When I am doing something, I can concentrate quite well

yes, that is true ☐☐☐☐☐☐☐ no, that is not true

9. I feel weak

yes, that is true ☐☐☐☐☐☐☐ no, that is not true

10. I don't do much during the day

yes, that is true ☐☐☐☐☐☐☐ no, that is not true

11. I can concentrate well

yes, that is true ☐☐☐☐☐☐☐ no, that is not true

12. I feel rested

yes, that is true ☐☐☐☐☐☐☐ no, that is not true

13. I have trouble concentrating

yes, that is true ☐☐☐☐☐☐☐ no, that is not true

14. Physically I feel I am in a bad condition

yes, that is true ☐☐☐☐☐☐☐ no, that is not true

15. I am full of plans

yes, that is true ☐☐☐☐☐☐☐ no, that is not true

16. I get tired very quickly

yes, that is true ☐☐☐☐☐☐☐ no, that is not true

17. I have a low output

yes, that is true ☐☐☐☐☐☐☐ no, that is not true

18. I feel no desire to do anything

yes, that is true ☐☐☐☐☐☐☐ no, that is not true

19. My thoughts easily wander

yes, that is true ☐☐☐☐☐☐☐ no, that is not true

20. Physically I feel in a good shape

yes, that is true ☐☐☐☐☐☐☐ no, that is not true

Scoring of the Checklist Individual Strength

Subscales

- **subscale 1: Fatigue Severity** **items 1, 4, 6, 9, 12, 14, 16, 20**
- subscale 2: Concentration items 3, 8, 11, 13, 19
- subscale 3: Motivation items 2, 5, 15, 18
- subscale 4: Activity items 7, 10, 17

For the items: 2, 5, 6, 7, 8, 11, 12, 15, 20, scoring is as follows:

yes, that is true

1	2	3	4	5	6	7

no, that is not true

For the items: 1, 3, 4, 9, 10, 13, 14, 16, 17, 18, 19, scoring is as follows:

yes, that is true

7	6	5	4	3	2	1

no, that is not true

Appendix B

Appendix B.2. Reference groups UCL

Males: 19-65 years, a-select sample from Dutch population and Dutch railway personnel during periodic medical check-up.
Females: 18-65 years, a-select sample from Dutch population, Dutch railway personnel during periodic medical check-up and Dutch nurses.

Appendix table B.1. Explanation UCL coping domains

UCL strategy	Description	Associated characteristics
Active approach (Act)	Keeping an overview of the situation, being focused on the problem and confidently solving the situation	Positive correlation with high self-esteem and adequate functioning. Negative correlation with depressive symptoms, neuroticism and fear
Palliative reaction (Pal)	Looking for distraction, getting away from the situation/ problem	Positive correlation with fear, neuroticism, psychopathology and inadequacy
Avoidance (Av)	Avoiding difficult situations, waiting to see what will happen	Positive correlation with low self-esteem, fear, mental and physical distress
Seeking social support (Soc)	Discussing the problem with someone or getting someone to help	Positive correlation with internal locus of control
Passive reaction (Pas)	Being overwhelmed by the situation, incapable of action, worrying	Positive correlation with fear, depressive symptoms and inadequacy. Related to reporting health complaints
Expression of emotions (E)	Positive correlation with anger, fear and neuroticism	Positive correlation with fear, depressive symptoms and inadequacy. Related to reporting health complaints
Reassuring thoughts (RT)	Capable of seeing positive things when problems occur, putting the situation in perspective	Positive correlation with somatic complaints. Negative correlation with neuroticism. Matches with an active approach but also a palliative reaction

Chapter 5

Correlation of C-reactive protein with severe fatigue in myasthenia gravis

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Abstract

Background and Objectives

The majority of myasthenia gravis (MG) patients suffer from fatigue, which can be defined as a subjective lack of energy and difficulty in initiating or sustaining voluntary activities. This is conceptually different from muscle weakness or muscle fatigability. Fatigue is one of the most reported symptoms in MG and has been hypothesized to be an innate mechanism to minimize muscle activity in order to protect muscles from (further) damage. The exact pathophysiology of fatigue remains unclear and it is very likely a multifactorial phenomenon. The aim of this study was to provide a better understanding on the pathophysiology of fatigue in MG.

Methods

We analyzed 38 serum biomarkers including various cytokines and myokines in a cohort of 116 anti-AChR positive MG patients. A multivariate linear regression analysis for each biomarker was performed in search for a correlation with fatigue. The following pre-selected covariates were included in the primary analysis: gender, age, disease severity, depression and anxiety scores, non-steroid immune suppressive medication and cumulative prednisone dosage in the past six months.

Results

Severe fatigue was present in 64% of patients. Results show a robust correlation between fatigue and C-reactive protein (CRP) in the primary analysis. This correlation persisted when additionally adjusting for BMI, strenuous physical activities and hemoglobulin levels.

Conclusions

Our findings suggest that chronic low grade inflammation, mediated by CRP, contributes to the pathogenesis of fatigue in MG. This aligns with the hypothesis that local peripheral inflammatory processes induce systemic inflammatory cascades responsible for fatigue.

Background

Autoimmune myasthenia gravis (MG) is a rare condition with antibodies targeted against key proteins of the neuromuscular junction (NMJ) of skeletal muscles[1]. Several types of antibodies have been identified, but the majority (approximately 80%) of patients have detectable antibodies against the acetylcholine receptor (AChR)[2]. Three mechanisms can contribute to the pathogenicity of AChR antibodies: 1) they block the binding of acetylcholine to AChRs, 2) they accelerate degradation of AChRs by crosslinking and internalization of the receptors or/and 3) they activate the complement system[2]. The predominant clinical phenotype of MG consists of fluctuating and fatigable weakness of skeletal muscles. Extraocular muscles are most frequently involved, leading to asymmetric and fluctuating ptosis and diplopia[1]. Although MG is considered to affect only NMJ, the majority of patients suffer from cognitive or mental fatigue as well[3, 4]. This type of fatigue is conceptually different from muscle weakness or fatigability and is often referred to as central fatigue, as it originates in the central nervous system[5]. Central fatigue can be best defined as a subjective lack of energy and difficulty in initiating or sustaining voluntary activities[5]. It is hypothesized that in neuromuscular diseases, central fatigue is a mechanism to minimize activities, in order to protect the muscles from further damage[5,6]. Disease-related muscle weakness may require higher levels of effort, which may contribute to central fatigue. Another hypothesis is that local peripheral inflammatory processes in auto-immune diseases may trigger systemic inflammatory cascades responsible for fatigue[6]. It has been proposed that inflammation reduces both internally and externally driven motivation states, to provide the body the opportunity to heal[7]. Several inflammatory biomarkers have previously been associated with central fatigue in numerous chronic auto-immune diseases, including rheumatoid arthritis (RA), Sjogren's disease and inflammatory bowel disease (IBD)[8]. Previous research has shown that in MG, fatigue is correlated with female gender, depressive symptoms, disease severity, body mass index (BMI) and physical activities[3, 9-12]. These data suggest that the pathophysiology of fatigue is multifactorial, although there is no evidence on causality.

Our aim was to gain a better understanding on the pathophysiology of fatigue in MG, by searching for one or more circulating serum biomarkers related to fatigue. Our second aim was to find longitudinal correlations between fatigue and potentially newly identified biomarkers.

Methods

This study was conducted between May 2022 and April 2023 at the Department of Neurology of the Leiden University Medical Center (LUMC), Leiden, the Netherlands.

Subjects

Anti-AChR positive MG patients (≥18 years) were recruited from the Dutch – Belgian Myasthenia Patient Registry[13]. Diagnosis of MG was based on clinical signs and symptoms characteristic for MG and a documented positive serological test for AChR. Exclusion criteria were: 1) a medical history of other active autoimmune disorders, with "active" being defined as having clinical symptoms or currently receiving immune modulating therapy and 2) a medical history of a neoplasm or receiving cytostatic treatment within the last year. If participants met the in- and exclusion criteria, they were scheduled in consecutive order of registration until the predetermined sample size (n = 116) was reached.

Procedures

The study consisted of two visits: one extensive baseline visit between May and August 2022 and a second shorter visit between March and April 2023. To limit the burden of study participation, the second visit was optional. After written informed consent, baseline characteristics (for more details see table 1) were collected and a baseline quantitative MG (QMG) test was performed by the researcher (A.R.) to document disease severity. Participants were asked to refrain from taking their morning pyridostigmine dosage until after the QMG test.

- ***Questionnaires at baseline and second visit***

Participants were asked to complete the following set of questionnaires on paper (baseline) or online (second visit): a) Checklist Individual Strength for fatigue (CIS-f) to assess central fatigue[14]. This is an 8-item self-assessment on experienced fatigue during the past two weeks and is scored on a 7-point Likert scale. It ranges from 8 to 56 points, with ≥35 indicating severe or clinically relevant fatigue. b) Hospital Anxiety and Depression Scale (HADS) for depressive symptoms[15] and c) MG-Activities of Daily Living (MG-ADL) for disease severity[16]. Consequently, participants were asked to provide an overview of their medication use over the past six months and information on their level of physical activities and height and weight.

- ***Baseline blood samples***

All blood samples were collected during the study visit between 10.30AM and 01.00PM to restrict potential circadian influences on biomarker results. Routine hematology (hemoglobin

(Hb), erythrocytes, thrombocytes, monocytes, lymphocytes, leukocytes + differentiation) and chemistry (C-reactive protein (CRP) and creatinine), together with components of the complement system (C1q, C3 and C4) and complement activation (classic pathway (CP) and alternative pathway (AP)) (directly stored on ice) were analyzed instantly by the clinical chemistry laboratory at the LUMC following standard operating procedures (eTable 1). All other biomarkers were analyzed by batch after collection of all samples, stored at -80°C. Serum AChR antibody titers and immunoglobulin G (IgG) were measured by the clinical chemistry laboratory at the LUMC using a radioimmunoprecipitation assay in accordance with standard operating procedures (for details see eTable 1).

- ***Serum protein measurements***

The following serum proteins-levels were determined with multiplex bead assays at the infectious disease laboratory at the LUMC[17] using Milliplex reagents (Merck, Amsterdam, The Netherlands). The assay makes use of a window of detection with a lower detection limit of <1pg/ml and the upper detection limit > 20000 pg/ml.
Brain-derived neurotrophic factor (BDNF), irisin, myostatin, fibroblast growth factor (FGF)21 were tested as part of the myokine panel (HMYOMAG-56K Human Myokine panel). Interferon γ (IFN-γ), interferon gamma-induced protein 10 (IP10), tumor necrosis factor α (TNF-α), fibroblast growth factor (FGF)2, interleukin (IL-) 1ß, 4, 6, 8, 10, 13, 15, 17a, 18 and 22 were selected as a custom made panel from the cytokine/chemokine/growth factor panel A (HCYTA-60K). Insulin-like growth factor 1 (IGF-1) and osteopontin were tested as single analytes (HIGFMAG-52K and HBNMAG-51K respectively). Assays were performed according to manufacturer's instructions with manufacturers specific standards and QC control samples. All samples were thawed and diluted in sample diluent according to manufacturer's instruction and run as single measurement. Samples were acquired on a Bio-plex 200 system and analyzed with Bio-plex Manager software v6.2.

- ***Blood samples at second visit***

A repeated sample of CRP was taken under similar circumstances 8.6 ±0.9 months later, between March and April 2023. The decision to only acquire repeated blood samples for CRP serum levels was made after the results of the primary multivariate regression analysis were available.

Statistical analyses

The sample size was based on an assumed correlation coefficient of 0.27 between a biomarker and central fatigue using a linear regression model with a significance of $p<0.05$, a power of 80% and a drop out rate of 10%.
Statistical analyses were performed using IBM SPSS Statistics version 25.0. Group data are

described by mean and standard deviation (±SD) for normally distributed data or median and interquartile range [IQR] for non-parametric data. Unpaired T-tests or Mann-Whitney U tests were used for comparison between two groups, one-way ANOVA for >2 groups. Pearson's *r* were used for bivariate correlations. A multivariate linear regression analysis was performed for each biomarker individually. If a biomarker was not detectable in 20 or more patients, it was not used for further statistical analyses. Data transformation for skewed biomarker data was not applied. Imputation for missing data was not applied. The pre-selected covariates as predictors for the regression analysis were as follows: gender, age, QMG, HADS-d, use of non-steroid immune suppressive medication yes/no and cumulative prednisone dosage in the past six months. This pre-selection was based on either a previously shown association or a supposed predictive or explanatory confounding effect on fatigue or biomarkers[3]. Significance was accepted at $p < 0.05$. Post hoc analyses were performed only on biomarkers of interest. Correction for multiple testing was only applied in post hoc analyses because of the exploratory design of the first part of the study. Biomarkers with values below calibration lines with the multiplex bead assay, were identified as undetectable with the current assay and set to zero for statistics.

Declarations

Ethics approval and consent to participate

The ethics committee of the LUMC approved the study protocol (NL72266.058.20). Written consent was obtained from all participants according to the declaration of Helsinki (2013) and in accordance with the Dutch Medical Research Involving Human Subjects Act (WMO), Good Clinical Practice (GCP) guidelines and General Data Protection Regulation (GDPR).

Results

Baseline characteristics

A study invitation was sent to 556 AChR positive MG patients. A total of 116 patients were included in consecutive order until the predetermined sample size was reached (Figure 1).

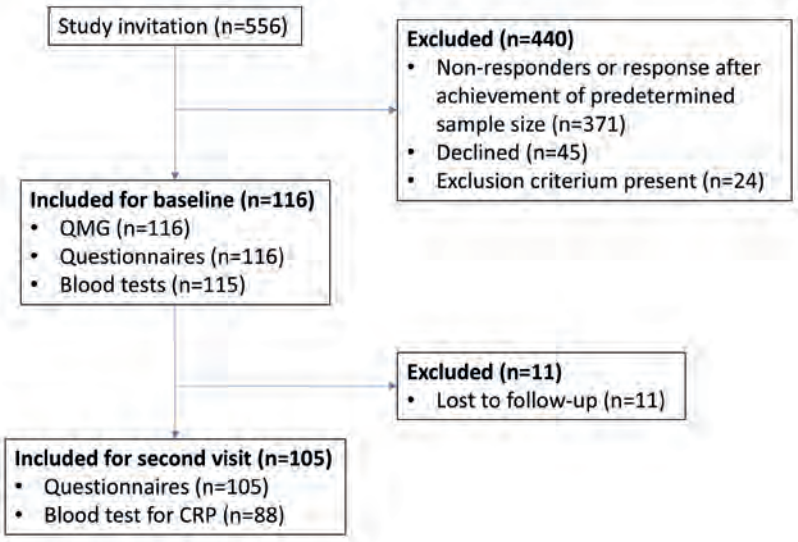

Figure 1. Study flow chart

Table 1 shows the clinical characteristics of the study population. The mean ±SD fatigue score (CIS-f) was 36.7 ±13.6 points. Severe fatigue (≥35 points) was present in 64% of patients. There was no difference in fatigue scores between males and females: 36.1 ±14.2 vs 37.3 ±13.0, p=0.65. Fatigue scores were positively correlated with disease severity: QMG: *r* 0.39 (p <0.001); R^2 0.15 and MG-ADL: *r* 0.55 (p <0.001); R^2 0.30. Figure 2 shows the median [IQR] fatigue scores and the percentage of patients with severe fatigue per MGFA (MG Foundation of America) class (score ≥35). There were no differences in fatigue scores between patients without MG medication, pyridostigmine monotherapy or steroids and/ or non-steroid immune suppressive medication (p = 0.21). Ten percent of all patients were in remission, as defined in the 2016 international consensus guidance[18], of whom 75% were in pharmacological remission.

Mean fatigue scores were significantly higher in patients with concomitant hypo- or hyperthyroid disease (n=10): 45.3 ±7.8 compared to 35.9 ±13.8 points in patients without thyroid disease (p=0.004). There was a positive correlation between BMI and fatigue scores:

Table 1. Clinical characteristics

Characteristic	Participants
Included, n (% female)	116 (48.3%)
Age, mean ±SD	62.7 ±13.0
Disease duration years, mean ±SD	11.6 ±10.6
QMG score, mean ±SD	6.2 ±5.0
MG-ADL score, mean ±SD	4.1 ±3.4
MGFA class, n (%)	.
· Remission	12 (10.3%)
· I	6 (5.2%)
· II	58 (50%)
· III	37 (31.9%)
· IV	3 (2.6%)
· V	0
Fatigue score (CIS-f), mean ±SD	36.7 ±13.6
Severe fatigue (CIS-f ≥35), %	63.8%
Depression score (HADS-d), mean ±SD	4.3 ±3.6
BMI, mean ±SD	27.6 ±5.3
Strenuous activities, n (%)	.
· None	86 (74.8%)
· Once a week	8 (7.0%)
· Twice a week	14 (12.2%)
· More than twice a week	7 (6.1%)
MG disease specific treatment, n (%)	
· No disease specific treatment	15 (12.9%)
· Pyridostigmine	67 (57.8%)
- Pyridostigmine monotherapy	24 (20.7%)
· Prednisolone	48 (41.4%)
- Prednisolone monotherapy[a]	19 (16.4%)
· Non-steroid immune suppressive medication	58 (50%)
· IV immune globulins	12 (10.3%)
· Plasmapheresis	1 (0.9%)
Non-steroid immune suppressive medication	
· Azathioprine	30 (25.9%)
· Mycophenolate mofetil	18 (15.5%)
· Cyclosporine	5 (4.3%)
· Methotrexate	4 (3.4%)
· Efgartigimod	3 (2.6%)
· Tacrolimus	2 (1.7%)

Abbreviations: [a] = with or without pyridostigmine; BMI = body mass index; CIS-f = checklist individual strength – fatigue; HADS-d = hospital anxiety depression scale – depression sub score; IV = intravenous; MG-ADL = myasthenia gravis – activities of daily living; MGFA = MG foundation of America; QMG = quantitative myasthenia gravis

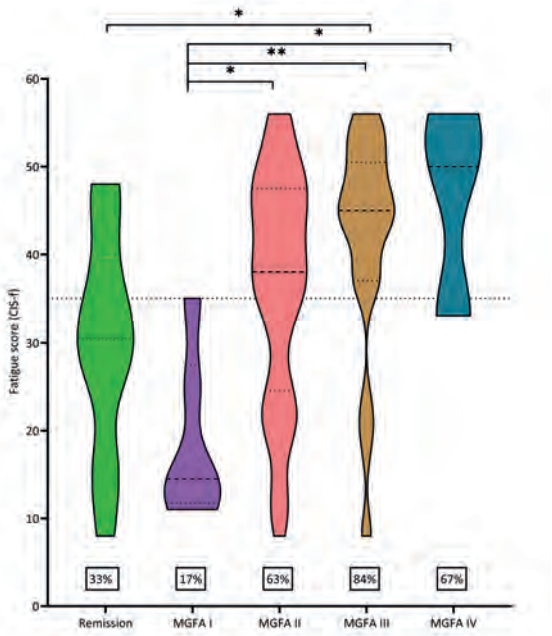

Figure 2. Median fatigue scores (CIS-f) per MGFA class. Horizontal line indicates cut-off for clinically relevant fatigue (≥35), boxes at bottom of figure shows percentage clinically relevant fatigue per MGFA class. Abbrevations: CIS-f = Checklist Individual Strenght – fatigue; *p<0.01; **p<0.001

r 0.23, p=0.015; R^2 0.05. Mean BMI scores did not differ between patients with or without steroids (currently or in the past six months): 27.6 ±4.7 versus 27.6 ±5.7 (p=0.99). A majority of patients (n=86, 74%) did not engage in strenuous physical activities, such as running, cycling, or engaging in sports. The mean fatigue score of patients who did not perform strenuous physical activities was 39.5 ±12.2, compared to 28.1 ±14.4 (p <0.001) in patients performing regular activities (≥ once a week). More than half of patients consumed ≥1 unit of alcohol a week, 31% reported never drinking alcohol and 14% less than one consumption a week. The mean fatigue score did not differ between patients who never consumed alcohol and those who consumed ≥1 unit a week (38.3 ±13.9 vs 35.4 ±14.0, p=0.32). A previous COVID infection was reported by 53% of patients. The mean fatigue score of patients with a previous COVID infection did not differ from patients without: 36.8 ±14.2 vs 36.1 ±13.1, p=0.77.

eTable 2 shows the results of a multivariate linear regression analysis for fatigue, adjusted for pre-selected baseline covariates (gender, age, QMG, HADS-d, use of non-steroid immune suppressive medication and cumulative prednisone dosage). Fatigue was positively correlated with the QMG score (ß 0.58, p=0.009) and the HADS-d (ß 1.99, p <0.001).

Fatigue and biomarkers at baseline

Table 2 shows mean serum concentrations of all investigated potential biomarkers. Separate multivariate linear regression analyses were performed to calculate adjusted correlations between fatigue and individual biomarkers (table 2). This analysis was repeated for each biomarker. After adjusting for the pre-selected baseline covariates a positive correlation with fatigue was found for CRP (ß 0.78, p=0.002). Fatigue was negatively correlated with Hb (ß -2.73, p=0.035) and basophilic granulocytes (ß -107.56, p=0.045).

Bivariate (unadjusted) analyses were performed for those biomarkers from the multivariate analysis that correlated with fatigue. This resulted in a negative correlation for fatigue with Hb (*r* -0.19, p=0.038; R^2 0.04). Anemia (Hb <7.5 (female) or <8.5 (male) mmol/l) was present in 17% of patients. The mean fatigue score in patients with anemia was 44.2 ±7.1 compared to 35.1 ±14.1 in patient without anemia (p <0.001). No bivariate correlation between fatigue and CRP (*r* 0.18, p=0.051) or basophilic granulocytes (*r* -0.12, p=0.20) was found. The major stimulus for CRP production is IL-6. A positive correlation was found between CRP and IL-6 (*r* 0.36, p=0.015; R^2 0.13), but no correlation was found between fatigue and IL-6 (*r* -0.10, p=0.49).

Post hoc analyses were only performed for CRP. With a post hoc Bonferroni correction for multiple testing, significance was accepted at p <0.0125. The positive correlation with fatigue persisted when additionally adjusting for BMI (ß 0.68, p=0.01), strenuous activities (ß 0.80, p=0.001) or Hb (ß 0.68, p=0.007). Adjusting for all post hoc variates simultaneously in the primary model also showed a positive correlation between CRP and fatigue (ß 0.60, p=0.021). CRP was not correlated with QMG score (*r* 0.06, p=0.56) or MG-ADL (*r* -0.02, p=0.84).

Longitudinal results

The majority (n=105, 91%) of patients participated in taking measurements at the second time point (figure 1), of whom 17 patients only completed the online questionnaires, resulting in a drop-out rate of 24% for blood withdrawals at the second time point. The mean interval between baseline and second visit was 8.6 ±0.9 months. The mean fatigue score at the second visit was 36.3 ±12.2 points, a decrease of 0.4 points compared to baseline.

Figure 3 shows the dichotomized fatigue results between baseline and the second visit. Of the patients with severe fatigue (CIS-f ≥35) at baseline, 18% had a decrease in fatigue scores below the cut-off for severe fatigue (CIS-f <35) and were now categorized as not severely fatigued. A total of 38% of patients without severe fatigue at baseline, had an increase of fatigue above the cut-off and were now categorized as severely fatigued.

Figure 4 shows the change (delta) between baseline and the second visit of MG-ADL and CRP plotted against the change in CIS-f scores. Compared to baseline, mean MG-ADL scores improved 0.5 points to 3.6 ±3.2 points. Median CRP at the second visit was 1.3 [0.7-2.3] mg/L, a decrease of 0.1 mg/L. There was a positive correlation between delta CIS-f and delta MG-ADL (*r* 0.40, p <0.001; R^2 0.16) but no correlation between delta CIS-f and delta CRP (*r* -0.02, p=0.88).

To replicate the observed correlation between fatigue and CRP at baseline, a repeated multivariate linear regression analysis was performed with data collected at the second visit, with adjustment for the same covariates as those used for the first analysis (n=88). The MG-ADL was used for disease severity. The observed correlation between fatigue and CRP at baseline was not found at the second visit (ß 0.37, p=0.20).

Next, to study the predictive potential of CRP for fatigue, a multivariate linear regression analysis was performed with CIS-f at the second visit as dependent covariate, together with the pre-selected baseline covariates (n=104). There was no correlation between CRP at baseline and fatigue at the second visit (ß 0.15, p=0.55). Additionally, fatigue at baseline was not correlated with CRP at the second visit (n=88, ß 0.06, p=0.93).

Table 2. Mean serum concentrations of all potential biomarker at baseline and results of multivariate linear regression analyses (statistically significant results in bold)

Biomarker	Result (mean ±SD / median [IQR])	Reference range and unit	Detectable in, n (%)	ß	SE	95% CI	Sig.
Hemoglobin	8.7 ±0.8	7.5 – 10[a] / 8.5 – 11[b] mmol/L	116 (100%)	-2.73	1.30	-5.3 – -0.19	0.035
Erythrocytes	4.6 ±0.4	4.0 – 5.0[a] / 4.5 – 5.5[b] x10*12/L	116 (100%)	-3.87	2.31	-8.40 – 0.67	0.10
Thrombocytes	243 ±65	150 – 400 x10*9/L	116 (100%)	0.01	0.02	-0.02 – 0.05	0.42
Monocytes	0.5 ±0.2	0.1 – 1.0 x10*9/L	116 (100%)	4.76	5.47	-5.97 – 15.48	0.39
Lymphocytes	1.3 ±0.7	1.0 – 3.5 x10*9/L	116 (100%)	-1.23	1.54	-4.25 – 1.80	0.43
Leukocytes	7.0 ±2.1	4.0 – 10.0 x10*9/L	116 (100%)	-0.34	0.55	-1.42 – 0.74	0.54
Neutrophil gran.	5.0 ±2.0	1.5 – 7.5 x10*9/L	116 (100%)	-0.26	0.65	-1.53 – 1.02	0.69
Eosinophil gran.	0.1 ±0.1	<0.5 x10*9/L	116 (100%)	-12.51	10.90	-33.87 – 8.85	0.25
Basophil gran.	0.04 ±0.02	<0.2 x10*9/L	116 (100%)	**-107.56**	**53.74**	**-212.88 – -2.24**	**0.045**
CRP	1.4 [0.7 – 3.2]	<0.5 mg/L	115 (100%)	**0.78**	**0.25**	**0.29 – 1.28**	**0.002**
Creatinine	76 ±17.8	49 – 90 µmol/L	116 (100%)	0.08	0.07	-0.06 – 0.22	0.25
C1q	118.0 ±17.7	102 – 171 mg/L	115 (100%)	0.06	0.06	-0.1 – 0.2	0.30
C3	1.1 ±0.2	0.9 – 2 g/L	115 (100%)	7.06	5.05	-2.8 – 16.9	0.16
C4	239.8 ±69.9	95 – 415 mg/L	115 (100%)	0.00	0.02	-0.03 – 0.03	0.77
AP	74.2 ±20.0	>39%	115 (100%)	0.07	0.05	-0.04 – 0.17	0.20
CP	107.3 ±20.4	>74%	115 (100%)	0.01	0.05	-0.09 – 0.11	0.85
Anti-AChR	15.0 [1.9 – 42.0]	0.0 – 0.5 nmol/L	114 (100%)	-0.01	0.01	-0.04 – 0.01	0.32
Total IgG	10.6 ±3.6	7 – 16 g/L	114 (100%)	-0.24	0.30	-0.82 – 0.34	0.41
IL1b	0.0 [0.0 – 0.0]	pg/ml*	10 (8.8%)	NP			
IL4	0.0 [0.0 – 0.0]	pg/ml*	20 (13.9%)	-0.10	0.13	-0.35 – 0.15	0.43
IL6	0.0 [0.0 – 1.5]	pg/ml*	46 (40.4%)	-0.22	0.27	-0.75 – 0.31	0.42
IL8	3.3 [2.2 – 5.2]	pg/ml*	113 (99.1%)	-0.01	0.02	-0.06 – 0.04	0.66
IL10	0.0 [0.0 – 4.0]	pg/ml*	40 (35.1%)	-0.13	0.11	-0.34 – 0.08	0.22
IL13	0.0 [0.0 – 0.0]	pg/ml*	17 (14.9%)	NP			
IL15	0.0 [0.0 – 0.0]	pg/ml*	13 (11.4%)	NP			
IL17a	0.0 [0.0 – 0.0]	pg/ml*	6 (5.3%)	NP			
IL18	0.0 [0.0 – 0.0]	pg/ml*	27 (23.7%)	-0.02	0.05	-0.13 – 0.08	0.67
IL22	0.0 [0.0 – 0.0]	pg/ml*	17 (14.9%)	NP			
TNF-α	0.0 [0.0 -13.0]	pg/ml*	56 (49.1%)	-0.01	0.01	-0.03 – 0.01	0.37

IGF-1	0.0 [0.0 – 0.0]	pg/ml*	25 (21.9%)	0.00	0.00	-0.00 – 0.00	0.85
FGF-2	0.0 [0.0 – 0.0]	pg/ml*	20 (13.9%)	-0.00	0.00	-0.01 – 0.01	0.53
FGF-21	0.0 [0.0 – 0.0]	pg/ml*	15 (13.2%)	NP			
Osteopontin	15.3 ±13.0	ng/ml*	114 (100%)	0.00	0.00	-0.00 – 0.00	0.74
Myostatin	0.0 [0.0 – 0.0]	pg/ml*	10 (8.8%)	NP			
Irisin	0.0 [0.0 – 0.0]	pg/ml*	15 (13.2%)	NP			
IFNγ	0.0 [0.0 – 0.0]	pg/ml*	20 (13.9%)	0.02	0.08	-0.16 – 0.31	0.83
IP10	269.7 ±149.9	pg/ml*	113 (99.1%)	0.01	0.01	-0.01 – 0.02	0.41
BDNF	11.9 ±4.4	ng/ml*	114 (100%)	0.00	0.00	-0.00 – 0.00	0.53

Abbreviations: aFemale reference range; bmale reference range; * = standardized reference range not available; AChR = acetylcholine receptor; BDNF = brain derived neurotrophic factor; CRP = C-reactive protein; FGF = fibroblast growth factor; IFN-γ = interferon γ; IGF-1 = insulin-like growth factor 1; IL = interleukin; IP10 = IFN-γ-induced protein 10; NP = not performed; TNF-α = tumor necrosis factor α;

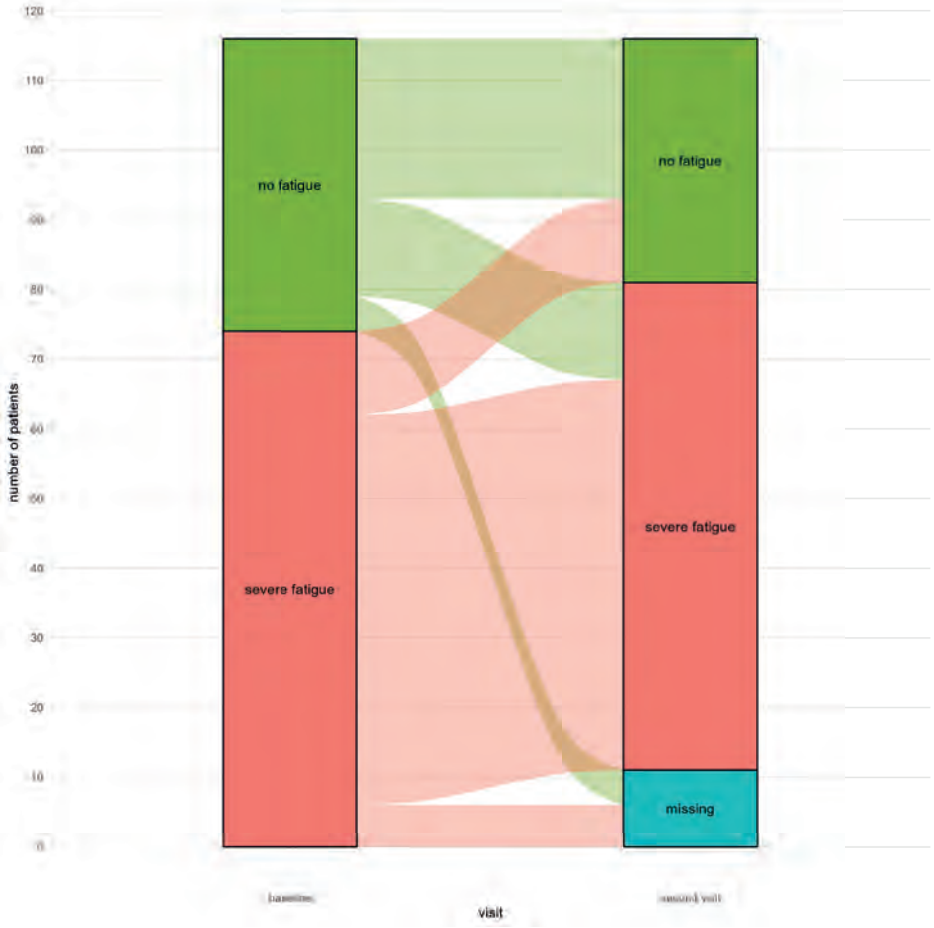

Figure 3. Switch from no severe or severe fatigue from baseline to the second visit.

Discussion

This study investigated circulating serum biomarkers for fatigue in MG. The results showed a robust correlation between fatigue and CRP at the first study visit. This correlation is independent of several covariates, including disease severity, cumulative prednisone dosage and Hb levels. This finding is in accordance with results found in other autoimmune diseases, such as rheumatoid arthritis (RA) and inflammatory bowel disease (IBD) and has also been observed in large, general population cohort studies[19-23]. This points towards a common, rather than an MG-specific fatigue pathway, possibly implicating low-grade inflammation as a cause of fatigue. However, the prevalence of fatigue in MG is much higher than in the

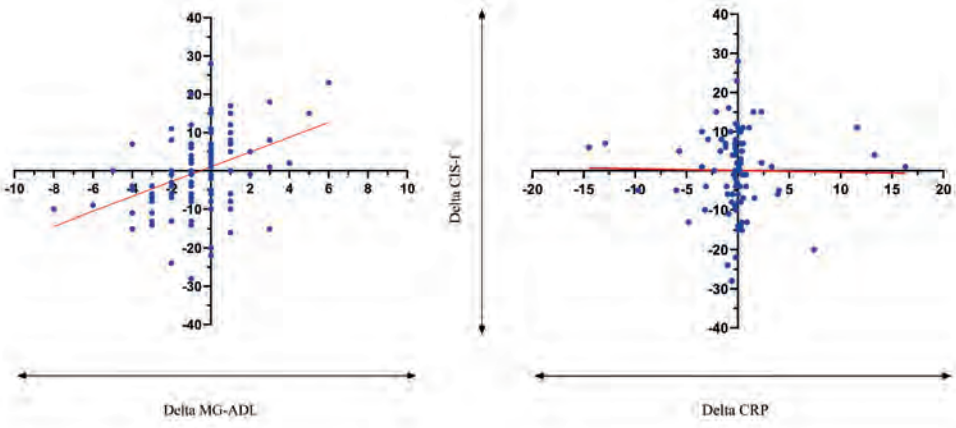

Figure 4. Change over time (delta) of disease severity (MG-ADL), fatigue (CIS-f) and C-reactive protein (CRP).

general population[24], suggesting that the threshold to develop fatigue is lower or that the exposure to fatigue-inducing triggers is higher in MG patients due to other, currently unknown contributing factors. Remarkably, increased CRP serum levels are not a typical feature in MG, unlike RA and IBD[25, 26]. This is supported by the absence of an association between CRP and clinical MG disease severity, as measured with QMG and MG-ADL. Indeed, CRP levels were within the normal range in most patients, and the median level for the entire study population was only slightly elevated: 1.4 mg/l [0.7 – 3.2] (reference <0.5 mg/l). Strikingly, even within these relatively low ranges, CRP was correlated with fatigue, suggesting low grade inflammation as part of the pathogenesis of fatigue in MG.

In accordance with previous research[3], fatigue is also strongly correlated with disease severity and depressive symptoms. Age and the female gender were not correlated with fatigue in the current study, most likely due to the smaller sample size[3]. When looking at the most frequently used immune suppressive therapies, there was no correlation of fatigue with the cumulative prednisone dosage over the past six months or the use of azathioprine.

CRP, IL-6 and fatigue

CRP is an acute phase pentameric protein, produced by hepatocytes in the liver[27]. Its production is mainly stimulated by IL-6 and, to a lesser extent by TNF-α and IL-1ß. IL-6 possesses both pro- and anti-inflammatory characteristics as a cytokine and a muscle produced myokine[28], and may play a key role in the pathogenesis of anti-AChR MG, but the exact mechanism is not yet fully understood[29-32].

Remarkably, despite the correlation with CRP, we did not find a correlation between IL-6 and fatigue. This could be due to the fact that IL-6 levels were below the detection threshold of the multiplex bead assay in 60% of patients, but these low levels may have been sufficient enough to stimulate CRP production. Another explanation could be the relatively small sample size or as a result of the high prevalence of immune suppressive therapy in the study population, which reduces serum IL-6 levels[31]. Finally, we did not measure the soluble IL-6 receptor (sIL-6R), which binds IL-6[34].

A previous study in a large sample of the working British population showed a correlation between fatigue, CRP and IL-6[21]. Furthermore, in RA, IL-6 inhibitors have shown to improve fatigue[21, 35, 36].

Immune-to-brain communication pathways

The exact mechanism by which peripheral circulating CRP or other cytokines contribute to central fatigue remains unclear. Central fatigue is a behavioral pattern that arises in the brain[5, 6], and several immune-to-brain communication pathways exist through which peripheral circulating proinflammatory cytokines communicate with the brain[7, 37, 38]. First, local cytokines can activate afferent nerves at the site of inflammation, for example the vagal or trigeminal nerve. A second route is the humoral pathway, in which pathogen-associated molecular patterns elicit pro-inflammatory cytokine production in the circumventricular organs[7, 37]. These cytokines can diffuse across the blood-brain barrier (BBB). A third pathway involves activation of IL-1 receptors in brain venules by circulating cytokines, causing local production of prostaglandin E2[7, 37]. A fourth pathway comprehends circulating cytokines gaining access across the BBB through saturable transport systems[7, 37]. There is also evidence that an increase in proinflammatory cytokines triggers a disruption of the BBB[38]. Through these pathways, activation of microglia cells takes place, resulting in the release of brain inflammatory cytokines and prostaglandins. These mediators may be responsible for the development of several behavioral responses, including fatigue[7].

Other biomarkers and fatigue

Fatigue is a well-known symptom in patients with anemia[41], therefore the observed negative correlation between fatigue and Hb levels was not unexpected. The exact mechanism of fatigue is unclear but could be related to lower oxygenation of body tissues[41]. There is no clear explanation for the negative correlation between basophilic granulocytes and fatigue. Basophilic granulocytes are white blood cells involved in allergic reactions and parasitic infections[42]. Previous research studying the association between white blood cell counts and fatigue in a large sample of the general population did not find any correlation between basophilic granulocytes and fatigue[43].

Except for Hb and basophilic granulocytes, we did not find any correlation between fatigue and the other studied biomarkers, including MG-specific AChR antibodies or the complement system. A recent randomized controlled trial in MG with zilucoplan, a complement inhibitor, demonstrated a significant reduction in fatigue scores in patients treated with zilocoplan compared to placebo[44]. However, these results were not adjusted for the improvement in disease severity due to zilucoplan and therefore this study does not provide information on the role of complement in the specific pathogenesis of fatigue.

Predictive value of CRP

Previous research demonstrated that CRP was a prospective and predictive biomarker for fatigue six months later in post stroke fatigue and three or five years later in the general population[21, 22, 49]. We could not reproduce a similar predictive value of CRP in MG patients, possibly due to our relatively small sample size (n=104) compared to these prior studies.

Limitations

The goal of this study was to search for a potential fatigue-biomarker to increase our understanding on the pathogenesis of fatigue specific to MG. Therefore, non-fatigued MG patients served as a control group. The lack of a healthy control group could be considered a limitation. Secondly, for some biomarkers the sensitivity of the assay was limited, which resulted in undetectable values. For IL-6 there was no difference in fatigue scores below or above the detection range of IL-6. Therefore, no additional (more sensitive) IL-6 assay was performed. The primary multiple linear regression analysis was repeated for each individual biomarker, we did not correct for multiple testing due to the exploratory nature of this experiment. With a post hoc Bonferroni correction ($p < 0.00167$), no biomarkers would reach significancy. Nonetheless, the main finding of this paper, the correlation between serum CRP concentrations and fatigue, remained significant after correcting for multiple testing. Although the results of the baseline visit are robust, we could not replicate this finding in a set of sera collected at a second visit. The number of participants at this second time point was lower than the predetermined sample size, due to a much higher drop-out rate of 24% than the predicted 10% as a result of the optional nature of the second visit. Due to this drop-out rate, the second measurement lacked statistical power and this is the most likely cause of the observed lack of correlation between fatigue and CRP. Furthermore, closer examination of the dataset at the second time point revealed two apparent outliers with very high CRP scores and low fatigue scores. Exclusion of these two subjects would have led to a significant correlation. Finally, CRP serum levels are affected by seasonal influences[50]. Data for the baseline visit was collected in summer, while data for the second visit was collected in March and April. Although median CRP serum levels did not differ between the two datasets, individual measurements may have been affected by seasonal changes.

Conclusion

The results of this study suggest that ongoing chronic low grade inflammation is part of the pathogenesis of fatigue in MG. This is supported by the results of previous research on fatigue both in autoimmune disorders and in the general population. However, low grade inflammation is probably only a partial contributor to fatigue and a multifactorial pathogenesis remains likely.

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Supplemental files

eTable 1. Standard operating laboratory procedures

Biomarker(s)	Method
Routine hematology	XN9000 analyser (Sysmex): colorimetry and flow cytometry
CRP	Roche COBAS 8000 Modular - c702: turbidimetry, antigen – antibody complex
Creatinine	Roche Cobas C8000 Modular - c702: enzymatic method, photometry wavelength 546/700 nm
Complement C1q	Siemens Prospec: nephelometry, antigen-antibody complex
Complement C3 and C5	Optilite (The Binding Site): turbidimetry
Complement activation routes	ELISA (Wieslab)
AChR antibodies	Radio immune assay (RSR)
Total IgG	Cobas 8000, C502 module: turbidimetry, antigen – antibody complex
Brain-derived neurotrophic factor (BDNF), osteopontin, irisin, myostatin, insulin-like growth factor 1 (IGF-1), fibroblast growth factor (FGF) 2,21, interferon γ (IFN-γ), interferon gamma-induced protein 10 (IP10), tumor necrosis factor α (TNF-α), interleukin (IL) 1ß,4,6,8,10,13,15,17a,18,22	Myokine panel, Human Bone Panel, Human IGF-I,II endocrine assay and the Human Cytokine/ Chemokine Immunology panel, Milliplex, Merck. Samples were acquired on a BioPlex 200 system and analyzed with BioPlex manager software (v6.2). Briefly, sera were diluted as suggested by the manufacturer for the different analytes and incubated with the specific antibody coated beads followed by biotin labeled detection antibodies and streptavidin-PE for quantification

eTable 2. Multivariate linear regression analysis of factors potentially associated with fatigue.

Dependent variable	CIS-f score			
Predictor	ß	SE	95% CI	Sig.
Female gender	1.16	2.35	-3.45 – 5.77	0.62
Age	-0.13	0.08	-0.29 – 0.03	0.12
QMG	0.58	0.22	0.14 – 1.02	0.009
HADS-d	1.99	0.30	1.38 – 2.58	<0.001
Non-steroid immune suppressive medication	0.84	2.01	-3.09 – 4.78	0.67
Cumulative prednisone dosage	0.00	0.00	-0.00 – 0.00	0.87

Part two

Faces of myasthenia gravis

Chapter 6

The face of myasthenia gravis

Neurology. 2020 Jul 14;95(2):89-90

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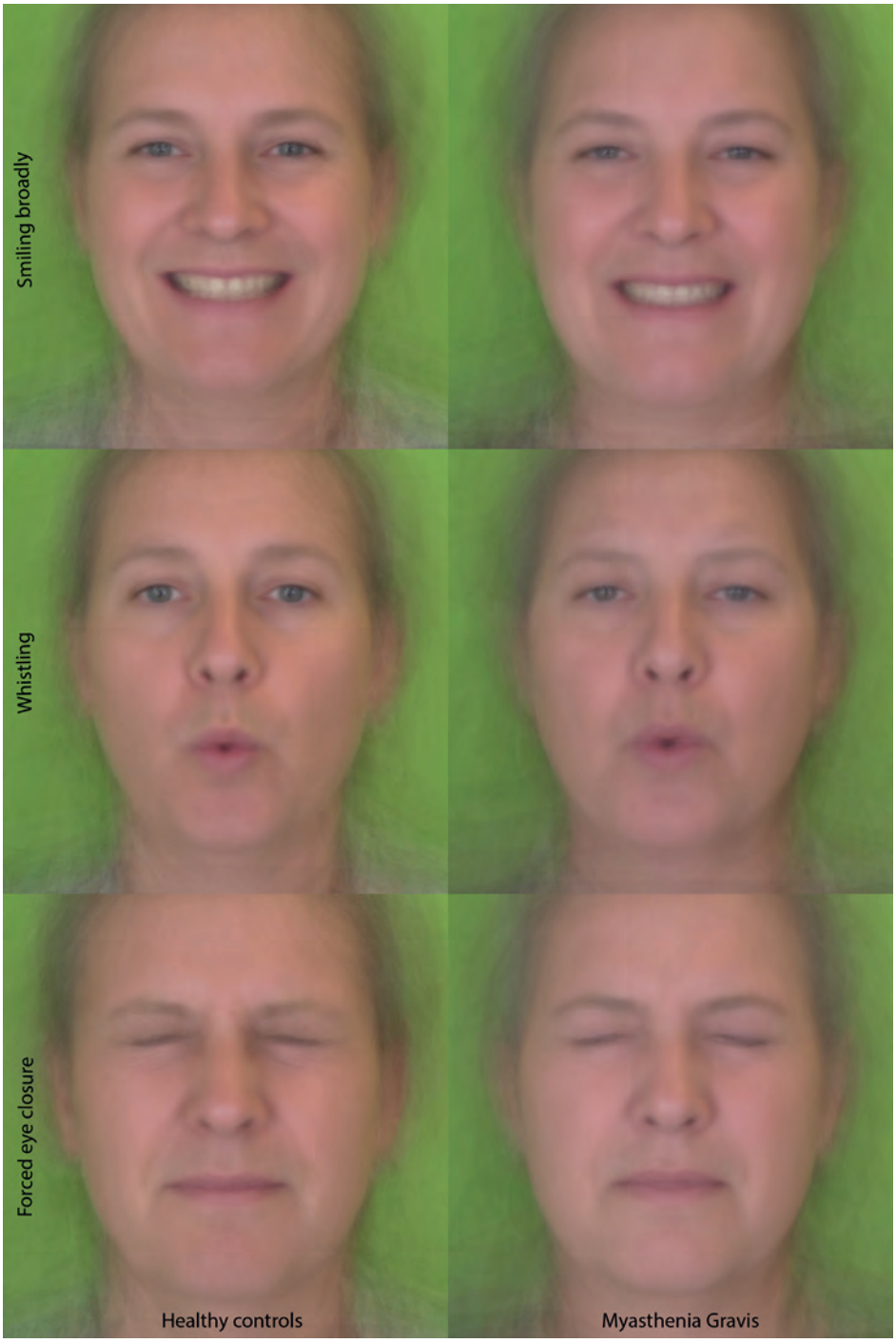
Smiling broadly
Whistling
Forced eye closure
Healthy controls
Myasthenia Gravis

Case summary

Myasthenia gravis (MG) is characterized by fluctuating weakness and fatigability of skeletal muscles. In most patients, extraocular and bulbar muscles are affected first, leading to diplopia, ptosis, and weakness of facial muscles[1]. We recorded the faces of 38 healthy controls and 52 patients with MG while performing a standardized set of facial expressions. These images were averaged using Python version 3.8.0. This resulted in highly similar faces with no recognizable individual features. The only discernible differences are typical clinical hallmarks of the disease: ptosis and generalized facial weakness around the eyes and mouth resulting in less vivid facial expression (figure).

References

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Chapter 7

Assessing facial weakness in myasthenia gravis with facial recognition software and deep learning

Ann Clin Transl Neurol. 2023 Aug;10(8):1314-1325

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Abstract

Background

Myasthenia gravis (MG) is an autoimmune disease leading to fatigable muscle weakness. Extra-ocular and bulbar muscles are most commonly affected. We aimed to investigate whether facial weakness can be quantified automatically and used for diagnosis and disease monitoring.

Methods

In this cross-sectional study we analyzed video recordings of 70 MG patients and 69 healthy controls (HC) with two different methods. Facial weakness was first quantified with facial expression recognition software. Subsequently, a deep learning (DL) computer model was trained for classification of diagnosis and disease severity using multiple cross-validations on videos of 50 patients and 50 controls. Results were validated using unseen videos of 20 MG patients and 19 HC.

Results

Expression of anger ($p=0.026$), fear ($p=0.003$) and happiness ($p<0.001$) was significantly decreased in MG compared to HC. Specific patterns of decreased facial movement were detectable in each emotion. Results of the DL model for diagnosis were as following: area under the curve (AUC) of the receiver operator curve 0.75 (95% CI 0.65-0.85), sensitivity 0.76, specificity 0.76 and accuracy 76%. For disease severity: AUC 0.75 (95% CI 0.60-0.90), sensitivity 0.93, specificity 0.63 and accuracy 80%. Results of validation, diagnosis: AUC 0.82 (95% CI: 0.67-0.97), sensitivity 1.0, specificity 0.74 and accuracy 87%. For disease severity: AUC 0.88 (95% CI: 0.67-1.0), sensitivity 1.0, specificity 0.86 and accuracy 94%.

Conclusions

Patterns of facial weakness can be detected with facial recognition software. Secondly, this study delivers 'proof of concept' for a DL model that can distinguish MG from HC and classifies disease severity.

Introduction

Myasthenia gravis (MG) is an autoimmune disease characterized by fluctuating muscle fatigability[1]. The prevalence of MG is low: approximately 1 to 2 per 10.000[1]. Although all striated muscles can be involved, the extra-ocular muscles are most commonly affected[1, 2]. Fluctuating asymmetric ptosis and diplopia are the first and predominant symptoms in a majority of patients, followed by weakness of bulbar muscles, resulting in facial weakness, amongst other symptoms[1, 3]. The diagnosis of MG is based on typical clinical features in combination with the presence of auto-antibodies against neuromuscular junction proteins, abnormal clinical neurophysiological tests or a positive neostigmine test[4]. Despite its typical semiology and a range of ancillary tests, diagnosing MG can be challenging. Up to 46% of MG patients do not receive the correct diagnosis within the first year of onset[5].

In addition, the fluctuating day-to-day severity of their muscle weakness is an issue of great concern for all patients with MG. Disease exacerbations, which can involve impaired breathing, leading to life-threatening situations, are almost always preceded by increased bulbar muscle weakness or worsening of primary symptoms[6]. These concerns are compounded by the fact that care for rare diseases is increasingly clustered in expert centers, leading to prolonged travel times to the hospital. A personal digital disease monitoring tool could improve outcomes by tracking symptoms and quality of life[7]. Such a tool could potentially be used for early detection or even prevention of exacerbations by allowing patients to making timely adjustments to their immune suppressant medication[8]. Artificial intelligence (AI) applications are an area of intense medical research, particularly to improve diagnosis and for home monitoring of disease severity[9]. However, to our knowledge AI applications are relatively scarce in the field of neuromuscular disorders so far and appear to be restricted to muscle imaging and gene profiling[10-14]. Assessment of facial weakness has thus far only been carried out in facial palsies and almost exclusively on photographs[15-20]. As facial features of MG patients are distinct from healthy subjects[21], we hypothesized that a short video recording of MG patients contains information that can be used both for diagnostic and monitoring purposes.

We aimed to 1) quantify and map patterns of facial weakness with the use of facial expression recognition software and 2) to develop a deep learning (DL) computer model for diagnosis and disease monitoring.

Material and Methods

This study was conducted between May 2019 and September 2020 at the Department of Neurology of the Leiden University Medical Center (LUMC), Leiden, the Netherlands and the Technical University Delft, the Netherlands.

Standard protocol approvals, registrations and patient consents
The ethics committee of the LUMC approved the study protocol. Written consent was obtained from all participants according to the declaration of Helsinki. A signed patient consent form has been obtained for publication of recognizable photographs.

Participants
Seventy MG patients and 69 age- and gender matched healthy controls (HC) were recruited from the Neurology outpatient clinic of the LUMC. Inclusion criteria were: age ≥ 18 years. Diagnosis of MG was based on clinical signs or symptoms supportive of MG and at least one of the following: a positive serologic test for AChR or MuSK antibodies and/or a diagnostic electrophysiological investigation supportive of the diagnosis myasthenia gravis and/or a positive neostigmine test. Participants were excluded if they were unable to give written informed consent or read Dutch or English video-instructions. Second, the presence of other medical conditions affecting the facial muscles was an exclusion criteria: e.g. active Graves' disease or unilateral facial paralysis. The use of corticosteroids was an exclusion criteria in the healthy control group.

All participants were included for quantification and mapping of facial weakness with the use of facial expression recognition software.
For the development of a DL model the first 50 MG patients and 50 HC were selected for training of the model. For external validation the remaining 20 MG patients and 19 HC were used.

Procedures
Videos were recorded with a 4K-camera in a standardized setting with assistance of the researcher, present during the entire recording. The subject was seated in front of a green screen with sufficient lighting. A computer screen displayed a slideshow of a model portraying 24 different facial expressions, which participants were asked to mimic with maximal intensity for the duration of each slide. The slideshow was accompanied by written Dutch instructions; verbal instructions were given when necessary. The slideshow contained the following expressions: neutral, eyes closed, right/left eye closed, eyes closed firmly, anger, fear, happiness, surprise, disgust, sadness, showing teeth, raising eyebrows, mouth

open, whistling, tongue protrusion (eight seconds each) and sustained gaze in eight directions (30 seconds for left and right gaze, four seconds for the other directions). Disease severity was measured using the quantitative MG score (QMG). All QMGs were performed approximately one hour before the recording by an experienced nurse who was trained and certified to perform QMG assessments. A QMG score of 0-9 was classified as mild and a QMG score >9 as moderate-severe disease[22]. The first five items of the QMG score were used as a degree of facial weakness (score 0-15). The current MG Foundation of America (MGFA) A (limb predominant) or B (bulbar predominant) classification was retrieved from patients' medical files. The cumulative prednisone dosage over the six months prior to participation was calculated because of the potential confounding effect of facial changes due to corticosteroids.

Quantification and pattern mapping of facial weakness

Quantification and mapping of facial weakness was assessed using FaceReader facial expression recognition software version 8 (Noldus Information Technology BV[23]) on the original video (resolution 3840x2160 pixels). This software classifies and quantifies the perceived expression of six emotions: anger, fear, happiness, surprise, disgust and sadness, by generating a numeric value between 0-1, every 80 ms throughout the video. Higher values correspond with more pronounced expression. The software also quantifies 20 individual action units (AUs, supplemental table 1) in a similar manner. AUs are part of the Facial Action Coding System (FACS)[24], developed for standardized assessment of facial behavior. Individual AUs correspond with a specific facial movement, which is related to activation of a certain muscle (E.g. AU1, movement: inner brow raiser, muscle: m. frontalis pars medialis. Supplemental table 1). The AU represents both the left and right side of the face.

For this study we compared mean scores between MG patients and HC of all six emotions, when prompted to mimic the corresponding emotion. Secondly, we compared median scores of all 20 individual AUs between the two groups during the expression of these six emotions. Both analyses were subsequently performed for disease severity.

Development of deep learning model

We used a three-dimensional (3D) convolutional neural network (CNN). The convolutional kernel size in a two-dimensional (2D) CNN is $N \times N$, corresponding to width and height ($W \times H$) of the image filter, where N determines the perception field in 2D. These 2D kernels can only capture spatial information in an image, which can be sufficient for many large-scale action recognition tasks[25, 26]. As muscle weakness temporally fluctuates in MG, we added temporal information as a third dimension, yielding a 3D CNN. We adopted an commonly used inflated 3D network (I3D)[27, 28] for capturing information in the temporal dimension T. The original settings were unaltered and available from the original sources[27, 28].

Different from a 2D CNN, the convolutional kernel in an I3D network is inflated to be $N \times N \times N$, which matches three dimensions: time, width and height ($T \times W \times H$). We evaluated three 2D CNN backbones into I3D architectures, namely ResNet-18, ResNet34 and ResNet-50[29]. The numbers indicate the depth of the ResNet architecture. Learned features of videos were flattened by a fully connected layer and a *Softmax* activation function was used to obtain the posterior probabilities $P(\hat{y}|x)$ of an input x. Posteriors are the output probabilities for each class of the neural network. The Cross-entropy loss measured the quality of prediction $\hat{y}$ by comparing it to the ground truth label y. The model was optimized by backpropagating the gradient of the Softmax loss function: $L_{\hat{y}y} = -\log(P(\hat{y}_c|x))$, where c indicates the class label and n the number of classes. y_c is the ground truth binary indicator of class c for input x.

To prevent overfitting, the model was pretrained using the Kinetics dataset[26]. Videos were cut into clips containing one specific expression manually by human visual assessment. Transition times between performed tasks were deleted. Duration of individual clips varied from two to thirty-three seconds, with a mean of six seconds. Resolution of clips was 256x256 pixels and no cropping was applied. Clips were processed per 16 batches (i.e., the gradients were aggregated and updated over 16 batches) and each batch contained 64 frames (frame rate video 32/sec), as the number of frames that can be processed in a batch is limited by the computational memory. Each batch contained frames from a single clip. Patient-level prediction was determined by the majority vote of all video clips per patient. Performance of the network is sensitive to hyperparameters[30-32]; we adopted the stochastic gradient descent with momentum as optimizer with the learning rate 0.01 and weight decay of 0.0001. We set the momentum parameter at 0.01.

For training, a three-fold cross-validation with a 2:1 split was applied for classification of diagnosis, with MG patients as the true class. For disease severity, a four-fold cross-validation with a 3:1 split was applied, with patients with QMG 0-9 as the true class. After completing the training phase, the model was applied to a completely new and unseen dataset of videos without any further modifications.

Comparison of the deep learning model and neurologists

Four neurologists specialized in neuromuscular disorders each rated 50 videos for diagnosis using a visual analog scale, resulting in a probability score between zero and one. They subsequently each rated 20 videos for disease severity (mild or moderate-severe disease) in a similar way. Videos were selected semi-randomized by the researcher: at least 20 healthy controls were included in the dataset for diagnosis. If a neurologist was familiar with a subject, this specific video was excluded. Datasets therefore differed between neurologists,

as different patients were excluded by each neurologist. The inter-rater reliability (IRR) scores were calculated for videos rated by two or all four neurologists. Mean scores were calculated if subjects were rated by more than one neurologist. Results of all four neurologists were pooled for comparison with the results of the deep learning model.

Statistical analysis

Quantification of six emotions and 20 different AUs every 80 ms with FaceReader resulted in 100 values between 0-1 for each individual AU and the emotion during the eight seconds that this expression was maintained. This data was transcribed to an expression-based time-weighted table in MATLAB (2020, MathWorks). To correct for potential outliers, 95th percentile scores of emotions were calculated during performance of the corresponding emotion, e.g. during expression of happiness, we calculated the 95th percentile score of the FaceReader-derived score for "happiness". Secondly, the 95th percentile score of each individual AU was calculated during the performance of each emotion. Per video, this resulted in one value for each emotion and six values for each of the 20 individual AU (one for each of the six emotions).

Statistical analyses were performed using IBM SPSS Statistics version 25.0. Group data are described by mean and standard deviation (±SD) for normally distributed data or median and interquartile range [IQR] for all other data. Unpaired T-tests or Mann-Whitney U tests were used for comparison between two groups, one-way ANOVA for >2 groups. Significance was accepted at $p<0.05$. Performance was determined by creating a receiver-operating characteristics (ROC) curve and determining the area under the curve (AUC), sensitivity, specificity and accuracy. A logistic regression analysis with all FaceReader variables that showed significant differences in the univariate analysis was performed to compare the overall performance of FaceReader with the deep learning model. The design of this study is exploratory, therefore we did not apply post-hoc correction for multiple testing.

Results

Baseline characteristics

Baseline characteristics are presented in table 1. Baseline characteristics of participants did not differ between videos assessed by the deep learning model and neurologists or between individual neurologists (supplemental table 2). Figure 1a-f shows screenshots of two expressions (neutral and left gaze) of three different participants: a HC, an MG patient with mild disease and one with moderate-severe disease.

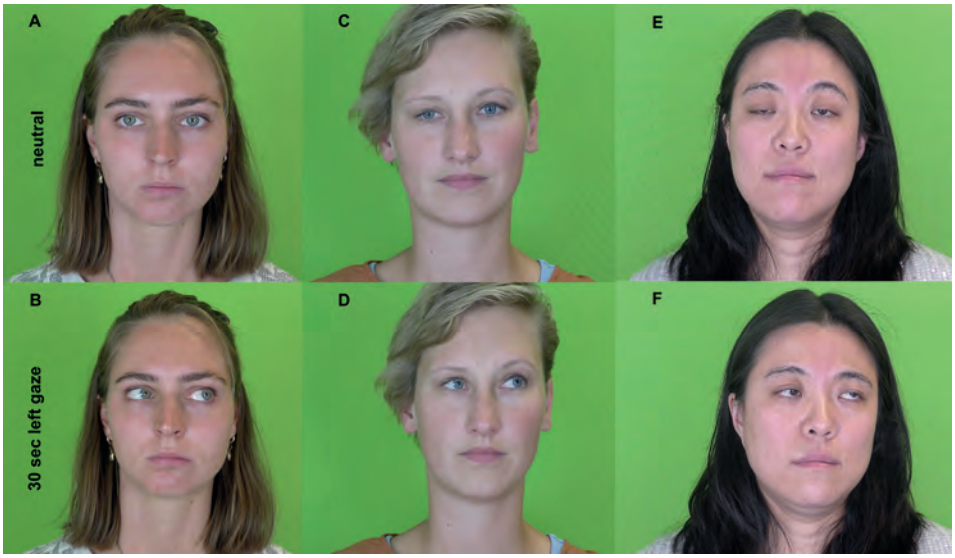

Figure 1. Video screen shots of three different participants.
a, b: Healthy control; c, d: MG patient with mild disease (total QMG score 9): slight right-sided ptosis; e, f: MG patient with moderate-severe disease (total QMG score 24): severe bilateral ptosis, compensatory raising of eyebrows, ophthalmoplegia, lower facial weakness.

Table 1. Baseline characteristics, grouped according to the groups used for the deep learning model.

	Patients training	Controls training	Patients validation	Controls validation	Sig.
Participants, n (% male)	50 (38%)	50 (38%)	20 (35%)	19 (42%)	-
Age, mean ±SD	54.7 ± 18.6[b]	49.4 ± 15.1[b]	55.3 ± 14.6[c]	48.1 ± 11.8[c]	0.910[a]; 0.731[a]
Disease duration in years, median [IQR]	5.8 [2.4-18.8]	-	4.5 [1.8-19.2]	-	0.687
Antibodies, %		-		-	-
· AChR	72%		90%		
· MuSK	14%		5%		
· Seronegative	14%		5%		
Total QMG score, mean ±SD	9.0 ± 4.8	-	11.0 ± 6.7	-	0.274
Missing, n	1		2		
Facial QMG score, mean ±SD	2.8 ± 2.4		3.4 ± 3.4		0.493
Facial QMG score 0-1 points, %	38%		41%		
Mild MG (QMG 0-9)					
· N, %	29 (59%)		10 (56%)		
· QMG, mean ±SD	5.6 ± 2.3		6.0 ± 1.9		0.648
· MGFA A/B, %	3% / 34%		20% / 10%		
Moderate-severe MG (QMG >9)					
· N, %	20 (41%)		8 (44%)		
· QMG, mean ±SD	13.8 ± 3.2		17.5 ± 4.2		**0.019**
· MGFA A/B, %	20% / 65%		25% / 63%		
Cumulative prednisone dosage (mg) past 6 months, median [IQR]		-		-	
· Mild	827.5 [0-2151][d]		0 [0-0][e]		**0.003**
· Moderate-severe	1471.6 [0-3758][d]		1362.5 [0-3356][e]		0.833

[a] first value represents between patient sets, second value between control sets
[b] no significant difference in age between patients vs controls in training set: p=0.117
[c] no significant difference in age between patients vs controls in validation set: p=0.099
[d] no significant difference in prednisone dosage between mild and moderate-severe patients in training set: p=0.323
e no significant difference in prednisone dosage between mild and moderate-severe patients in validation set: p=0.079
Abbreviations: QMG = quantitative myasthenia gravis score; MG = myasthenia gravis; MGFA = myasthenia gravis foundation of America (A = limb predominant, B = bulbar predominant) at the time of the video recording.

Quantification and mapping of facial weakness

The mean FaceReader-derived scores of the emotions anger, fear and happiness were significantly lower during the expression of these specific emotions in patients with MG, compared to HC (table 2, figure 2).

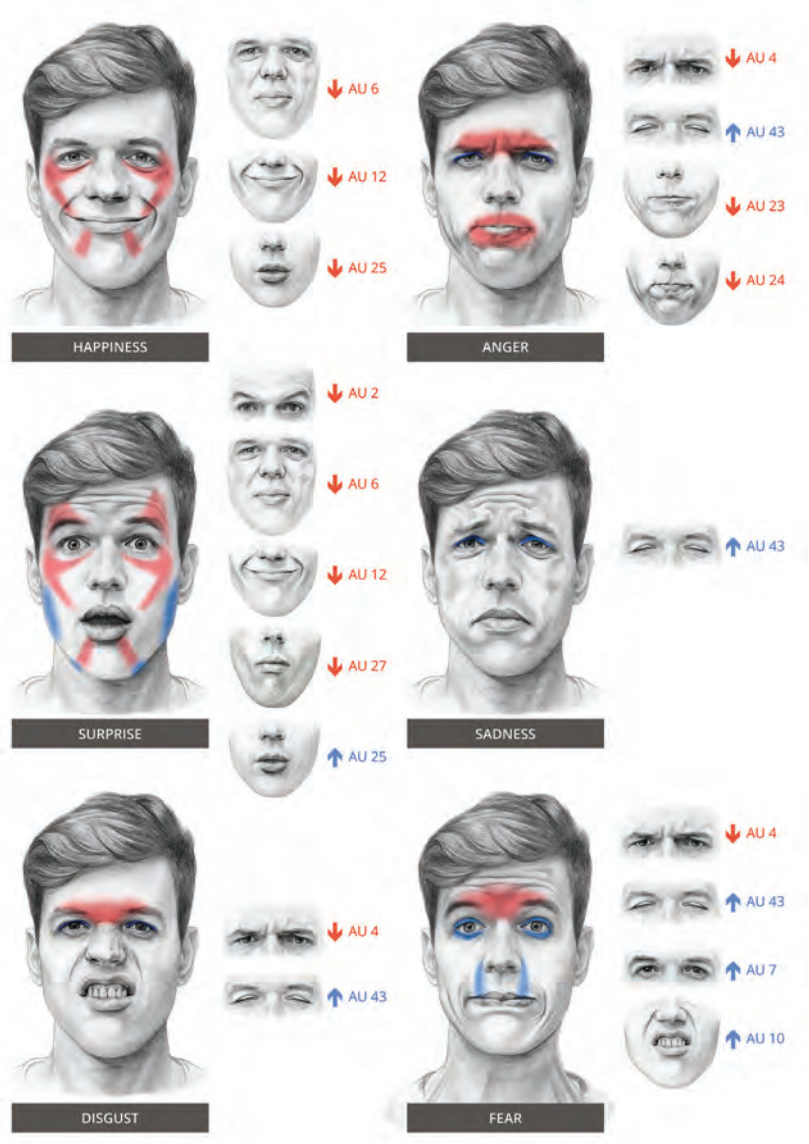

Figure 2. Receiver-operating curves of emotions anger, fear and happiness in myasthenia gravis compared to healthy controls.

Table 2. Quantification of six emotions in myasthenia gravis patients versus healthy controls (upper part table) and mild versus moderate-severe disease (lower part table).

Facial expression	Myasthenia gravis patients (n=70)	Healthy controls (n=69)	p-value
Anger, mean ±SD	0.33 ±0.26	0.45 ±0.32	0.026
Fear, mean ±SD	0.12 ±0.21	0.24 ±0.27	0.003
Happiness, mean ±SD	0.59 ±0.35	0.80 ±0.27	<0.001
Surprise, mean ±SD	0.44 ±0.34	0.40 ±0.34	0.422
Disgust, mean ±SD	0.42 ±0.31	0.45 ±0.35	0.604
Sadness, mean ±SD	0.42 ±0.34	0.49 ±0.34	0.263
Facial expression	Mild disease (n=39)	Moderate severe disease (n=28)	p-value
Anger, mean ±SD	0.32 ±0.24	0.33 ±0.28	0.906
Fear, mean ±SD	0.15 ±0.25	0.09 ±0.14	0.224
Happiness, mean ±SD	0.65 ±0.32	0.53 ±0.37	0.160
Surprise, mean ±SD	0.46 ±0.35	0.43 ±0.35	0.750
Disgust, mean ±SD	0.49 ±0.29	0.34 ±0.32	0.054
Sadness, mean ±SD	0.41 ±0.33	0.43 ±0.34	0.837

The AUC of the ROC curve of “anger” was 0.60 (95% CI 0.51-0.70). Sensitivity was 0.66, specificity was 0.54 and accuracy reached 58%. For “fear” the AUC of the ROC curve was 0.64 (95% CI 0.55-0.74). Sensitivity was 0.62, specificity 0.62 and accuracy reached 54%. For “happiness” the AUC was 0.71 (95% CI 0.62-0.79). Sensitivity was 0.75, specificity was 0.65 and accuracy reached 70%. There were no significant differences in mean scores of expressed emotions between patients with mild and moderate-severe disease (table 2). There was, however, a significant negative correlation between the expression of “disgusted” and the QMG score (-0.28, p=0.045).

Figure 3 summarizes all AUs that differed significantly between MG and HC during expression of the six emotions. Raw scores are available in supplemental table 3.
The following differences were observed between MG patients and HC. During expression of anger, brows were lowered less (AU4), lips were pressed and tightened less (AU23, 24) and eyelids were lowered more (AU43). During the expression of fear: brows were lowered less (AU4), eyelids were tightened more (AU7), the upper lip was raised more (AU10) and eyelids were lowered more (AU43). During the expression of happiness: cheeks were raised less (AU6), lip corners were pulled up less (AU12) and lips parted less (AU25). During the expression of surprise: the outer parts of the brows were raised less (AU2), cheeks were raised less (AU6), lip corners were pulled up less (AU12), lips parted less (AU25) and the mouth was stretched more (AU27). During the expression of disgust: brows were lowered less (AU4) and eyelids were lowered more (AU43). Sadness: eyelids were lowered more

(AU43). Logistic regression on the emotions and AUs with a significant difference between MG and HC, yielded a accuracy of 77%. The extensive output table, including individual odd ratios is available as supplemental table 4.
When comparing patients with moderate-severe disease to mild disease (supplemental table 3): during expression of happiness: cheeks raised less (AU6) and lip corners pulled up less (AU12). Disgust: lips parted less (AU25). Sadness: cheeks raised less (AU6). A negative correlation with QMG score was present for: anger AU6 (-0.274, p=0.025), happiness AU6 (-0.326, p=0.007), surprise AU6 (-0.293, p=0.017), sadness AU6 (-0.306, p=0.012). Logistic regression on the AUs with a significant difference between the two severity groups, yielded an accuracy of 77%. The extensive output table, including individual odd ratios is available as supplemental table 5.

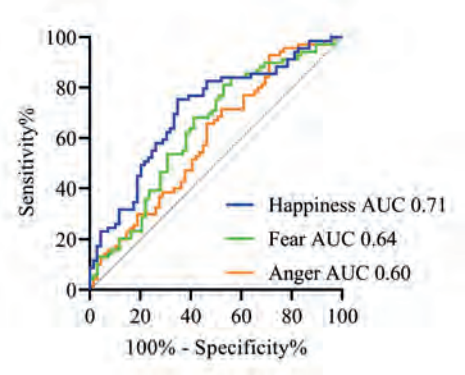

Figure 3. Patterns of weakness in myasthenia gravis during the expression of six emotions.
In red: decrease in AU compared to HC, in blue: increase in AU compared to HC. Smaller images on the right of each emotion show the individual AU with the corresponding movement.

Deep learning model

Highest performance was achieved using a 3D ResNet-50 for diagnosis and a 3D ResNet-34 for disease severity. Results of the multiple cross-validation on the training set were as follows: for diagnosis, the AUC was 0.75 (95% CI 0.65-0.85). Sensitivity and specificity were both 0.76, accuracy reached 76%. For disease severity, the AUC was 0.75 (95% CI 0.60-0.90). Sensitivity 0.93, specificity 0.63 and accuracy reached 80%. Of the incorrect classifications, 78% was incorrectly classified as mild disease severity. According to their MGFA classification: 1 (14%) was class I (pure ocular), 29% class A and 57% was class B (p=0.828). The remaining 22% was incorrectly classified as moderate-severe disease severity. All were MGFA class 0 (remission) according to their medical file. Overall, 44% of incorrect classifications occurred in MGFA class B, 33% in class 0/1 and 22% in class B (p=0.450).

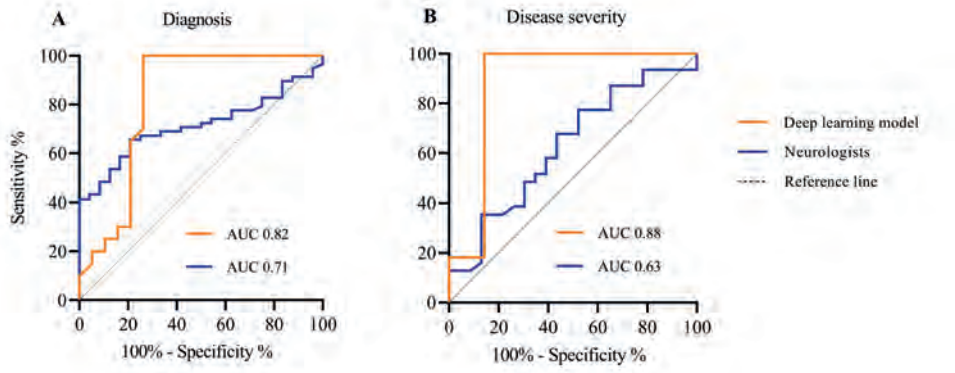

Figure 4. ROC curves deep learning model and neurologists. A: results for diagnosis, B: results for disease severity.

Application of the trained model to an unseen validation set of MG patients and HC yielded the following results (figure 4): for diagnosis, AUC was 0.82 (95% CI: 0.67-0.97). Sensitivity 1.0, specificity 0.74 and accuracy reached 87%. AUC for disease severity was 0.88 (95% CI: 0.67-1.0); sensitivity 1.0, specificity 0.86 and accuracy reached 94%. Only one incorrect classification occurred in the moderate-severe disease severity group, which was an MGFA class A patient.

Neuromuscular neurologists

Mean probability scores were calculated from results of all four neurologists combined. For diagnosis, AUC was 0.71 (95% CI: 0.61-0.82) (figure 4). Sensitivity and specificity were 0.69 and 0.67, respectively; accuracy reached 68%. AUC for disease severity was 0.63 (95% CI: 0.47-0.78). Sensitivity and specificity were 0.68 and 0.57, respectively, accuracy reached 63%. Of the incorrect classifications, 71% was incorrectly classified as mild disease severity. According to their MGFA classification: 35% was class 0 or I, 20% was class A and 45% was class B (p=0.368). The remaining 29% was incorrectly classified as moderate-severe disease severity. According to their MGFA classification: 50% was class 0 or I, 13% was class A and 38% was class B (p=0.461). Overall, 43% of incorrect classification occurred in MGFA class B, 39% in class 0/I and 18% in class B (p=0.125)

Performances of individual neurologists are displayed in figure 5 and supplemental table 6. For classification of diagnosis, only 18 videos were assessed by all four neurologists. The IRR for this subset, was 0.214. For disease severity, no videos were rated by all four neurologists. IRR for videos rated by two neurologists are displayed in table 3.

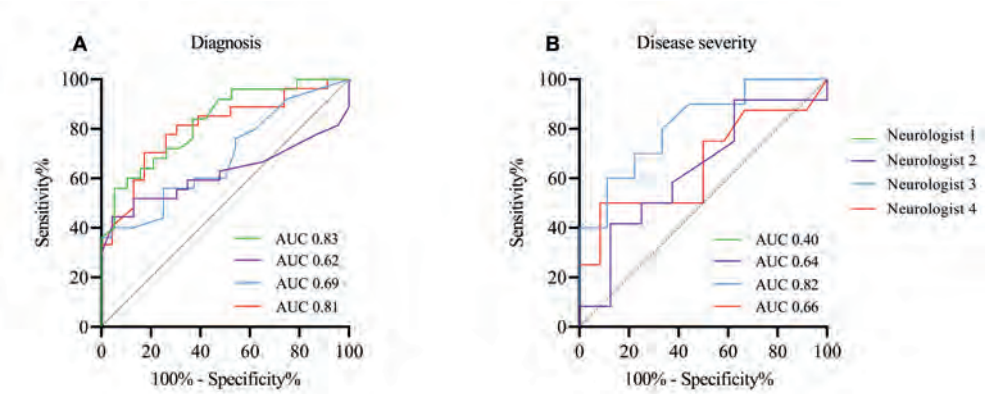

Figure 5. ROC curves individual neurologists. A results for diagnosis, B: results for disease severity. Neurologist 1 not plotted in B because of AUC <0.5.

Table 3. interrater-reliability scores for videos rated by two neurologists. Upper right part for classification of diagnosis, lower left part for classification of disease severity. N = the number of videos rated by both neurologists.

	Neurologist 1	**Neurologist 2**	**Neurologist 3**	**Neurologist 4**
Neurologist 1		0.418 (n=29)	0.138 (n=21)	0.528 (n=29)
Neurologist 2	NA (n=1)		0.513 (n=29)	0.592 (n=50)
Neurologist 3	NA (n=0)	-0.244 (n=4)		0.584 |(n=29)
Neurologist 4	NA (n=1)	0.316 (n=20)	0.239 (n=4)	

Discussion

To our knowledge, this is the first study using advanced computer analytics to quantify facial weakness in MG. We show that in a large group of chronic MG patients with relatively low QMG scores, overall weakness of facial muscles is a common occurrence. This is in accordance with previous research, which showed that more than 60% of MG patients reported facial weakness, dysarthria, and/ or weakness with chewing or swallowing[3]. This weakness can be quantified with the use of facial expression recognition software[23] and could potentially serve as a diagnostic tool and a biomarker for disease severity. In addition, a DL model trained on video data of facial expressions was capable of recognizing patients with MG and classifying disease severity with high accuracy.

Quantification of facial weakness

MG patients had a reduced expression of the emotions anger, fear and happiness, most likely due to weakness of the facial muscles. Facial expression is similarly affected in facial

palsies and facioscapulohumeral dystrophy and affects social interaction[15, 33, 34]. The effect of impaired facial expression on non-verbal communication in MG has not been previously studied. However, a negative impact on daily interaction and communication is to be expected. In five out of six emotions, activity was decreased in one or more AUs necessary to maintain that specific expression, e.g.: pulling of the lip corners was less pronounced during expression of happiness (AU12). Typical MG facial weakness was present in two-third of emotions: an increase in AU43 (eye closure), which was likely affected by ptosis. Unfortunately, FaceReader was not able to provide information on gaze deviations, which typically occur during persistent gaze[35].

FaceReader showed few differences between mild and moderate-severe disease, but AU6, corresponding to action of the cheek raiser or m. orbicularis oculi was significantly lower during two emotions in moderate-severe compared to mild patients and negatively correlated with the QMG score in four emotions. Weakness of the m. orbicularis oculi is common in MG[2] and AU6 could therefore be a potential biomarker for disease severity. However, we did not correct for multiple testing in this exploratory study and this would require further validation in a longitudinal study.

Comparison with previous studies on facial weakness

Previous publication on facial imaging in neuromuscular disorders have focused on facial palsies[15-18, 36] and commonly used photographs. Only one study used facial expression analysis software on video data for the quantification of basic facial expressions[15]. Facial imaging has also been used for diagnosis of genetic disorders[37-39]. In these studies, photographs were used to identify specific disorders based on typical facial dysmorphias. To our knowledge, no previous published study has made use of the temporal dimension in videos for the development of a DL model to assess facial movement and weakness in neuromuscular disorders. The main benefit of using videos over photos is the ability to capture typical effort-dependent weakness of facial muscles in MG. Ptosis is present in 66% of MG patients. It takes, on average, 28 seconds to develop[35] and it is more likely to become apparent on video recordings than on photographs.

For both diagnosis and classification of disease severity, the DL model performed better than four specialized neuromuscular neurologists, combined or individually. It should be noted that we asked both the model and the neurologists to assess an isolated phenomenon without any clinical context: a video of facial expressions. This differs significantly from clinical practice, in which a detailed history, physical examination and ancillary tests are essential elements for establishing the diagnosis. This is both a strength and weakness of our study. On one hand, it shows that assessment of a facial video alone could potentially

be sufficient to reach a diagnosis or estimate disease severity, although this would likely require an improved model, based on data from different populations and centers and a model trained to distinguish MG patients from disease controls. On the other hand, the inclusion of additional information would likely lead to even better results and would result in a more relevant comparison of the diagnostic performance of the DL model and clinical experts.

Interestingly, the diagnostic accuracy of the neurologists assessing facial videos was lower and showed more variation than expected. This may have been caused by the fact that neurologists were asked to perform an unfamiliar task: to assess an isolated phenomenon on a standardized video with no sound without any clinical context such as the patients history, physical examination or results from additional investigations. There were no differences in the occurrence of incorrect classifications between patients with a limb or bulbar predominant disease pattern, not for the DL model as well as the clinical experts. Applying a multivariate logistic regression analysis on all emotions and AUs with a significant difference, yielded similar results as the DL model during training: 77% versus 76% for diagnosis and 77% versus 80% for disease severity. However, validation of the DL model on unseen videos achieved higher accuracies. It would be of interest if the FaceReader software could detect differences in weakness over time.

Potential clinical applications

For diagnostic purposes, results of the DL model should be considered a 'proof of concept' as we included a relatively small number of MG patients from one center and no patients with other causes of facial weakness or 'MG-mimics', with and without steroids, such as facial nerve palsy, oculopharyngeal muscular dystrophy, motor neuron disease and chronic progressive external ophthalmoplegia. These limitations preclude the use of the current model in clinical practice.

For monitoring purposes, automated classification of disease severity that could be used by patients themselves in addition to standard care, could potentially lead to an improvement of clinical care. It could reduce the need to travel long distances for control visits, assist patients in adapting the dose of their maintenance medication according to prespecified personalized rules and potentially avoid hospitalization by immediately starting emergency treatment in case of an alarming deterioration. In this exploratory study have demonstrated that facial weakness is quantifiable in videos using facial expression recognition software. In addition, results of the DL model show that binary classification of disease severity is possible with high accuracy. Our results suggest that the degree of facial movements, as a reflection of facial weakness, is a good predictor for overall muscular weakness. This

supports the use of facial weakness to assess disease severity in MG. However, two important limitations remain: our results were obtained on a dichotomous classification instead of the continuous QMG score. Furthermore, these results should be validated in a longitudinal study to investigate the ability to detect changes within individuals.

Limitations

Identifying the elements in the data used by convolutional neural networks for classification is not straightforward. Although techniques such as class activation mapping have been developed to identify areas of interest in still images[40], class activation mapping algorithms for video data were not available at the time of these experiments. However, as all external factors (lighting, green screen, camera angle) were standardized, classification could not have been based on anything other than the face and its movement. In addition, automated analyses with FaceReader demonstrated quantifiable differences in facial expressions on which a deep learning could be trained.

Unexpectedly, performance of the current DL model was better on the unseen validation set than on the original training set. This is a surprising finding because the purpose of the additional validation set was to test whether training results could be reproduced with unseen data, not to further improve the model. There are two possible explanations for this finding. The QMG score of patients with moderate-severe disease was significantly higher in the dataset used for validation compared to the training set. This potentially made it more easy to detect a difference in disease severity or to discriminate patients from HC in this dataset. Alternatively, the observed higher diagnostic yield may have been a spurious result caused by the relatively small sample-size of the validation set. Given the rarity of MG, this was the maximum number of patients we were able to include within a reasonable time frame.

Finally, because of the exploratory nature and relatively small sample size of this study, no covariates were used in the analysis. However, several covariates that affect the performance of facial recognition algorithms have been identified[41]. These covariates include age, sex and ethnicity. In short, older people are easier to recognize by facial recognition software than younger people[41]. There is also an effect of sex on the accuracy of facial recognition software but there is disagreement whether men or women are more easily recognized, and this effect decreases with increasing age[41]. Ethnicity can affect the performance of facial recognition software as ethnic differences in facial characteristics may lead to different results. Furthermore, an algorithm trained on a specific ethnic group may yield incorrect results when applied to people of a different background[41]. Participants included in this study were a reflection of the Dutch population and therefore mostly of European descent,

although this was not formally assessed. Therefore, caution is advised when extrapolating these results to patients groups of other ethnicities.

Due to the exploratory nature of this study, we did not apply a post-hoc correction. A post hoc Bonferroni correction on the quantification of facial weakness (FaceReader) would have resulted in significance levels of 0.0004 for the AUs and 0.008 for the six emotions.

Acknowledgments
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Supplemental files

Supplemental table 1. Action units according to Facial Action Coding System.

Action unit	Name	Corresponding facial muscle(s)
01	Inner brow raiser	M. frontalis (pars medialis)
02	Outer brow raiser	M. frontalis (pars lateralis)
04	Brow lowerer	M. depressor glabellae, m. depressor supercilli, m. corrugator supercilli
05	Upper lid raiser	M. levator palpebrae superioris, m. superior tarsalis
06	Cheek raiser	M. orbicularis oculi (pars orbitalis)
07	Lid tightener	M. Orbicularis oculi (pars palpebralis)
09	Nose wrinkler	M. levator labii, superioris alaeque nasi
10	Upper lip raiser	M. levator labbii superioris (caput infraorbitalis)
12	Lip corner puller	M. zygomaticus major
14	Dimpler	M. buccinator
15	Lip corner depressor	M. depressor anguli oris
17	Chin raiser	M. mentalis
18	Lip pucker	M. incisivus labii superioris/inferioris
20	Lip stretcher	M. risorius, m. platysma
23	Lip tightener	M. orbicularis oris
24	Lip pressor	M. orbicularis oris
25	Lips part	M. depressor labii inferioris; **relaxation** m. mentalis/ m. orbicularis oris
26	Jaw drop	**Relaxation** m. masseter, m. temporalis, m. pterygoid internus
27	Mouth stretch	M. pterygoids, m. digastricus
43	Eyes closed	**Relaxation** m. levator palpebrae superior

Supplemental table 2. Baseline characteristics of patients assessed by neurologists versus deep learning model (validation)
a first p-value represents deep learning model versus pooled neurologists, second value represents between individual neurologists

	Deep learning model	Pooled neurologists	Neurologist 1	Neurologist 2	Neurologist 3	Neurologist 4	P-value[a]
Age, mean ±SD	51.7 ± 13.6	53.8 ± 16.5	52.9 ± 16.2	53.8 ± 17.3	54.7 ± 15.3	53.8 ± 17.3	0.461; 0.961
Total QMG score, mean ±SD	11 ± 6.7	9.3 ± 4.4	9.2 ± 4.6	9.0 ± 3.8	10.2 ± 5.6	9.0 ± 3.8	0.329; 0.810
· % mild	55.6%	55.7%	55%	60%	47.4%	60%	0.991; 0.840

Supplemental table 3. Significant individual action units (median [IQR]) per basic emotion

Emotion	Action unit	MG median [IQR]	HC median [IQR]	Activity action unit in MG	P-value
Anger	4	0.32 [0.13-0.54]	0.50 [0.23-0.76]	Decrease	0.002
	23	0.00 [0.00-0.00]	0.00 [0.00-0.03]	Decrease	0.032
	24	0.00 [0.00-0.00]	0.00 [0.00-0.02]	Decrease	0.028
	43	0.01 [0.00-0.13]	0.00 [0.00-0.04]	Increase	0.042
Fear	4	0.01 [0.00-0.16]	0.06 [0.00-0.19]	Decrease	0.041
	7	0.00 [0.00-0.10]	0.00 [0.00-0.01]	Increase	0.024
	10	0.00 [0.00-0.02]	0.00 [0.00-0.00]	Increase	0.025
	43	0.00 [0.00-0.01]	0.00 [0.00-0.00]	Increase	0.002
Happiness	6	0.10 [0.02-0.21]	0.15 [0.07-0.32]	Decrease	0.024
	12	0.30 [0.09-0.53]	0.55 [0.38-0.72]	Decrease	<0.001
	25	0.25 [0.00-0.84]	0.77 [0.23-0.93]	Decrease	0.001
Surprise	2	0.13 [0.00-0.37]	0.27 [0.01-0.62]	Decrease	0.045
	6	0.00 [0.00-0.00]	0.00 [0.00-0.02]	Decrease	0.022
	12	0.00 [0.00-0.10]	0.07 [0.00-0.20]	Decrease	0.002
	25	0.34 [0.22-0.65]	0.56 [0.25-0.83]	Decrease	0.046
	27	0.00 [0.00-0.09]	0.00 [0.00-0.00]	Increase	0.050
Disgust	4	0.11 [0.00-0.24]	0.17 [0.07-0.39]	Decrease	0.039
	43	0.00 [0.00-0.09]	0.00 [0.00-0.00]	Increase	<0.001
Sadness	43	0.01 [0.00-0.14]	0.00 [0.00-0.03]	Increase	0.006
Emotion	**Action unit**	**Mild disease median [IQR]**	**Moderate-severe disease median [IQR]**	**Activity action unit in moderate-severe MG**	**P-value**
Happiness	6	0.18 [0.08-0.25]	0.06 [0.00-0.16]	Decrease	0.002
	12	0.43 [0.21-0.56]	0.20 [0.01-0.44]	Decrease	0.032
Disgust	25	0.30 [0.00-0.74]	0.03 [0.00-0.25]	Decrease	0.030
Sadness	6	0.04 [0.00-0.10]	0.00 [0.00-0.01]	Decrease	0.011

Supplemental table 4. Variables in logistic regression myasthenia gravis versus healthy controls

Variable	B	S.E.	Wald	Sig.	Exp(B)
Anger	-0,357	1,013	0,124	0,725	0,700
Fear	-0,901	1,113	0,655	0,418	0,406
Happiness	1,609	1,549	1,078	0,299	4,995
Anger AU4	0,051	1,084	0,002	0,962	1,053
Anger AU23	0,575	4,337	0,018	0,894	1,778
Anger AU24	-1,391	4,06	0,117	0,732	0,249
Anger AU43	-0,166	2,545	0,004	0,948	0,847
Fear AU04	-6,637	2,775	5,719	**0,017**	0,001
Fear AU07	8,554	3,373	6,432	**0,011**	5184,996
Fear AU10	5,366	4,262	1,585	0,208	213,905
Fear AU43	6,493	6,578	0,974	0,324	660,772
Happiness AU06	2,422	2,122	1,302	0,254	11,263
Happiness AU12	-7,806	2,807	7,736	**0,005**	0,000
Happiness AU25	-0,439	0,885	0,246	0,620	0,645
Surprise AU02	-0,696	0,859	0,657	0,418	0,499
Surprise AU06	-18,493	8,955	4,265	0,039	0,000
Surprise AU12	-0,295	2,53	0,014	0,907	0,745
Surprise AU25	1,026	1,111	0,853	0,356	2,790
Surprise AU27	2,22	1,395	2,531	0,112	9,207
Disgust AU04	2,139	1,519	1,984	0,159	8,493
Disgust AU43	11,676	5,809	4,04	**0,044**	117697,142
Sadness AU43	-1,242	3,229	0,148	0,700	0,289
Constant	1,486	0,732	4,124	0,042	4,418

Supplemental table 5. Variables in logistic regression patients with mild versus moderate-severe disease

Variable	B	S.E.	Wald	Sig.	Exp(B)
Happiness AU06	-8,646	3,811	5,147	0,023	0,000
Happiness AU12	-0,448	1,337	0,112	0,738	0,639
Disgust AU25	-2,764	1,215	5,175	0,023	0,063
Sadness AU06	-4,892	5,554	0,776	0,378	0,008
Constant	1,888	0,671	7,912	0,005	6,605

Supplemental table 6. Performance of neuromuscular neurologists.

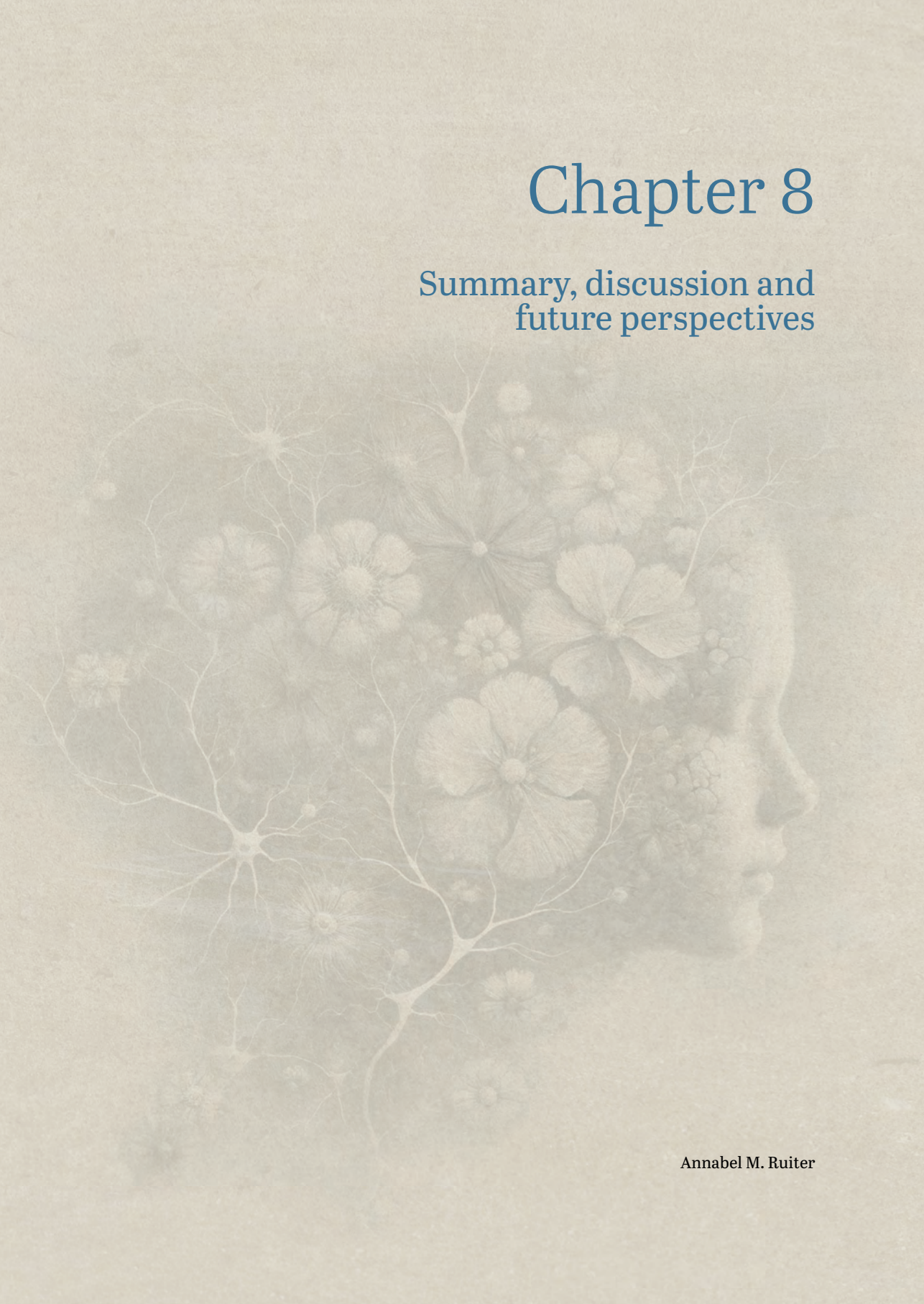
	Accuracy Sensitivity; specificity	
	Diagnosis	**Disease severity**
Neurologist 1	72.7% 0.72; 0.74	57.9% 0.63; 0.44
Neurologist 2	64.0% 0.63; 0.52	60.0% 0.58; 0.63
Neurologist 3	61.2% 0.60; 0.63	73.7% 0.78; 0.70
Neurologist 4	76.0% 0.78; 0.74	75.0% 0.92; 0.50

Part three

Epilogue

Chapter 8

Summary, discussion and future perspectives



Annabel M. Ruiter

Summary, discussion and future perspectives

Myasthenia gravis (MG) is an antibody-mediated autoimmune disease affecting the neuromuscular junction (NMJ) of the peripheral nervous system, leading to fatigable muscle weakness. MG with acetylcholine receptor antibodies is the most prevalent form of MG. Its most typical hallmark a fluctuating asymmetric weakness of the eyelids and diplopia[1].
This thesis consisted of two parts. The first part covers the subject of central fatigue in MG: its prevalence, pathophysiology and burden for patients. The second part focusses on artificial intelligence for diagnosis and disease monitoring of MG through automated assessment of facial weakness.

Part one: Fatigue in myasthenia gravis

Chapter two describes the first results of the Dutch – Belgian Myasthenia Gravis registry. This registry uses a unique combination of patient-reported annual questionnaires together with physician-reported medical information, which is requested once at entry of the registry. Results of 565 MG patients and 38 Lambert-Eaton myasthenic syndrome patients provided insights on disease course which were in line with previously published data[2, 3]. We presented several novel insights regarding the Dutch MG cohort. One important finding was the high rate of potentially steroid-induced comorbidities, including hypertension and type 2 diabetes. This finding emphasizes the importance of starting non-steroid immune suppressive medication early. Besides longitudinal information on disease course, the registry also provided insights in disease burden. Surprisingly, the most limiting symptom reported by MG patients was not muscle weakness or diplopia, but fatigue. In neuromuscular diseases in general, a differentiation has been proposed between peripheral fatigue (muscle weakness and fatigability) and central fatigue (mental or cognitive fatigue)[4, 5]. Central fatigue is defined as an experienced lack of energy to sustain physical of mental tasks. A common hypothesis is that central fatigue serves to protect muscles from (further) damage by down-regulating activities, physically as well as mentally[4, 5]. Unfortunately, this patient-reported questionnaire did not refer to a specific type of fatigue. Along with other symptoms, patients were asked if they suffered from fatigue: 'yes', 'no' or 'I don't know'. Subsequently, they could indicate which symptom was experienced as the most limiting. In the following chapters we further focus on central fatigue.

Chapter three is a systematic review summarizing the MG-related literature on all aspects of central fatigue. Of 445 studies produced by the initial systematic search, 21 remained for final inclusion. Most studies were case control or case series. The sample sizes varied between 8 and 257 participants. In all studies, fatigue was assessed with the use of a variety of patient-reported questionnaires. The prevalence of fatigue in MG patients ranged between

44 and 82%. Secondly, fatigue was also highly prevalent in patients with a low disease severity, for example in patients in remission. In the control groups, the prevalence of fatigue was significantly lower and varied between 18-40%. The prevalence of fatigue in MG was in concordance with other chronic neuromuscular disorders, such as facioscapulohumeral muscular dystrophy (FSHD) and myotonic dystrophy, but also with other autoimmune diseases, like rheumatoid arthritis (RA) and inflammatory bowel disease (IBD)[6-8]. In MG, fatigue was strongly associated with disease severity, depressive symptoms and female gender, and correlated with a lower quality of life. Literature on the treatment of fatigue in MG is scarce. There is limited evidence on the benefits of a physical and psychological training program, however this was a very small study (n=9)[9]. To date, there is no evidence supporting the treatment of fatigue as a separate symptom besides muscle weakness. Previous studies found a significant improvement in patients treated with eculizumab as well as with intravenous immunoglobulins, plasma-exchange or prednisone[10, 11]. However, adjustment for disease severity was not applied, therefore the observed improvement of fatigue may have been the results of improvement of MG symptoms.

In **chapter four** we focussed on a large representative cohort of Dutch MG patients to assess the relationship between central fatigue and several factors, including disease severity and quality of life but also physical activities and coping strategies. A total of 420 MG patients participated in this study, making this the largest MG fatigue study so far. Severe fatigue was present in 62% of MG patients. We found that fatigue was positively correlated with disease severity, female gender, body mass index (BMI) and depressive symptoms. This was comparable with previous fatigue studies in MG, described in the systematic review but also with other autoimmune diseases, like rheumatoid arthritis (RA)[7]. These findings suggest a common pathophysiology for fatigue in chronic (autoimmune) disorders in contrast to a MG specific pathway. Next, we found that performing strenuous activities, such as running or cycling at an increased pace, was strongly correlated with lower fatigue scores. Although most patient (75%) did not perform any form of strenuous activities, this correlation was even found in patients performing this type of activity only once a week. One could suggest that patients with a low disease severity would probably be able to perform these type of activities due to better muscle condition, whereas a higher disease severity leads to a more inactive lifestyle. However, this correlation presented in **chapter four** is after adjustment for disease severity, BMI and age. This beneficial effect was not found for moderate physical activities, such as walking or cycling on a regular pace, even when these activities were carried out more than 150 minutes daily. This effect of moderate and strenuous physical activity on fatigue has not been systematically assessed in previous MG studies but comes as no surprise, since physical training programs are beneficial to fatigue in other neuromuscular disorders, including FSHD and chronic inflammatory

demyelinating polyneuropathy[12, 13]. Another novel finding in this study was the difference in coping styles between fatigued and non-fatigued patients. Fatigued patients scored significantly higher on passive coping strategies compared to non-fatigued patients. Passive coping strategies are linked to expressing physical and mental distress, as well as denying the presence of stressful events, abandoning efforts to achieve goals in stressful situations, and intensifying feelings of stress[14]. To our knowledge, coping strategies have not been previously studied in relation to fatigue in MG or other chronic/ autoimmune diseases. There is very limited literature on coping strategies associated with fatigue. In a systematic review on breast cancer patients, among other things, subjective/ perceived stress was associated with higher levels of fatigue in different stages of breast cancer[15]. This is in line with our findings.

In **chapter five** we aimed to gain a better understanding on the pathophysiology of central fatigue in MG by searching for a potential circulating serum biomarker. One hypothesis is that central fatigue in autoimmune diseases is caused by systemic inflammatory cascades which are triggered by a local peripheral inflammatory process[5]. In case of MG, local inflammation at the NMJ might trigger these systemic cascades. In a cohort of 116 anti-acetylcholine receptor (AChR) positive patients, we found a robust positive correlation between fatigue and C-reactive protein (CRP), after adjustment for several pre-selected covariates like disease severity and cumulative prednisolone dosage and the use of non-steroid immune suppressive medication. This correlation continued to exist after post-hoc adjustment for BMI and strenuous physical activities. Our findings suggest that low grade inflammation is part of the pathogenesis of central fatigue and supports the hypothesis that local peripheral inflammation is a trigger for central fatigue. There is no previous literature on biomarkers for central fatigue in MG but the results of our study are in accordance with results on biomarkers for fatigue in other autoimmune diseases. Both in RA and IBD, a positive correlation between fatigue and CRP levels has been observed[16, 17]. In addition, this correlation has also been observed in very large general population cohort studies[18, 19], and there are even reports that CRP can be predictive for the development of future fatigue[20]. A repeated blood sample was collected in 88 MG patients (76% of the initial cohort), but unfortunately we could not reproduce our initial correlation between fatigue and CRP. This is most likely due to a lack of statistical power for the repeated measurement: the number of participants at the second visit was below the predetermined sample size. Secondly, the repeated measurement contained two apparent outliers with high CRP values and low fatigue scores, which due to the relatively small sample size had a substantial influence on the correlation. Longitudinal fatigue scores were collected in 104 MG patients (90% of the initial cohort). We could not replicate the predictive value of CRP for the development of future fatigue in this subgroup as demonstrated in a previous large general

cohort study of almost 3000 participants[20]. CRP production is mostly stimulated by interleukin 6 (IL-6). Previous research in different cohorts of non-MG patients, including a large general population cohort, cancer and chronic haemodyalisis[18, 21, 22], showed a correlation between fatigue and IL-6. Studies in RA even showed an improvement of fatigue in patients treated with IL-6 inhibitors[23, 24]. In addition, there is evidence that IL-6 plays a key role in the pathogenesis of MG[25-28]. This supports the relevance of our observed correlation between fatigue and CRP, which is a product of IL-6 stimulation. In **chapter four** we found that an active lifestyle including regular strenuous physical activities, was correlated with significant lower fatigue scores. Under normal circumstances, baseline IL-6 levels are higher in persons who have an inactive lifestyle compared to persons who frequently engage in physical activities[29, 30]. Although this has not been studied in MG or other diseases, we hypothesize that the beneficial effect of physical training programs on fatigue could at least partly be caused by a decrease in baseline levels of IL-6 as a result of these training programs.

Future perspectives

Central fatigue is an important and prevalent symptom in MG, that contributes to a lower quality of life. Consequently, its acknowledgement is of utmost importance. A significant amount of evidence points towards a role of physical activities for the improvement or even prevention of central fatigue. Therefore, a detailed clinical trial should be executed to study the effects of a strenuous training program in an adequately sized cohort. Due to different coping strategies in fatigued patients, it is essential to combine this with some sort of cognitive behavioral therapy, especially to provide tools to incorporate a more active lifestyle in their daily life after the trial has finished. During this trial repeated blood samples could provide more insights in the serum levels of CRP, IL-6 and others, before, during and after physical training sessions, and the effect of strenuous activity on baseline IL-6 levels in MG. Next, future research should explore the effect of immunosuppressive therapy, in severely fatigued patient, even when their condition is clinically stable or in (pharmacological) remission. While previous studies have demonstrated that improvements in disease severity often correlate with reduced fatigue, the question remains if it is appropriate to initiate or intensify treatment solely to address fatigue.

Part two: Faces of myasthenia gravis

Chapter six presents an image of the face of myasthenia gravis. Photos of 52 patients with MG and 38 healthy controls were averaged to compose one face expressing different facial expressions. The averaged images resulted in highly similar faces in which the MG face shows the typical clinical hallmarks of MG: ptosis and generalized facial weakness. This

averaged images formed the basis for **chapter seven**, in which we aimed to quantify and map patterns of facial weakness with facial expression recognition software. The second aim was to develop a deep learning (DL) computer model to classify diagnosis and disease severity. In this cross-sectional study we made video-recordings of 70 MG patients and 69 healthy controls (HC). A short video was recorded of each participant expressing a standardized set of 24 different facial expressions. An analysis of these videos with facial expression recognition software demonstrated a significantly decreased expression of anger, fear and happiness in MG patients compared to HC. This indicated that specific patterns of facial weakness are detectable in MG with facial analysis software. Impairments in facial expressions are also seen in other disorders affecting facial muscles, like facial palsies and facioscapulohumeral dystrophy and are associated with a lower quality of life due to their effects on social interactions[31-33]. Although we did not assess secondary outcomes such as quality of life or depressive symptoms, it is reasonable to assume that this decrease in facial expressiveness may significantly influence an individual's social well-being. A more recent study reported that MG patients performed worse on facial expression recognition compared to HC[34]. The authors hypothesize, speculatively, that MG patients might exhibit impairments in their ability to recognize emotions, potentially due to disruptions in the integration of bodily sensations and sensory inputs crucial for emotional processing. Our DL model was able to classify diagnosis and disease severity as a dichotomous outcome with considerable specificity and sensitivity. These results are promising and provide a first 'proof of concept', however further finetuning is required before this model can be used as a clinical tool.

Future perspectives

Chapter seven provides a 'proof of concept' that advanced computer technologies can be used for quantification and classifying diagnosis and disease severity of MG. Given the invasive and laborious nature of advanced diagnostic techniques now available for MG, including antibody testing and repetitive nerve stimulation, a user-friendly application for tracking disease fluctuations would be significantly more valuable. Such an application could contribute to better disease insights for patients, fewer unnecessary consultations and enhance the efficiency of MG care. Usual care typically involves outpatient consultations two or possibly three times a year, depending on the severity of the patient's disease and their response to treatment. A smartphone application could be used to conduct daily or weekly evaluations of disease severity, providing highly detailed information that is currently unavailable through standard care. This data may teach us more on the natural course of MG, potentially enabling us to predict exacerbations and tailor immunosuppressive therapy

more precisely to individual patients. Our study provides a first step, but further training and development of the model is necessary to result in more detailed information on disease severity. The model should incorporate an assessment of facial expressions, ideally focusing on a limited number of core-expressions. Additionally, future studies need to determine whether integrating patient-reported data and a brief strength assessment would enhance the model's effectiveness as an intuitive monitoring tool. Recent research demonstrated that an artificial intelligence model was able to successfully distinguish MG patients from healthy controls by analysing specific parts of the MG-core examination conducted via telemedicine[35]. This indicates that specific MG examinations could have added value for the effectiveness of our model as well.

Overall conclusion

The work presented in this thesis contributed to the knowledge of central fatigue in MG, which is a prevalent and important symptom leading to a diminished quality of life. A multifactorial origin is likely, due to the strong correlation with several factors, like disease severity, female gender and depressive symptoms, similar to fatigue in other autoimmune or neuromuscular diseases. In this multifactorial pathogenesis, low-grade inflammation plays a role of which the degree of contribution must be further determined. The results discussed in this thesis showed significant lower reported fatigue scores in patients who engaged in strenuous physical activities. This offers possibilities for a potential treatment of fatigue in MG and as a result of these findings, the MG research team of the LUMC is currently conducting a clinical trial to further study the effects of both a physical training program and cognitive behavioural therapy on central fatigue. This possible beneficial effect of training may be partially be explained by reduction of low-grade inflammation through physical activities, though this hypothesis requires further investigation.
In the second part of this thesis we demonstrated that typical patterns of facial weakness in MG can be captured visually in photos and videos. Novel in MG, we showed a 'proof of concept' of a DL model for classification of diagnosis and disease severity. The results of this study are promising for the development of a future (smartphone based) monitoring tool with an intuitive user interface, aligning with the broader trend of utilizing digital technologies to improve MG care.

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Chapter 9

Nederlandse samenvatting

Annabel M. Ruiter

Nederlandse samenvatting

Myasthenia gravis (MG) is een antilichaam-gemedieerde auto-immuunziekte, die de neuromusculaire overgang van het perifere zenuwstelsel aantast, wat leidt tot inspanningsgebonden spierzwakte. MG veroorzaakt door antistoffen tegen de postsynaptische acetylcholine receptor is de meest voorkomende vorm. Het meest kenmerkende symptoom van AChR MG is een fluctuerende asymmetrische zwakte van de oogleden en diplopie (dubbelzien)[1]. Dit proefschrift bestaat uit twee delen. Het eerste deel behandelt het onderwerp centrale vermoeidheid bij MG: de prevalentie, pathofysiologie en impact voor patiënten. Het tweede deel richt zich op het gebruik van kunstmatige intelligentie voor het stellen van de diagnose en ziektemonitoring van MG op basis van gelaatszwakte.

Vermoeidheid in myasthenia gravis

Hoofdstuk twee beschrijft de eerste resultaten van het Nederlands – Belgisch myasthenia gravis register. Dit register maakt gebruik van een unieke combinatie van patiënt gerapporteerde jaarlijkse vragenlijsten aangevuld met medische informatie beschikbaar gesteld door de behandelend arts. Deze medische informatie werd eenmalig bij de aanmelding voor het register opgevraagd. De resultaten van 565 MG patiënten en 38 patiënten met het Lambert-Eaton myastheen syndroom (LEMS) geven inzicht in het beloop van de ziekte en is deels in lijn met resultaten uit andere studies. We hebben een aantal nieuwe bevindingen beschreven met betrekking tot het Nederlandse MG-cohort. Een belangrijke bevinding was het hoge aantal potentieel steroïde-geïnduceerde comorbiditeiten, zoals hypertensie en diabetes mellitus type 2. Deze bevinding benadrukt het belang van het vroegtijdig starten met steroïdsparende immunosuppressieve medicatie. Naast longitudinale informatie over het ziektebeloop, bood het register ook inzicht in de ziektelast. Verrassend genoeg was de meest beperkende klacht die door MG-patiënten werd gerapporteerd niet spierzwakte of dubbelzien, maar vermoeidheid. **Hoofdstuk drie** is een systematische review waarin alle aspecten van vermoeidheid in MG worden samengevat. De initiële zoekvraag leverde 445 MG studies op waarvan er uiteindelijk 21 overbleven voor de definitieve inclusie. De meeste studies waren case-control of case-series met een groepsgrootte variërend tussen de 8 en 257 deelnemers. In alle studies werd vermoeidheid beoordeeld met behulp van verschillende door patiënten gerapporteerde vragenlijsten. De prevalentie van vermoeidheid bij MG-patiënten varieerde tussen 44 en 82%. Opvallend genoeg was vermoeidheid ook vaak aanwezig bij patiënten met een lage ziekteactiviteit, bijvoorbeeld bij patiënten in remissie. In de controlegroepen was de prevalentie van vermoeidheid aanzienlijk lager en varieerde deze tussen 18-40%. Vermoeidheid in MG was sterk geassocieerd met ziekte-ernst, depressieve symptomen, het vrouwelijk geslacht en zorgt daarnaast voor een lagere kwaliteit van leven. Er was weinig literatuur over de

behandeling van vermoeidheid te vinden, maar er zijn aanwijzingen, dat fysieke training en/of cognitieve gedragstherapie vermoeidheid kunnen verbeteren. Enkele studies vonden tevens verbetering van vermoeidheid na behandeling met eculizumab, intraveneuze immuunglobulines, plasmaferese of prednison. In **hoofdstuk vier** hebben we in een grote groep Nederlandse MG patiënten onderzoek gedaan naar de relatie tussen vermoeidheid en diverse factoren, waaronder ziekte-ernst en kwaliteit van leven, maar ook de mate van fysieke activiteit en copingstrategieën. In totaal namen 420 MG-patiënten deel aan deze studie, waarmee dit de grootste vermoeidheidsstudie bij MG tot nu toe is. Ernstige vermoeidheid was aanwezig bij 62% van de MG-patiënten. Ernstige vermoeidheid was gecorreleerd met de ernst van de ziekte, het vrouwelijk geslacht, body mass index (BMI) en depressieve symptomen. Daarnaast vonden we dat intensieve lichamelijke activiteit, zoals hardlopen of racefietsen, sterk gecorreleerd was met lagere vermoeidheidsscores. Hoewel de meeste patiënten (75%) geen enkele vorm van intensieve lichamelijke activiteit uitvoerden, werd deze correlatie zelfs aangetoond bij patiënten die dit type activiteit slechts één keer per week deden. Dit gunstige effect werd niet gevonden bij matige fysieke activiteiten, zoals wandelen of fietsen op een normaal tempo, zelfs niet wanneer deze activiteiten meer dan 150 minuten per dag werden uitgevoerd. Een andere nieuwe bevinding in deze studie was het verschil in copingstijlen tussen vermoeide en niet-vermoeide patiënten. Vermoeide patiënten scoorden significant hoger op passieve copingstrategieën in vergelijking met niet-vermoeide patiënten. Een voorbeeld van een passieve copingstrategie is piekeren en snel overweldigd zijn door een situatie, waardoor iemand geen grip kan krijgen op de situatie. Bij een actieve copingstijl gaat iemand probleemgericht te werk en kan hierdoor juist goed het overzicht houden. In **hoofdstuk vijf** deden we onderzoek naar de pathofysiologie van vermoeidheid bij MG door te zoeken naar potentiële biomarkers in het bloed, welke geassocieerd zijn met vermoeidheid. Een hypothese is dat vermoeidheid bij auto-immuunziekten wordt veroorzaakt door systemische inflammatoire cascades die worden getriggerd door een lokaal perifeer inflammatoir proces[2]. In het geval van MG zou inflammatie van de neuromusculaire overgang deze systemische cascades dan kunnen activeren. In een cohort van 116 anti-acetylcholinereceptor (AChR) positieve MG patiënten vonden we een robuuste correlatie tussen vermoeidheid en C-reactief proteïne (CRP). De correlatie bleef overeind na correctie voor verschillende vooraf geselecteerde covariabelen zoals ziekte-ernst, cumulatieve prednisondosering en het gebruik van steroïdsparende immunosuppressieve medicatie. Deze correlatie bleef tevens bestaan na post-hoc correctie voor BMI, intensieve fysieke activiteit en/of hemoglobinegehalte. Onze bevindingen suggereren dat laaggradige ontsteking deel uitmaakt van de pathogenese van vermoeidheid en ondersteunen de hypothese dat lokale perifere ontsteking een trigger is voor het ervaren van hinderlijke vermoeidheid. Bij een herhaalde bloedafname, welke werd uitgevoerd bij 88 MG-patiënten (76%) van het initiële cohort, konden wij helaas onze initiële correlatie

tussen vermoeidheid en CRP niet reproduceren. Dit is waarschijnlijk het gevolg van onvoldoende statistische power voor de herhaalde meting: het aantal deelnemers bij de tweede meting was lager dan de vooraf bepaalde benodigde groepsgrootte. Ten tweede bevatte de herhaalde meting twee duidelijke uitschieters met hoge CRP-waarden en lage vermoeidheidsscores, die door de relatief kleine steekproefomvang een aanzienlijke invloed hadden op de correlatie.

Toekomstperspectieven

Vermoeidheid is een belangrijk en veelvoorkomend symptoom bij MG dat bijdraagt aan een lagere levenskwaliteit. Erkenning hiervan is daarom van belang. Een aanzienlijke hoeveelheid bewijs wijst op de mogelijkheid om middels fysieke activiteit de vermoeidheidsklachten te verbeteren of op de preventieve werking, waardoor vermoeidheid zelfs voorkomen kan worden[3-6]. Deze bevindingen pleiten er voor om een gedetailleerd klinisch onderzoek uit te voeren om de effecten van een intensief trainingsprogramma in een voldoende grote cohortgroep te bestuderen. Vanwege verschillende copingstrategieën bij vermoeide patiënten is het essentieel dit te combineren met een vorm van cognitieve gedragstherapie, met name om tools te bieden om na afloop van het onderzoek een actievere levensstijl in het dagelijks leven te integreren. Tijdens dit onderzoek kunnen herhaalde bloedmonsters meer inzicht geven in het gedrag van CRP en IL-6 (als belangrijkste activator van CRP) vóór, tijdens en na fysieke trainingssessies.
Daarnaast, zou in een vervolgonderzoek moeten worden gekeken naar het eventuele effect van aanpassing van de MG-specifieke medicamenteuze behandeling bij ernstig vermoeide patiënten, zelfs wanneer hun conditie klinisch stabiel is of in (farmacologische) remissie verkeert. Hoewel eerdere studies aantoonden dat verbetering van ziekte-ernst ten gevolge van behandeling vaak samenhangt met een afname van vermoeidheid, blijft de vraag of het gepast is om de behandeling te starten of te intensiveren uitsluitend om vermoeidheidsklachten te verminderen.

Gezichten in myasthenia gravis
Hoofdstuk zes toont een afbeelding van het gezicht van myasthenia gravis. Hiervoor werden foto's van 52 MG patiënten en 38 gezonde vrijwilligers 'gemiddeld' tot 1 foto waarop een bepaalde gelaatsuitdrukking zichtbaar is. Deze gemiddelde foto's resulteerden in zeer vergelijkbare gezichten, waarbij het MG-gezicht de typische klinische kenmerken van MG vertoont: ptosis (hangende oogleden) en algehele gelaatszwakte. Deze gemiddelde foto's vormden de opmaat voor **hoofdstuk zeven**, waarin we probeerden patronen van gelaatszwakte te kwantificeren en in kaart te brengen met software gericht op het herkennen

van gelaatsuitdrukkingen. Het tweede doel van het onderzoek beschreven in **hoofdstuk zeven** was het ontwikkelen van een deep learning (DL)-computermodel voor de classificatie van diagnose en ziekte-ernst bij patiënten met MG. In dit cross-sectionele onderzoek maakten we video-opnames van 70 MG-patiënten en 69 gezonde vrijwilligers. Van elke deelnemer werd een korte video opgenomen terwijl ze een gestandaardiseerde set van 24 verschillende gelaatsuitdrukkingen nadeden. Een analyse van deze video's met de gezichtsherkenningssoftware toonde een significant verminderde expressie van boosheid, angst en vreugde bij MG-patiënten vergeleken met de controlegroep. Dit gaf aan dat specifieke patronen van gelaatszwakte door de software te detecteren is. Het DL-model kon diagnose en ziekte-ernst als dichotome uitkomst classificeren met een aanzienlijke gevoeligheid. Deze resultaten zijn veelbelovend en vormen een eerste 'proof of concept', maar verdere verfijning is nodig voordat dit model als klinisch hulpmiddel kan worden ingezet.

Toekomstperspectieven

Hoofdstuk zeven biedt een 'proof of concept' dat geavanceerde computertechnologieën kunnen worden gebruikt voor het kwantificeren en classificeren van diagnose en ziekte-ernst bij MG. Gezien de huidige mogelijkheden voor het stellen van de diagnose MG, zoals antilichaamtests in bloed en repetitieve zenuwstimulatie, zou een gebruiksvriendelijke applicatie voor het volgen van ziekteprogressie zeer waardevoll zijn. Zo'n applicatie kan bijdragen aan betere ziekte-inzichten voor patiënten, minder onnodige ziekenhuisafspraken en een efficiëntere MG-zorg. Standaardzorg omvat doorgaans frequente poliklinische afspraken, afhankelijk van de ernst van de ziekte en het effect van behandeling. Een smartphone applicatie kan dagelijkse of wekelijkse evaluaties van ziekte-ernst uitvoeren en gedetailleerde informatie bieden die momenteel niet beschikbaar is via standaardzorg. Deze gegevens kunnen meer inzicht geven in het natuurlijke beloop van MG, waardoor mogelijke exacerbaties voorspeld kunnen worden en immunosuppressieve therapie preciezer op individuele patiënten kan worden afgestemd. Onze studie is een eerste stap, maar verdere ontwikkeling van het DL-model is nodig zodat het gedetailleerdere informatie over de ziekte-ernst kan geven. Toekomstige studies moeten bepalen of integratie van door patiënten gerapporteerde gegevens, zoals een korte vragenlijst, en een korte spierkrachtsbeoordeling, de effectiviteit van het model als intuïtief monitoringsinstrument verder zouden verbeteren.

Conclusies

Het werk dat in dit proefschrift wordt gepresenteerd, heeft bijgedragen aan de kennis over vermoeidheid bij MG, een veelvoorkomend en belangrijk symptoom dat leidt tot een verminderde kwaliteit van leven. Een multifactoriële oorsprong wordt vermoed vanwege de sterke correlatie met diverse factoren zoals ziekte-ernst, vrouwelijk geslacht en depressieve symptomen, vergelijkbaar met vermoeidheid bij andere chronische auto-immuun- of neuromusculaire aandoeningen. In deze multifactoriële pathogenese lijkt laaggradige ontsteking een belangrijke rol te spelen. Het beschreven onderzoek geeft mogelijke biomarkers, die in een grotere groep verder onderzocht kunnen worden. De resultaten die in dit proefschrift worden besproken, laten significant lagere vermoeidheidsscores zien bij patiënten die intensieve fysieke activiteiten uitoefenen. Dit biedt mogelijkheden voor een potentiële behandeling van vermoeidheid bij MG. Naar aanleiding van deze bevindingen voert het MG-onderzoeksteam van het LUMC momenteel een klinische studie uit om de effecten van een fysiek trainingsprogramma om het effect op de vermoeidheid verder te onderzoeken. Een mogelijk gunstige effect zou verklaard kunnen worden door een vermindering van laaggradige ontsteking door fysieke activiteiten, hoewel deze hypothese verder onderzoek vereist.

In het tweede deel van dit proefschrift hebben we aangetoond dat typische patronen van gelaatszwakte bij MG visueel kunnen worden vastgelegd in foto's en video's. Nieuw in MG is dat we een 'proof of concept' hebben laten zien van een DL-model voor classificatie van diagnose en ziekte-ernst. De resultaten van deze studie zijn veelbelovend voor de ontwikkeling van een toekomstig intuïtief monitoringinstrument, in lijn met de bredere trend om digitale technologieën te benutten voor meer efficiëntie en ter verbetering van MG-zorg.

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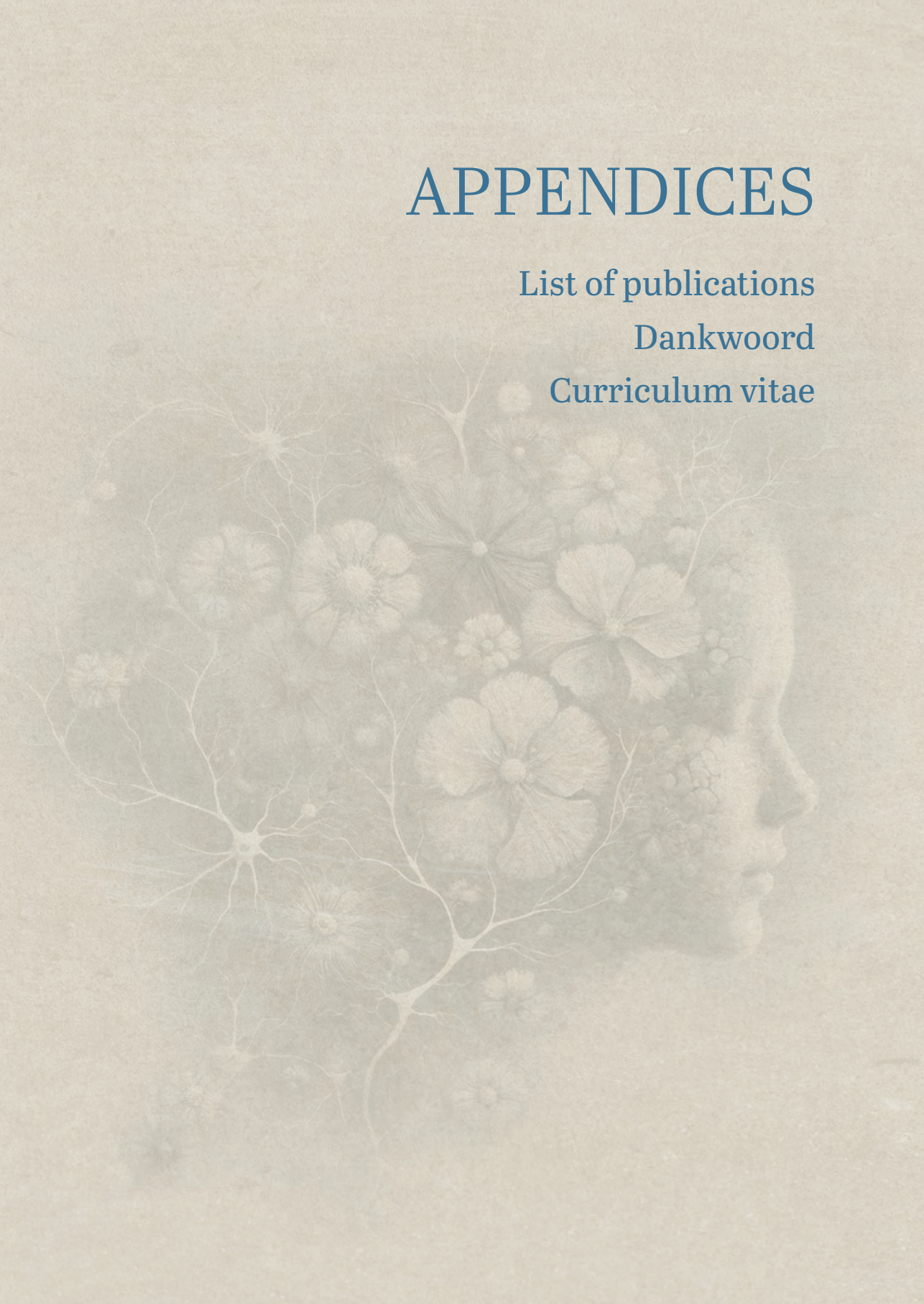
APPENDICES



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Curriculum vitae

Annabel Ruiter werd op 17 maart 1987 geboren te Hoogwoud. Na het behalen van haar VWO diploma aan het Tabor College Oscar Romero (Hoorn) in 2005, startte zij met de bachelor biomedische wetenschappen aan de Universiteit van Amsterdam. In 2006 startte zij met haar opleiding geneeskunde aan de Vrije Universiteit. Tijdens haar studie was zij in 2009 lid van het bestuur van de studievereniging (MFVU) in de functie van sponsorcoördinator. In 2009 en 2010 deed zij tijdens haar wetenschappelijke stage onderzoek naar visuele hallucinaties bij de ziekte van Parkinson.

Na het behalen van haar artsenexamen in mei 2013 is zij gestart als ANIOS op de spoedeisende hulp in het UMC Utrecht en vervolgens in oktober 2013 als ANIOS neurologie in het Onze Lieve Vrouwe Gasthuis in Amsterdam. In oktober 2015 begon zij haar opleiding tot neuroloog in het Leids Universiteit Medisch Centrum (opleiders Prof. dr. J.J. van Hilten en dr. C.S.M. Straathof). Zij startte met haar promotieonderzoek ['Faces and fatigue in myasthenia gravis'] in april 2019, zoals beschreven in dit proefschrift, onder leiding van dr. M.R. Tannemaat en Prof. J.J.G.M. Verschuuren. Per 1 april 2025 is zij gestart als neuroloog in het Onze Lieve Vrouwe Gasthuis in Amsterdam.

Annabel woont in Overveen met haar man Tijmen Hommes en twee zoons Jippe (2020) en Olle (2022).