Epidemiology, comorbidity and clinical course of myasthenia gravis

A registry-based study

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Contents

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- The Bergen Myasthenia Gravis Research Group at the Department of Clinical Medicine, University of Bergen, Norway

- The Muscle Study Group at the Department of Neurology, Duke University Medical Center, North Carolina, USA

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Abstract

Background: Autoimmune myasthenia gravis (MG) is a rare neuromuscular transmission disorder. The pathophysiologic mechanisms are well-studied, but the etiology remains unknown. The clinical course of MG is still characterized by remissions and exacerbations. Identifying prognostic factors may be helpful when choosing treatment strategies in MG patients. Studies on MG epidemiology report increasing prevalence and incidence of the disease. However, the reported data on MG epidemiology and of prognostic factors vary considerably, reflecting differences in study design, case ascertainment and measurements of clinical severity and outcome.

Objectives: First, we aimed to determine the prevalence, incidence and gender specific characteristics of MG patients needing drug treatment in a well-defined population cohort. Second, we examined the total drug treatment and comorbidity in MG patients. Finally, we aimed to assess the clinical course of MG, and to identify prognostic factors that may contribute to a good outcome in MG patients.

Materials & Methods: Patient information in papers I and II was retrieved from the Norwegian Prescription Database, which contains information on all prescription drugs expedited in Norway since 2004. In paper III, comprehensive clinical information on MG patients treated in a consistent fashion for over three decades was obtained from the Myasthenia Gravis Patient Database at Duke University Medical Center (North Carolina, USA).

Results & Conclusions: The point prevalence of symptomatic MG in a complete Norwegian cohort 1 January, 2008 was 131 per million inhabitants, and the incidence rate for the year 2007 was16 per million. Our calculated prevalence and incidence is in agreement with other population-based studies. MG patients are more often treated with non-MG prescription drugs than patients using drugs for most other conditions, reflecting frequent comedication and medical comorbidity. The prognosis of MG is favorable for the majority of patients, regardless of age, maximum disease severity and antibody status.

List of publications

Paper I Andersen JB, Engeland A, Owe JF, Gilhus NE. *Myasthenia gravis requiring pyridostigmine treatment in a national population cohort.* European Journal of Neurology. 2010; 17(12): 1445-50.

Paper II Andersen JB, Owe JF, Engeland A, Gilhus NE. *Total drug treatment and comorbidity in myasthenia gravis; a population-based cohort study.* European Journal of Neurology. 2014; 21(7): 948-55.

Paper III Andersen JB, Gilhus NE, Sanders DB. *Factors affecting outcome in myasthenia gravis.* 2015; submitted.

Abbreviations

ACh	Acetylcholine
AChE-I	Acetylcholine esterase inhibitor
AChR	Acetylcholine receptor
ATC	Anatomical Therapeutical and Chemical Classification
CI	Confidence interval
CSR	Complete stable remission
CT	Computer tomography
DDD	Defined Daily Dose
ECG	Electrocardiography
EFNS	European Federation of Neurological Societies
EOMG	Early onset myasthenia gravis
EPP	Endplate potential
HLA	Human leukocyte antigen
HR	Hazard ratio
ICD-10	International Classification of Diseases, version 10
ICPC-2	International Classification of Primary Care Codes, version 2
IgG	Immunoglobulin G
IVIG	Intravenous immunoglobulin
LEMS	Lambert-Eaton myasthenic syndrome
LOMG	Late onset myasthenia gravis
LRP4	Lipoprotein receptor-related protein 4
MGFA	Myasthenia gravis Foundation of America

MG	Myasthenia gravis
MRI	Magnetic resonance imaging
MuSK	Muscle-specific tyrosine kinase
MM	Minimal manifestation
MMF	Mycophenolate mofetil
NCPR	Norwegian Central Population Registry
NIPH	Norwegian Institute of Public Health
NMJ	Neuromuscular junction
NorPD	Norwegian Prescription Database
PE	Plasma exchange
PIN	Personal Identification Number
PR	Pharmacologic remission
RIA	Radioimmunoprecipitation assay
RNS	Repetitive nerve stimulation
RyR	Ryanodine receptor
SFEMG	Single-fiber electromyography
SIR	Standardized incidence ratio
SLE	Systematic lupus erythematosus
USA	United States of America
VATS	Video-assisted thoracoscopic surgery

1. Introduction

1.1 Brief historical outline

The historical report from 1644 of Chief Opechankanough, a Native American Indian, was probably the first described case of myasthenia gravis (MG):[1]

> "*The excessive fatigues he encountered wrecked his constitution; his flesh became macerated; his sinews lost their tone and elasticity; and his eyelids were so heavy that he could not see unless they were lifted up by his attendants. (...) He was unable to walk; but his spirit rising above the ruins of his body directed from the litter on which he was carried by his Indians.*"

Later, in 1672, the characteristic variability of weakness in the limbs and bulbar muscles was described in a patient by the English physician Thomas Willis.[2] Willis is accredited the recognition of the disease as a distinct clinical entity.[3]

The Greek words for muscle (*myo*) and weakness (*asthenia*) and the Latin word for severe or grave (*gravis*) have given name to myasthenia gravis.[4] The *gravis* part of the word refers to the severity of the disease before any treatment was available.[5] The name was first introduced in 1895 by Friedrich Jolly, describing two cases under the title *myasthenia gravis pseudo-paralytica.*[3] Until then, the disease was known as the Erb-Goldflam symptom-complex, after the two physicians who first characterized the distinct clinical features of MG.[6]

For the patients with a severe disease, the mortality was high (Figure 1). As mechanical ventilation was not invented until 1929 (Philip Drinker's "the iron lung"),[5] no remedy for patients with fatal weakness of the respiratory muscles was available. The effect of physostigmine, an cholinesterase inhibitor, on MG symptoms was first successfully applied in MG patients by Dr. Mary Walker in 1934.[7] This discovery drastically improved the quality of life for MG patients,[8] and

anticholinesterase drugs are still the principal drugs of choice in symptomatic management of MG today.

The involvement of the thymus was already observed in the late 1800s,[9] and the link was established in the early 1900s from several autopsy reports.[10-12] In the 1930s, convincing evidence of improvement of myasthenic symptoms following thymectomy with long-lasting remission in patients both with and without thymic tumors,[13;14] established thymectomy as a therapeutic intervention in the management of MG patients.

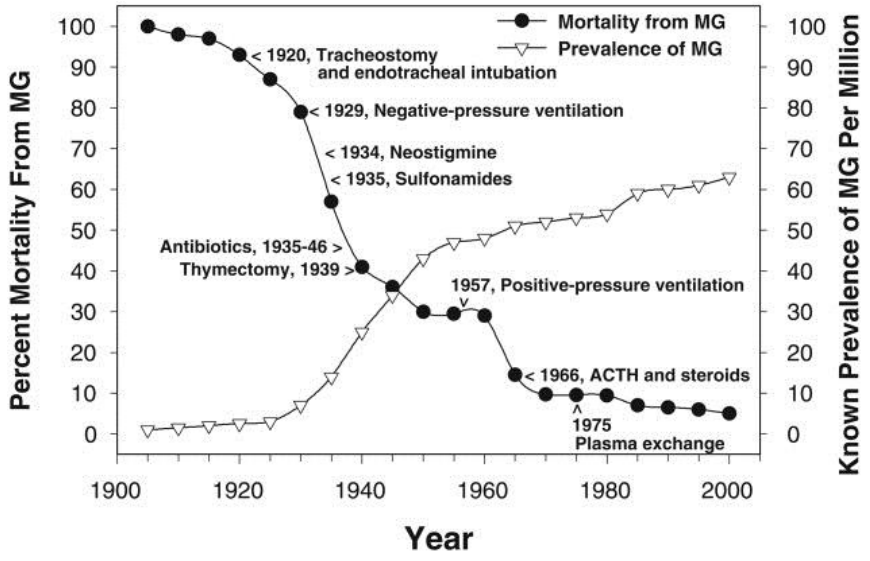
Over the next two decades, the understanding of the underlying mechanisms causing myasthenic symptoms was rapidly progressing. At the end of the 1950s, several observations of the autoimmune etiology in MG were emerging.[4] Simpson was the first to propose this novel hypothesis that MG was an autoimmune disorder caused by an antibody directed at a specific protein in the neuromuscular junction (NMJ).[15]

By 1973, Patrick and Lindstrom managed to demonstrate the autoimmune response to the acetylcholine receptor (AChR).[16] They discovered that rabbits immunized with purified muscle-like AChR developed muscle weakness which was reversible with acetylcholinesterase inhibitors (edrophonium or neostigmine). This model became known as experimental autoimmune MG and was later widely used to study various aspects of the disease, including new immunotherapies.[15]

With the understanding of the impairment of the neuromuscular transmission being caused by anti-AChR antibodies against the NMJ, immunosuppression became a fundament in MG therapy.[4] Prednisone and azathioprine have been the mainstay in the management of MG since the 1970s, together with plasma exchange for acute exacerbations after experiments showing remarkable improvement in MG symptoms.[17] The beneficial effect of plasma exchange was inversely correlated with the level of AChR antibodies, confirming that MG symptoms were caused by circulating antibodies.[18]

As advances in diagnosis and therapy have been evolving, the prevalence of known cases of MG has correspondingly increased (Figure 1). Acute exacerbations and myasthenic crisis with life-threatening respiratory failure are today effectively handled at modern intensive care facilities,[19] and MG-related deaths are now under 10%.[20;21] Spontaneous remissions are still rare, but long-lasting remissions are observed in about 20% of the patients, usually after thymectomy.[20;21] More common are pharmacologic remissions (PR), defined as absence of MG symptoms while on immunosuppressive therapy.[22] From being regarded as a severe, disabling disease, with optimal treatment, the long-term prognosis in the majority of MG patients is good and the life-expectancy normal.[23;24]

Figure 1. Known prevalence and mortality from MG during 1900 to 2000.



From: Grob D, *et al* (2007): Lifetime course of myasthenia gravis.[20]

1.2 Epidemiology of MG

Studies on MG epidemiology have been conducted worldwide over the past 60 years, some population-based, but mostly on hospital-based populations and case series.[25;26] Geographically, a substantial contribution of population-based studies derives from European countries, especially United Kingdom, Scandinavia, Italy and Holland.[25] With the advent of modern computer technology and accelerated computer capacity and memory, comprehensive databases are rapidly becoming a major resource in all fields of research. Studies using nationwide clinical and/or administrative databases to assess different aspects of MG, including epidemiology are increasing, most recently from Scandinavia, Australia and Taiwan.[27-30]

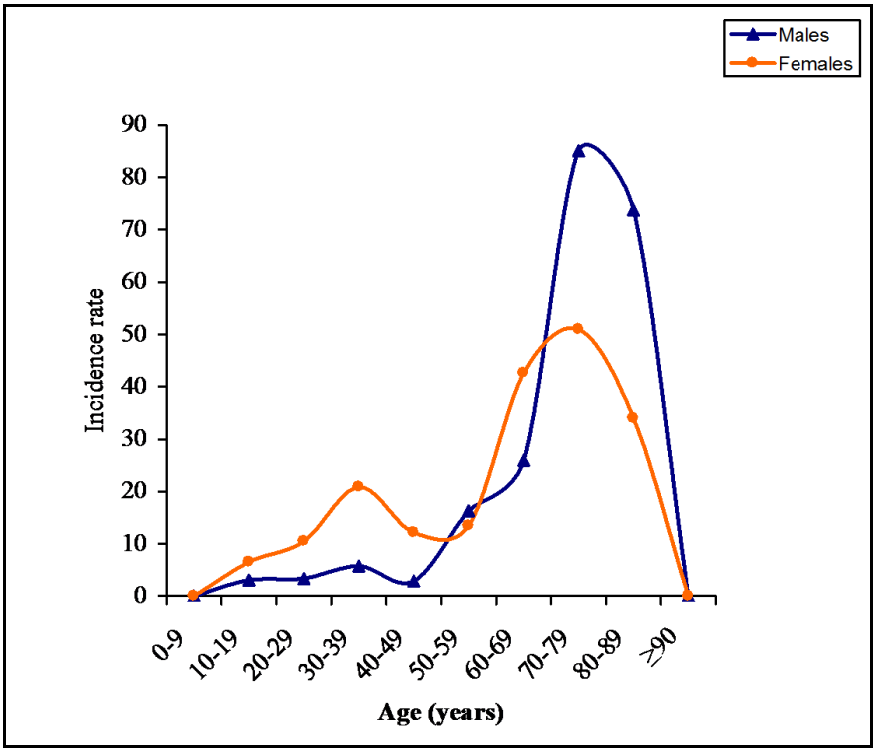
MG is an uncommon neurological disorder. Reported annual incidence has gradually increased from 1.4-9.1 per million inhabitants in the1950s-80s[31-39] until 24.9 per million in 2012.[29] This increasing trend is particularly profound in the elderly.[40;41] Consequently, over the past six decades, MG prevalence has risen from less than 30 per million[42] to over 300 per million in 2014.[43] In Norway, the prevalence has increased from 21 per million in 1951[42] to 90 per million 30 years later.[34] Another three decades later, the prevalence of MG in Norway is around 130-145 per million.[44;45] Factors explaining these trends are increased recognition of MG due to better diagnostic tools, enhanced awareness among neurologists, increased longevity of the population and changes in lifestyle, environmental or genetic factors. Improved treatment with no increased death rate in MG patients influences prevalence heavily, but not incidence.

MG can develop in both men and women, at all ages and in all races. Disease onset is influenced by age and gender (Figure 2). MG onset after the age of 50 years used to be considered rare.[46] Differences in age at onset between the two genders were noticed already in the 1900s; females were younger at onset than males,[47] with three times more incident female than male cases before the age of 50 years. After which, males are more often affected than females.[20;47]Somnier *et al* demonstrated a bimodal

distribution for both genders in 1991 and postulated that early and late onset MG were two distinct disease entities.[38]

There are distinct age and gender differences in disease onset between populations. Childhood MG with onset under the age of 15 years is more common in Chinese and Japanese populations, in up to 30% of the cases, most of these with purely ocular manifestations. Interestingly, only 10-15% childhood MG was found in the Taiwanese population, similar to European and North American populations.[30;48] Only a few studies have assessed racial differences in Caucasians and African-Americans. In these studies a more severe form of MG in African-Americans were observed.[49;50]

Figure 2. Age and sex specific incidence of symptomatic MG in Norway.



From: Andersen JB, *et al* (2010): Myasthenia gravis requiring pyridostigmine treatment in a national population cohort.[44]

1.3 Pathophysiology, antigenic targets and autoantibodies in MG

1.3.1 Structure and function of the neuromuscular junction

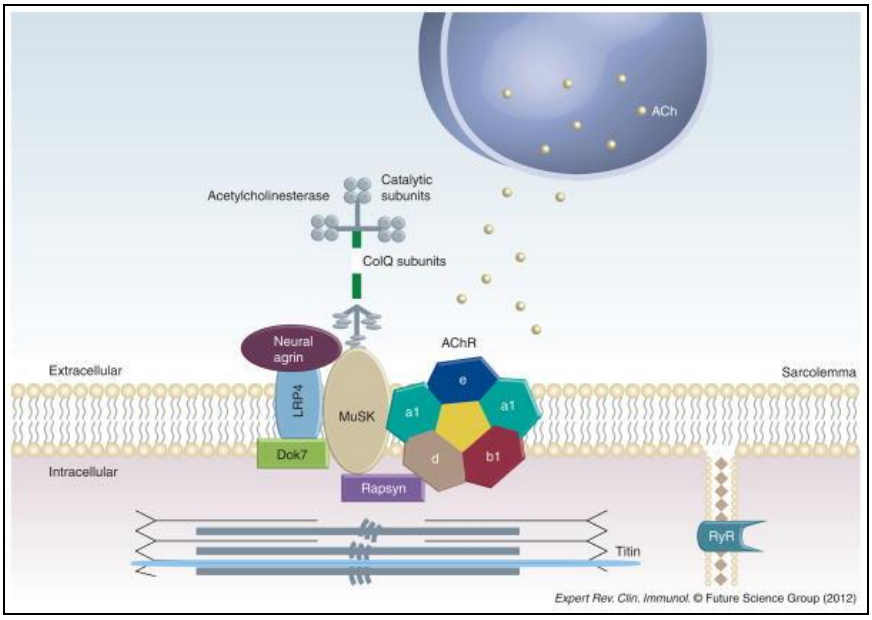
The neuromuscular junction (NMJ) is made up of three main parts:[4]

1. The presynaptic motor nerve terminal, responsible for synthesis, storage and release of the neurotransmitter acetylcholine (ACh).

2. The synaptic cleft containing acetylcholine esterase (AChE).

3. The postsynaptic membrane, with deep folds and densely packed with AChR at the top of the fold. Proteins involved in clustering of the AChR are also on the muscle membrane, close to the AChR, including Rapsyn, muscle-tyrosine kinase and agrin.

Figure 3. Muscle autoantigens in MG



ACh: Acetylcholine; AChR: Acetylcholine receptor; ColQ: Collagen Q; LRP4: Lipoprotein-related protein receptor 4; MuSK: Muscle-Specific tyrosine kinase; RyR: Ryanodine receptor.

From: Meriggioli, MN *et al* (2012): Muscle autoantibodies in myasthenia gravis: beyond diagnosis?[51]

Neuromuscular transmission is precipitated by calcium influx to the motor nerve terminal through voltage-gated calcium channels (VGCCs). Acetylcholine (ACh) is then instantly released into the synaptic cleft, where it diffuses to reach and bind the AChR. This interaction triggers the opening of the AChR ion channel, resulting in depolarization of the muscle membrane and generation of the muscle action potential with subsequent muscle contraction. The ACh is rapidly destroyed by the AChE. Repolarization of the motor nerve terminal is induced by opening of the voltage-gated potassium channels (VGKCs). Several proteins on the postsynaptic membrane are antigenic targets in MG (Figure 3).[15]

1.3.2 AChR and anti-AChR autoantibodies

The nicotinic AChR at the muscle endplate is a transmembrane protein, consisting of five subunits forming a pentameric, fast-reacting ion channel. There are two isoforms of this oligomeric protein; an embryonic form made up of two identical α-subunits, which contain the binding site of the ACh, and three different subunits; β, ε and δ. In the adult form, the ε-subunit has been substituted for the γ-subunit. Other characteristics of the maturation of the NMJ are folding of the muscle membrane, the increasing number and density of the AChRs, a slower receptor turnover rate and decreased channel opening time.[52;53]

The AChR-antibodies are polyclonal and invariably immunoglobulin G (IgG). All four subclasses may be found in MG patients, but most commonly are IgG1and IgG3, both effective complement activators. IgG2 and IgG4 are only found in very low concentrations and activate complement poorly (IgG2) or not at all (IgG4).[54] The AChR-antibodies bind preferentially to the main immunogenic region of the α-subunit.[55] The pathogenicity of the AChR-antibodies is mediated through three main mechanisms:

(A) Binding and crosslinking of the AChRs, resulting in an increased endocytosis and degradation of the AChRs.[56]

(B) Binding and activation of complement factors causing destruction of the muscle membrane and leading to altered membrane morphology, which becomes flat instead of folded.[57]

(C) A more unusual effector mechanism is the binding of AChR-antibodies to the binding site of the ACh, blocking the function of the AChR.[58]

This results in reduced number of functional AChRs and neuromuscular transmission failure. AChR-antibodies can bind both the adult and embryonic isoforms of the AChR, usually with different affinity. In maternally mediated neonatal MG, high-affinity antibodies towards the embryonic isoform cross the placenta, causing neuromuscular transmission failure in the fetus.[59]

1.3.3. The agrin/LRP4/MuSK signaling pathway

MuSK is localized at the postsynaptic membrane of the NMJ and is the main autoantigen identified in MG patients without AChR-antibodies (ref). Together with the low-density lipoprotein receptor-related protein 4 (LRP4), MuSK functions as a receptor for agrin.[60] Agrin is a large extracellular protein which is released by the nerve terminal during synapse development. A tetrameric complex is formed from the binding of agrin to LRP4, which interacts and activates MuSK, resulting in the clustering of AChRs.[61] Ablation of genes encoding for agrin, MuSK or LRP4 prevents NMJ formation.[62-65]

MuSK-antibodies

Autoantibodies against MuSK was first discovered in 2001 in patients without AChR-antibodies (termed 'seronegative MG').[66] MuSK-antibodies and AChR-antibodies are never present at the same time.[67] Antibodies against MuSK are mostly of the IgG4 subclass and do not activate complement.[68] The pathological mechanisms in which MuSK-antibodies induce MG have until recently been unclear. Several observations these recent years have led to the unravelling of the myasthenogenic effect of anti-

MuSK IgG4: IgG4 levels in MuSK patients correlate with disease severity,[69] and the IgG4 from MuSK sera alone may induce MG directly.[70] In contrast, AChR-antibodies cause MG mainly through complement activation and accelerated internalization of the AChRs.[4]

Studies using immunized MuSK-MG mice provided evidence of electrophysiological disruptions both pre- and postsynaptically.[71;72] In 2012, MuSK IgG4 was proven directly pathogenic, without additional immune components, causing both pre- and postsynaptic dysfunction.[73] In vitro electrophysiological and histological studies using muscle biopsies from MuSK-MG patients confirm these findings showing low levels of presynaptic ACh release, small miniature endplate potentials, partially denervated postsynaptic areas and degradation of postsynaptic folds.[74] However, further studies are needed to fully understand the pathological mechanisms of IgG4 in MuSK-MG patients.

LRP4-antibodies

LRP4-antibodies in MG sera without AChR- or MuSK-antibodies were first identified in 2011,[75] and also on rare occasions in AChR- and MuSK-positives.[75-77] The pathogenicity of LRP4-antibodies includes: Inhibition of the agrin/LRP4/MuSK pathway, and thereby clustering of AChRs; complement activation and lysis of the postsynaptic membrane as LRP4-antibodies are mainly IgG1 and IgG3 with ability to activate complement;[78] compromised release of ACh from presynaptic vesicles.[78] The latter could explain why such antibodies are found in patients with Lambert-Eaton myasthenic syndrome (LEMS),[75] which is caused by autoantibodies against the VGCC at the presynaptic membrane.[79]

Agrin-antibodies

In 2014, two independent groups reported that antibodies against agrin were identified in MG patients without detectable AChR-, MuSK- or LRP4-antibodies.[80;81] Some patients were double or even triple positive with both anti-agrin and anti-AChR/MuSK/LRP4 in their sera, suggesting multiple antigenic targets with more

severe disease in these patients.[70;77] The clinical significance of these findings is not yet clear, but as agrin is crucial in the development and maintenance of the NMJ,[82] interference with the agrin/LRP4/MuSK pathway by anti-agrin might lead to a reduction of functional AChRs and impairment of neuromuscular transmission.[83]

1.3.4 Thymoma-associated autoantibodies

Thymoma-associated autoantibodies are important as diagnostic and prognostic tools in MG, as their presence is correlated with disease severity and presence of thymoma.

Titin-antibodies

Titin is a large intracellular protein of the skeletal and cardiac sarcomere. Together with other muscle proteins, titin is important for muscle cell elasticity. Antibodies against titin, discovered in 1990 by Aarli *et al*,[84] are found in 95% of MG patients with thymoma,[85] and in 30-50% of late onset MG, usually in patients older than 60 years.[86;87] The presence of titin-antibodies in MG patients with a late onset correlates to disease severity.[88] Titin-antibodies are rarely seen in MG patients with an early onset, and the presence of such antibodies in these patients is highly suggestive of a thymoma.[89]

RyR-antibodies

The RyR is a calcium channel of the sarcoplasmic reticulum. The release of calcium from the sarcolemma through the receptor into the cytoplasm is essential in muscle contraction. The RyR-antibodies were described by Mygland *et al* in 1992,[90] but their pathogenic role is not yet established. Presence of RyR-antibodies serves as a marker of a more severe and prolonged disease, with poorer chance of a favorable outcome after thymectomy in MG patients with a late onset, and strongly indicates the presence of a thymoma.[91;92] When testing for RyR- and titin-antibodies in combination, the sensitivity and specificity is 95%, yielding a positive predictive value for a thymoma in MG of 70%.[85]

VGKC KCNA4

The VGKC is a transmembrane ion channel, mainly found in the brain, peripheral nerves and skeletal and heart muscles. Channel opening repolarizes the nerve terminal after an action potential. Antibodies to the KCNA4 (formerly Kv1.4) subfamily of VGKCs are seen in up to 30% of MG patients. In the Japanese population, KCNA4-antibodies are associated with a more severe form of MG, with bulbar symptoms, presence of thymoma and myasthenic crisis.[93] This was not the case in Caucasians, who had mild clinical presentation.[94] The clinical role of these antibodies therefore needs further clarification. The KCNA4-antibodies are useful as markers of the potential development of severe autoimmune myocarditis and response to calcineurin inhibitors.[95]

1.4 Classification and subgroups of MG

The heterogeneity of MG is reflected by the antibody diversity, clinical expression, thymic pathology, age at onset and associations to human lymphocyte antigen (HLA). The different subgroups have distinct clinical features with implications for treatment. There is no consensus regarding classification of MG subgroups. In this thesis, we classify MG by the presence or absence of AChR-antibodies.

1.4.1 MG with AChR-antibodies

AChR-antibody positive MG (AChR-MG)

85-90% of MG patients have detectable antibodies against the nicotinic AChR-antibodies, representing the largest MG subgroup.[96;97] Four distinct subtypes of AChR-MG are recognized:

1. Early onset MG (EOMG)

This is the classic form of MG, most often seen in younger women with a female to male ratio being three to one. An early onset of MG used to be defined as onset of MG symptoms before the age of 40 years. This age cut-off was first introduced by Compston *et al* as an arbitrary age limit to study non-thymoma patients.[98] They found a gender bias in disease presentation according to age; females were younger at disease onset than males. Later studies on the age and gender specific incidence of MG suggested 50 years of age as a cut-off to better reflect pathogenetic differences in disease onset.[38;47;99] The EOMG subgroup used to constitute 60% of AChR-MG. Over the past decade, however, the rate of patients with onset after 50 years has increased, while the rate of patients with onset before 50 years has been stable or even decreasing.[40;100;101]

In addition to AChR-antibodies, titin-antibodies may be detected in about 10% of EOMG patients, while RyR-antibodies are rarely present.[85] The thymus in EOMG is usually hyperplastic with germinal centers, which are sites of B-cell response against the AChR.[102] There is a strong association with the HLA-DR3, and B8 alleles in this subgroup.[98;103] Other autoantibodies or autoimmune diseases, such as autoimmune thyroid disease and systemic lupus erythematosus (SLE), occur more often in these patients than in patients with a later disease onset.[104;105]

2. Late onset MG (LOMG)

LOMG is defined as onset of MG symptoms after the age of 50 years, the female to male ratio being near equal. [46;106] With emerging epidemiologic evidence of increasing incidence of the very old MG patients, the age cut-off of 60 and 70 years has been proposed for LOMG.[106;107] This subgroup now constitutes over 50% of AChR-MG.[107]

In most patients thymus is atrophic, that is normal for age, and thymus hyperplasia is unusual.[107] In addition to AChR-antibodies, 60% have titin-antibodies, and 15% RyR-antibodies.[85] The presence of titin-antibodies seem to correlate with a more severe disease.[88] LOMG seldom has other autoimmune diseases.[108] There is an association

with HLA-B7, -DR2, -DR7 and -DRB1*15:01 in this patient group,[98;103] HLA-DR7 is particularly found in LOMG patients with anti-titin antibodies.[109] LOMG may present with ocular or generalized weakness, with lower disease activity than EOMG and a favourable prognosis.[46] However, complete stable remissions (CSRs) are rare, and mortality is higher compared to EOMG, most likely attributable to age and comorbid conditions.[106]

3. Ocular MG

Patients with purely ocular manifestations constitute about 10-20% of AChR-MG patients, and may present at any age and in both genders.[110] When the symptoms manifest themselves to other parts of the body, the disease is termed 'generalized'. Ocular MG is localized to the extra-ocular muscles. Ptosis and diplopia are the first signs of the disease in up to 85% of MG patients. If the disease has not generalized within the first two years after symptom onset, which is the case in about 80% of the patients, it is likely to stay purely ocular.[111] There is an ongoing debate whether or not early corticosteroid treatment limits the conversion from ocular to generalized disease.

Half the patients with ocular MG have detectable antibodies against the AChR in routine assays, whereas additional patients have AChR-antibodies detected when using a cell-based assay.[112] MuSK-antibodies are rarely found in ocular MG, and thymic histology is largely unknown.[51;110] Reported rate of spontaneous remission is about 15-18%, but the clinical course varies. [113]

4. Thymoma-MG

Thymomas are seen in 10-15% of MG patients, and MG is the most common thymoma-associated autoimmune disease; 30-50% of patients with thymoma have MG.[114] Thymomas are neoplasms derived from thymic epithelial cells. These cells are mixed with non-neoplastic T-cells. Thymoma-MG occurs in both males and females at all ages, but is typically diagnosed at 40-60 years, and rarely during the two first decades of life.[115] Clinically, the disease tends to be more severe compared to EOMG

with more frequent weakness of the oropharyngeal muscles. The long-term prognosis regarding muscle weakness is similar to LOMG without thymoma.[116]

Thymoma-MG patients have an array of antibodies. Virtually all of them have AChR-antibodies, such antibodies also occurring in 25% of patients with a thymoma but with no clinical symptoms of MG. 95% have titin-antibodies and over half have RyR-antibodies, but not antibodies against MuSK.[102] Antibodies associated with paraneoplatic syndromes, such as anti-VGKC and -VGCC may be present.[48] Thymoma-MG patients do not have a specific HLA profile, and are seldom DR3 positive.[47]

AChR-MG with low-affinity AChR-antibodies

Some AChR-antibodies can bind divalently to adjacent AChRs only when they are expressed in dense clusters. Such antibodies are not detectable by the commercially available radioimmunoprecipitation assays (RIAs), but can be found when using a cell-based assay and indirect immunofluorescence.[117] About 60% of MG patients without AChR- or MuSK-antibodies detected in routine assays have such low-affinity AChR-antibodies.[117] The pathogenicity of these antibodies is likely to be the same as for regular AChR-antibodies.[118] Clinically, these patients are indistinguishable from AChR-MG patients, with similar prognosis and response to treatment.

1.4.2 MG without AChR-antibodies

MuSK-positive MG (MuSK-MG)

MuSK-antibodies are reported in 5-60% of MG patients without AChR-antibodies, and MuSK-MG is the largest subgroup of MG patients seronegative for AChR-antibodies, representing 5-8% of all MG cases.[119] Differences in clinical manifestations, immune parameters, and therapeutic responses clearly establish MuSK-MG and AChR-MG as two distinct disease entities. MuSK-MG can occur in all ages, but the onset tends to be earlier, and there is a female predominance.[120] The

occurrence of MuSK-MG seems to differ worldwide, with latitudinal correlations. The highest frequency of MuSK-MG is found in the Mediterranean countries and at similar latitudes in North America and Asia, and decreasing further north.[121] Only five cases have been reported in Norway with a population of five millions.[122] The rate of MuSK-MG is higher in African-Americans than in whites.[50]

Most MuSK-MG patients have a generalized disease, often with a more severe symptomatology with bulbar, facial, neck and respiratory weakness.[120] Antibody titer and disease severity is highly correlated in this MG subgroup.[123;124] The role of the thymus in MuSK-MG is not fully understood. Thymomas do not have an increased frequency, and thymus histology is usually normal.[102] There are rarely other autoantibodies present in MuSK-MG patients, and they seldom have additional autoimmune diseases.[67;125] There is a strong association with HLA-DR14 and -DQ5.[126;127] The long-term prognosis is usually less favorable than in AChR-MG, with a lower rate of remission and a higher rate of refractory disease.[119]

LRP4-MG

The rate of LRP4-MG varies between 7% and 32% in MG patients without AChR-antibodies.[77] This subgroup may occur in all ages, and there is a female preponderance. Clinical presentation ranges from purely ocular manifestations to myasthenic crisis.[75;76] In some patients, these autoantibodies were found together with antibodies against the AChR or MuSK. Patients with a combination of antibodies presented with more severe symptoms. Thymic changes identified so far include hyperplasia only.[77]

MG without known autoantibodies ('Triple negative MG')

This subgroup consists of patients lacking antibodies against the AChR, MuSK and LRP4. Clinical presentation is heterogeneous, representing the entire spectrum of disease severity. As new pathological autoantibodies are discovered (anti-agrin, -Collagen Q and -rapsyn), and more sensitive antibody assays are becoming available

(low-affinity antibody testing for AChR, MuSK and others), the true prevalence of this MG subgroup is probably very low.[128]

1.5 Diagnosis of MG

The diagnosis of MG is based on typical MG symptoms and signs, response to pharmacological tests, positivity of antibody assay testing, electrophysiological examinations and radiological imaging.

Clinical manifestations

Fluctuating fatigable weakness of specific muscle groups, improving with rest and worsening with activity, is the clinical hallmark of MG.[48] In 85% of the cases, weakness of the extra-ocular muscles causing ptosis and diplopia is the first signs and symptoms of MG.[20] Generalization usually occurs within the first two years in the majority of patients, most commonly affecting the facial, limbs and axial muscles. In 15%, the initial symptoms are bulbar weakness with chewing, swallowing and speaking difficulties. On rare occasions, the respiratory muscles are affected, requiring immediate medical attention. Pathological fatigue not subsiding with rest is another clinical characteristic of MG.[129] This phenomenon is described in other autoimmune diseases such as multiple sclerosis and rheumatoid arthritis as well.[130;131]

There is a wide range of differential diagnosis, such as motor neuron disease, multiple sclerosis, muscular dystrophy, polymyositis, mitochondrial myopathies, general fatigue, psychiatric conditions and hyperthyroidism.[132] Ocular myopathies or neuropathies such as neuromyelitis optica should be considered in patients with purely ocular symptoms.[133] Also, excluding other neuromuscular diseases including LEMS and congenital myasthenic syndromes is important. MG may easily be overlooked or misdiagnosed, especially in elderly patients. Ageing may cause sagging of the lower eyelids and make ptosis difficult to identify. Diplopia might not be detected due to vision impairment from macular degeneration or cataract formation, and dysarthria and dysphagia in elderly persons may be caused by several other conditions, such as cerebrovascular diseases.[46] Elderly patients with MG symptoms

are in general practice often misdiagnosed as having a brain stem lesion, usually stroke.

The patient history is essential in MG diagnostics, particularly the identification of any day-time variability in muscle weakness. Clinical assessments should include: Ocular muscles by testing the ability to maintain an upward or lateral fixed gaze for about 30 seconds (*'ptosis test'*); facial muscles, especially eye closure; bulbar features such as speech and swallowing; axial muscles, i.e. neck extension and flexion; proximal limbs, e.g. by asking the patient to keep an arm stretched for one minute. Weight loss could be a sign of bulbar affection. Tachypnea or orthopnea may be signs of respiratory involvement and the risk of developing a myasthenic crisis is increased.

Pharmacology

After a diagnosis of MG is suspected following a careful clinical examination, the response of the suspected muscles to AChE-inhibiting drugs may be tested. Oral administration of pyridostigmine can be used. AChE-I available, edrophonium, (*'Tensilon test'*) which has a rapid onset and short half-life, is preferred. The drug is administrated intravenously, and a marked objective improvement is considered a positive response, and highly specific of MG.[134] This test is now used only in the assessment of suspected MG cases without detectable antibodies.

Antibody testing

The link between neuromuscular impairment and circulating AChR-antibodies was firmly established in 1973 by Patrick and Lindstrom.[16] Three years later, Lindstrom *et al* developed an assay using radioimmunoprecipitation (RIA) to measure AChR-antibodies in MG sera.[96] AChRs prepared from humans were labelled with ^{125}I-α-bungarotoxin, a snake venom that binds specifically and practically irreversibly to the receptors. The concentration of AChRs was quantified by measuring bound toxin to the receptors. Next, the receptor-toxin complex was precipitated with serum from a suspected MG case. Different preparations of the AChR could vary in the capacity to bind both the toxin and the antibody. Today's commercial kit using a radio-receptor

assay (RIA technique) with AChR as an antigen has high sensitivity and better reproducibility. The concentration of AChR-antibodies is measured in nmol/L, and a raised value above the cut-off (0.5nmol/L) is considered nearly 100% specific for MG.[97]

The AChR-antibodies are detectable in only about 85% of the MG patients with generalized disease and about 50% of patients with purely ocular weakness. More sensitive cell-based antibody assays are being developed, but are not yet commercially available. This technique enables detection of antibodies that only bind to AChRs in clusters, i.e. low-affinity AChR-antibodies.[117]

In patients with MG clinic, but without a positive AChR-antibody assay, MuSK-antibodies should be assessed. As of yet, no commercial tests are available for the identification of LRP4-antibodies. Testing for other autoantibodies such as anti-titin may be important, as their presence indicates a more severe disease. Although the presence of AChR-antibodies is specific for MG, they may on rare occasions be found in patients with other autoimmune diseases, graft-versus-host disease in allogeneic bone marrow transplantation, thymoma without MG and neuromyelitis optica.

Electrophysiology

Repetitive nerve stimulation (RNS) and single-fiber electromyography (SFEMG) are the two most important electrophysiological tests in MG diagnostics. The decreased number of AChRs and the reduction of sodium channels due to altered post-synaptic membrane morphology result in electrophysiological abnormalities. Both factors contribute to a reduction in the endplate potential (EPP), which normally is larger than the threshold needed to generate an action potential. This difference between the EPP and the threshold potential is called the safety factor in neuromuscular transmission, and is reduced in MG.[4] The release of ACh is reduced after repetitive activity, and the EPP may fail to reach the necessary threshold to trigger the action potential. The resulting decrement in amplitude of the compound muscle action potential seen after RNS is called the decremental response. The decrease in ACh

release reaches maximum after delivery of the first four stimulations of a high-frequency train of stimuli (2-5 Hz). A decremental response >10% is considered abnormal. An abnormal result of the RNS is highly specific for MG with 95% specificity, depending on the operator and the muscles tested. The sensitivity is about 75% in generalized MG and <50% in ocular MG.[48]

The time it takes for the EPP to reach the threshold for muscle action potential varies. This variability may be measured as neuromuscular jitters by SFEMG. In SFMG, a specially designed concentric needle electrode is used to record two muscle fibers' action potentials generated by a single motor neuron at the same time. Neuromuscular jitters occur due to the difference in time between the firing of the two muscle fibers. In MG, the jitter is increased in about 95-99% if the appropriate muscles are tested, with a high negative predictive value.[135] The specificity is lower than for repetitive testing. Neurophysiological tests are crucial to diagnose antibody-negative MG, but unnecessary when AChR- or MuSK-antibodies have been detected.

Imaging

Thymus pathology is common in MG, and all patients with confirmed or suspected MG should undergo a chest CT or MRI to exclude the presence of a thymoma. Thymic hyperplasia and thymoma may be indistinguishable on imaging, but thymoma is seen as a homogeneous lobulated mass in the anterior mediastinum. A normal-sized thymus gland does not exclude hyperplasia, which is often impossible to diagnose by imaging. Contrast-enhanced CT-scan is the modality of choice for evaluation of thymomas,[136] with high sensitivity (89%). The specificity is, however, only 77%.[137] MRI can give additional information if suspicion of tumor infiltration or invasion of adjacent organs or metastasis.

1.6 Treatment of MG

The basis of MG management is symptomatic treatment in combination with immunotherapies (Figure 4). The treatment is life-long for most patients. The goal of treatment is to induce and maintain remission while minimizing the side-effects and risk of exacerbations.

1.6.1 Symptomatic treatment

The first line of treatment in MG is oral acetylcholine esterase - inhibitors (AChE-I), most commonly pyridostigmine bromide (Mestinon ©), but also neostigmine and ambenonium are in lesser degree used.[138] The drug blocks the function of the enzyme AChE, thereby enhancing the time and amount of the neurotransmitter ACh in the synaptic cleft. This treatment is only symptomatic and does not affect the course of the disease. In some patients, usually in those with a mild disease, oral AChE-I is sufficient to control the symptoms.

AChE-Is are usually well-tolerated when given in standard doses up to 60 mg five times a day. Increased cholinergic stimulation may cause side-effects, mostly muscarinic symptoms, typically stomach cramp, diarrhea and increased sweating,[139] but also nicotinic symptoms, such as muscle fasciculations and cramps.[140] The side-effects are dose-dependent, and the optimal dosage should be adjusted accordingly to maximize the therapeutic benefit and minimize the side-effects. Hypersalivation, bradycardia, excessive sweating and miosis are muscarinic symptoms of cholinergic overdose.[139]

Patients with MuSK-MG usually respond poorly to AChE-I, or even experience worsening of MG symptoms. In some MuSK-mouse models, there is evidence of AChE deficiency, possibly explaining the hypersensitivity to AChE-I seen in MuSK-MG patients.[141] Further studies are needed to confirm this hypothesis.

1.6.2 Long-term immunotherapies

Corticosteroids are the first immunosuppressive drugs of choice when symptomatic therapy is insufficient. Prednisolone is the preferred steroid in most European centers, while prednisone is the standard steroid used in the USA. The drug is taken orally, and the start-dose should be high enough to induce remission (up to 60-80 mg alternate days, or 30-60 mg daily), after which the dose is slowly tapered to the minimum dose required to maintain remission. A temporary worsening may be seen at high doses of prednisolone ('*steroid dip*'), and a close observation of the patient is urged.[138] This is the reason why some centers prefer to increase the prednisolone slowly in MG.

The anti-inflammatory effects of corticosteroids are complex and not fully understood. These include induced apoptosis of T-cells and blocked transcription of inflammatory cytokines.[142] Although efficient, there are serious side-effects associated with both short- and long-term use of corticosteroids; osteoporosis, diabetes, hypertension, weight gain, fluid retention, insomnia, cataract, peptic ulcer disease and increased risk of infections.[138;142]

Azathioprine is the first drug of choice whenever long-term immunosuppression is required in MG.[138] The drug is recommended used in combination with corticosteroids as a steroid-sparing agent. The combination is also more effective than corticosteroids alone.[143] There is a delayed onset of action, and maximum effect is usually achieved first after 6-24 months. Azathioprine and corticosteroids are therefore often initially given as combination therapy for a more rapid therapeutic effect, after which corticosteroids are slowly reduced unless relapse occurs.

Azathioprine inhibits DNA- and RNA-synthesis, and thereby T- and B-cell proliferation. The drug is well-tolerated. Flu-like symptoms and gastrointestinal disturbances develop in about 10%. The potential development of leukopenia and hepatoxicity requires careful monitoring of the blood count and liver enzymes during the first few months. Discontinuation of the drug usually reverses these effects.[138]

Mycophenolate mofetil (MMF) is a second-line immunosuppressant, usually reserved for MG patients who do not tolerate or respond to azathioprine.[138] MMF inhibits purine synthesis specifically in lymphocytes, and has documented effects in inflammatory conditions such as psoriasis and SLE.[144] In MG, retrospective studies suggest a therapeutic and steroid-sparing effect of MMF, but these effects have not been confirmed by randomized controlled trials.[145;146] In these two trials, the patients were followed for 6 months only, which may have been too short considering the biologic effect of MMF. In the largest of the retrospective studies with follow-up time of 2-3 years, the beneficial effect of MMF was demonstrated, both as monotherapy and in combination with corticosteroids.[147] These effects were evident after 6 months in both groups. The side-effects are usually mild, most commonly diarrhea, nausea, headache and infections, but there is a possible increased risk for lymphoma.[148]

Ciclosporin has a well-documented steroid-sparing and therapeutic effect in MG.[149] The drug inhibits calcineurin signaling and thereby T-cell functions. Ciclosporin is a second-line immunosuppressant in MG due to side-effects of nephrotoxicity and hypertension, and is only considered when azathioprine cannot be used, or the effect of azathioprine is inadequate.[138]

Methotrexate is proven safe and beneficial in other autoimmune diseases, but is poorly documented in MG. The drug is a structural analogue of folic acid and inhibits its metabolism, thereby inhibiting DNA synthesis. Due to the lack of beneficial effect in MG, methotrexate is only recommended when first-choice immunosuppressants cannot be used or are inadequate.[138]

Cyclophosphamide has a well-documented effect on MG. The drug interferes with DNA replication by adding an alkyl group to the guanine base of DNA, affecting both B- and T-cells at high doses. Poor side-effect profile including febrile neutropenia, bone-marrow suppression, bladder toxicity, opportunistic infections, and carcinogenic and teratogenic effects, limits the use of cyclophosphamide. The drug is therefore only considered in patients who are intolerant or unresponsive to several other immunosuppressants.[138]

Tacrolimus (FK506) is proven efficient in MG at a low dose in one open trial and several case reports,[150;151] with an additional effect in anti-RyR antibody positive patients.[152] The drug binds to the FK506-binding protein and inhibits the T-cells through the calcineurin-mediated pathway. Tacrolimus also enhances the release of RyR-related sarcoplasmic calcium. Side-effects are dose-dependent and include paraesthesias, tremor, hypertension, hyperglycemia, renal insufficiency and possible risk of malignancy.[148] The drug is recommended in patients with a poorly controlled disease, particularly in patients with anti-RyR antibodies.[138]

Rituximab is reported efficient in MG in several uncontrolled studies, both in AChR-MG and, particularly, in MuSK-MG.[153-156] Rituximab is a monoclonal antibody targeted against the CD20 antigen expressed in pre-B-cells, and subsequently depletes B-cells. Interestingly, only the short-lived plasma cells are affected. These cells produce IgG4, and may explain the efficacy of rituximab in MuSK-MG, although the mechanism of action in MG is not fully clear.[156] Rituximab may also influence T-cell responses. The drug is well-tolerated, and is approved in treatment of B-cell lymphoma and rheumatoid arthritis. Side-effects are related to the intravenous administration of the drug, but cases of infections, prolonged B-cell depletion, heart failure and progressive multifocal leukoencephalopathy have been reported.[148;156] The patients should therefore be carefully monitored. Rituximab is often recommended for severe and moderate to severe MG where first line immunosuppressive therapy has failed.

Eculizumab has demonstrated promising results in severe and refractory MG in one randomized controlled trial.[157] Eculizumab is a recombinant humanised monoclonal antibody that binds to C5, preventing C5 cleavage, blocking the formation of a complement complex. Unlike other immunosuppressive therapies available in MG, this drug targets at the innate immune system. With the proven efficacy and safety from this multicenter-trial, eculizumab represents a new therapeutic approach in MG patients with severe and refractory disease.[157]

Etanercept, belimumab, granalocyte-macrophage colony-stimulating factor, are some of the emerging immunotherapy options in MG. These drugs represent more specific therapy, targeted at different elements of the immune system. Target-specific drugs are currently approved for other autoimmune disorders, but these are not yet well-documented in MG.[158] Future immunotherapeutics should aim at specific targets related to MG pathogenesis.

1.6.3 Thymectomy

Since Sauerbruch's first successful thymectomy in 1911,[3] several observational studies and controlled studies have documented the beneficial effect of thymectomy in MG patients without thymomas.[159;160] The procedure is therefore recommended in MG, but not without controversy as there are no prospective studies or randomized controlled trials of the effect of thymectomy in non-thymoma MG. In reviewing studies showing positive associations between thymectomy and MG remission, Gronseth and Barohn demonstrated confounding differences of prognostic importance in the baseline characteristics between thymectomy and non-thymectomy patients. [160] Thus, there is still a need for prospective studies to conclusively establish the benefit of thymectomy in non-thymoma MG.

The chance of remission is enhanced when thymectomy is performed early rather than later in the course of the disease, and an early intervention is preferred.[138] The procedure is performed either transsternally or by a video-assisted thoracoscopic technique (VATS). Both techniques appear equally effective, but there is better cosmetic result and less need of postoperative medication after VATS.[161] The effect of thymectomy usually occurs within two years, after which immunosuppressive drugs often given prior to thymectomy can be tapered off.[138]

EOMG patients often have an enlarged thymus, and long-lasting remission is observed in up to 30% after thymectomy.[159] Early thymectomy is usually

recommended in all patients with anti-AChR-positive, early onset, generalized disease, with or without thymic hyperplasia.[138;160]

In LOMG patients with an atrophic thymus, thymectomy is not recommended.[162] However, patients with a debut before the age of 60 years and those with thymic hyperplasia should be considered for thymectomy.[162] The presence of titin-, or RyR-antibodies suggests that thymectomy may be less beneficial.

In ocular MG, there is no evidence of better clinical outcome after thymectomy, and thymectomy is therefore not recommended in this group.[113] There are some conflicting results of thymectomy in MuSK-MG, but thymectomy is not recommended in this group based on the evidence available.[138] In AChR- and MuSK-antibody negative patients with an early onset, thymectomy is recommended if presence of low-affinity AChR-antibodies is detected or suspected.[138]

Thymoma is an absolute indication for thymectomy. Patients with thymoma often show no clinical improvement after thymectomy, although younger patients may have some benefit. The goal of thymectomy in these patients is therefore to treat the cancer.[138]

1.6.4 Acute treatment

Plasma exchange (PE) and intravenous immunoglobulin (IVIG) represent two immunosuppressive therapies with a rapid onset of action and are used when acute intervention is required, e.g. exacerbations or myasthenic crisis, and also to prepare for thymectomy. Both are proven effective and safe in MG.[163] During PE, autoantibodies are removed from sera by membrane filtration or centrifugation. PE is used also in patients without detectable autoantibodies. IVIG is a concentrated solution of immunoglobulins, mostly IgG. The mode of action is complex and includes cytokine inhibition, neutralization of activated complement and autoantibodies.[164] Both PE and IVIG are short-term treatments. The therapeutic effect

occurs within 2-5 days and lasts for about 4-12 weeks. Both randomized and non-randomized evidence show equal efficacy for both treatment modalities in MG exacerbations.[165;166] IVIG has a better side-effect profile and is easier to administer than PE, and is thus the preferred option in many centers.[138;166] The two treatments can be given sequentially.

Myasthenic crisis

Myasthenic crisis is defined as severe weakness that requires intubation or delayed extubation following surgery.[5] Up to 20% of MG patients experience a myasthenic crisis over the course of the disease.[19] Risk factors include surgery and drugs that may worsen MG symptoms, but in 70% of the cases, the crisis is precipitated by an infection.[167] In many cases, the cause is unknown. MG may start with severe respiratory failure, and in MG patients with a confirmed disease, deterioration to myasthenic crisis is easily recognizable.

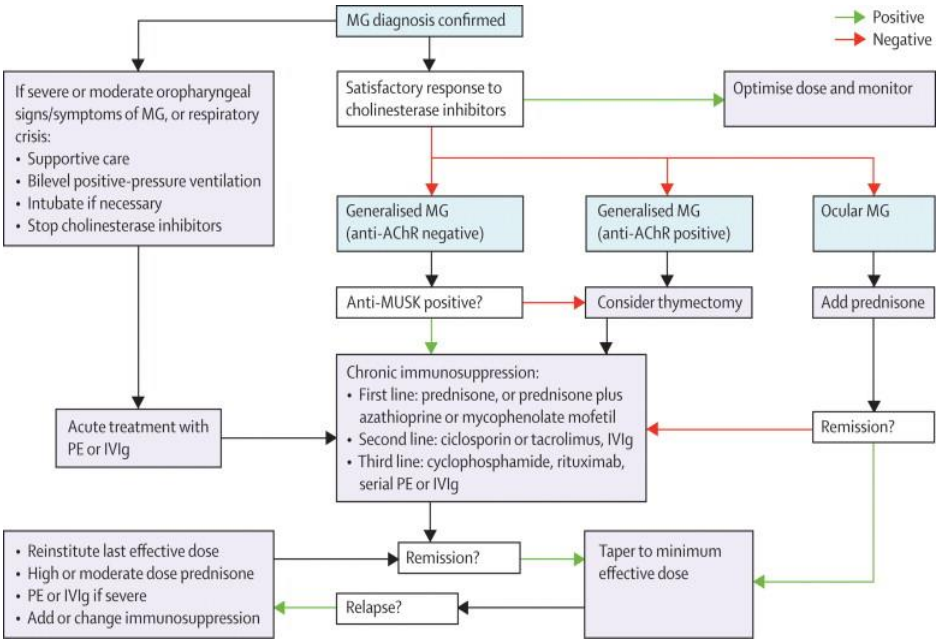
Prompt recognition and intervention with respiratory support is life-saving. AChE-Is increase bronchial secretion, temporary discontinuation is therefore often recommended.[168] PE or IVIG should be given. Available evidence may indicate a slightly better effect of PE over IVIG in myasthenic crisis.[163] The chosen modality should be combined with high dose steroids during the recovery. Long-term immunosuppressive treatment is recommended to maintain the effect of PE or IVIG.[169] AChE-Is should be reintroduced early.[169] The mortality of myasthenic crisis is less than 5%.[19]

1.6.5 Supplementary treatment

A serious complication of long-term steroid treatment is osteoporosis, and bisphosphonate and supplements with calcium and vitamin D should be considered in all patients.[142] A sedentary lifestyle due to fatigue and muscle weakness combined with weight gain and increased glucose tolerance as results of steroid treatment

contribute to an increased risk of cardiovascular diseases. Lifestyle modifications such as weight reduction may be important, although the scientific basis is lacking.[138] Physical training improves muscle strength and is safely recommended in mild and moderate MG.[138] Respiratory muscle training may strengthen the respiratory muscles and improve lung function.[170] Infections should be treated aggressively, and seasonal flu vaccination should be recommended in MG patients.[138]

Figure 4. Treatment flowchart.



From: Meriggioli MN, *et al* (2009): Autoimmune myasthenia gravis: emerging clinical and biological heterogeneity.[48]

1.7 Comorbid conditions in MG

Muscle weakness in MG is in most patients effectively treated, but the management of MG may be complicated by comorbid conditions, consequently affecting the quality of life and outcome. Comorbidity are linked to MG subgroups.[125]

Autoimmune comorbidity

The risk of a second autoimmune disorder is increased in MG patients compared to non-MG, with a frequency of 15%, most frequently in EOMG, but with a higher frequency also in LOMG compared to the non-MG population.[171] In a systematic review, autoimmune thyroid disease, SLE, diabetes mellitus and rheumatic arthritis were identified as the most frequent autoimmune disorders associated with MG.[172] More recently, a population-based study from Sweden found the strongest association between MG and polymyositis/dermatomyositis, SLE and Addison's disease, especially for EOMG. All these disorders are associated with the HLA-B8-DR3 haplotype.[173] This shared haplotype is confirmed in genome-wide association studies in EOMG.[174]

Cardiac disease

Cardiac involvement in MG is recognized, especially in association with thymoma through growth and local invasion.[171;175] MG-specific cardiac muscle antibodies were shown by Mygland *et al* in 1991.[176] Reactive cardiac autoantibodies have been described in thymoma-MG and LOMG. About half of all MG patients and nearly 97% of all thymoma-MG patients have antibodies against titin and RyR, targeting both striated and cardiac muscle in vitro. More recently, antibodies against the β-adrenergic receptors, muscarinic AChRs and VGKC (KCNA4) have been reported. The latter has been suggested as a possible marker for cardiac involvement in MG, especially myocarditis, which occurs more frequently in MG compared to other autoimmune disorders, particularly in thymoma-MG.[95;171] Minor cardiac dysfunctions have been shown in functional imaging studies[177;178] and electrocardiographic findings such as T-wave abnormalities and QT-prolongation have been reported.[175]

The clinical significance of these findings is not yet determined. Deaths from cardiac diseases are not increased in MG patients.[23]

Respiratory tract disease and infections

The vital capacity is reduced in 39% of MG patients, and the risk of severe respiratory tract disease is increased in MG.[20;23] Modern day intensive care facilities have aided the decline of deaths due to respiratory disease in MG to near normal.[23] Infections may precipitate MG worsening, and should be treated aggressively, especially of the respiratory tract, as respiratory muscle weakness can lead to myasthenic crisis.[171]

Cancer

Studies regarding cancer risk in MG are limited and conflicting. Most existing case-control studies have methodological limitations, and MG subgroups are rarely assessed.[171] In two large Taiwanese population-based studies, the risk of cancer in non-thymoma MG was overall increased, specifically for lymphoma.[179;180] This overall increased cancer risk was not confirmed by Pedersen *et al* in a national case-control study, combining several nationwide registries.[181] The Danish authors did, however, find a slight increase in the risk of overall cancer in patients with long-term use of azathioprine. In patients with both long-term use and high cumulative doses of azathioprine, the risk of lymphomas was also increased.[182] Unfortunately, the type of lymphoma could not be evaluated. Also, the risk estimates were based on small numbers. Long term use and high doses of azathioprine was also associated with highly increased risk of non-melanoma skin cancer.[183] Among Norwegian MG patients, cancer was not overrepresented as a cause of death.[23] The lymphoepithelioma in thymoma-MG increases the risk of cancer, and is not related to autoimmunity.[184]

Drugs

Several drugs may cause worsening of MG symptoms, or even unmask latent MG or cause transient myasthenic symptoms, due to increased neuromuscular blocking.[185]

Such drugs include several antibiotics, cardiovascular drugs, analgesics, anticonvulsants, psychotropics, anti-rheumatics, antimalarials, eyedrops and endocrine thyroid replacement therapy.[186] The initiation and maintenance of such drug therapy in MG should therefore be carefully monitored, and the patients informed of potential worsening of symptoms. D-penicillamine is contraindicated in MG.[187] Cases of D-penicillamine-induced MG have been reported. Discontinuation reverses the MG symptoms.[188]

Pregnancy and delivery

Complications during pregnancy and surgical interventions during delivery in MG women occur slightly more frequently compared to non-MG women.[189] Spontaneous abortion is not increased, and vaginal delivery is safe in most cases. During pregnancy, the women should be followed by a team of obstetricians, neurologists and pediatricians. The course of MG during pregnancy is variable. About 30% experience MG worsening, usually during the first trimester. Puerperal infections may increase the risk of MG exacerbation, requiring prompt treatment. In 20-40%, symptom improvement is observed during the second and third trimester, probably due to the reinstated immunosuppression.[190] The clinical course varies between different pregnancies. The long-term outcome of MG is not worsened by pregnancy, and conception is not discouraged.[190]

Transient neonatal MG occurs in 10-20% of infants of MG mothers, due to placental transmission of IgG antibodies. The antibodies are cleared naturally postpartum. Symptoms of weak sucking and crying, dysphagia, hypotonia and respiratory distress are in these babies evident within the first two days after delivery and can persist during the first four weeks of life, often only for days, however. Maternal MG can in rare cases cause arthrogryposis multiplex congenita, which is defined as non-progressive congenital contractures and malformations, due to lack of fetal movement in utero. Maternal MG severity is not correlated with occurrence of neonatal MG or arthrogryposis multiplex congenita. Breastfeeding is normally encouraged, but should be avoided in newborns with severe neonatal MG.[190]

1.8 The course of MG

Despite advances in therapy, the course of MG remains variable. Spontaneous remissions are rare. The long-term outcome is generally good, with a life-expectancy near normal. Deaths due to MG exacerbations or crisis are very rare. Nevertheless, long-lasting remissions are only obtained in about 20% of the patients, usually after thymectomy. Prognostic factors include age at onset and time of diagnosis from onset.[191]

The clinical course of MG seems to be determined within the first two to three years after disease onset, with maximum disease severity occurring within this time period. 70% reach the maximum level of severity during the first year of onset, and 85% during the first three years.[111] In subsequent years, the patients usually improve or attain a more stable disease.

In about 85% of all patients, the disease becomes generalized. 66% of the patients with only ocular manifestations at disease onset were found to develop a generalized disease.[111] If generalizations have not occurred within the first two years in patients with a debut of ocular MG, the disease is most likely to remain purely ocular.

1.9 Registry-based epidemiological research

Epidemiology is defined as "*the study of the distribution of disease and determinants of health-related states or events in specified human populations, and also the application of such studies to the control of human health problems*."[192] The aims of epidemiology are to elucidate disease etiology and to determine the risk factors for the disease, and thereby ultimately to develop strategies for prevention of the disease. In clinical settings, epidemiology seeks to "*make predictions about individual patients by counting clinical events in similar patients, using strong scientific methods for studies of groups of patients to ensure that predictions are accurate*".[193]

National health registries represent a new opportunity of studying health problems on complete populations, with enhanced power, less bias, and are less time- and cost-consuming for the individual researcher. On the other hand, detailed clinical information is often missing. Linkage of different central health registries may to some degree overcome this issue. Most central health registries are based on '*silent consent*,' i.e. any individual whom do not wish to have their health information stored in central health registries have to actively reserve themselves. This may represent an ethical challenge for researchers and the providers of the registries. Access to data is therefore subject to an extensive, and often times lengthy, application process. Clinical patient registries usually contain more detailed information about individual patients, and are based on written, informed consent, but are usually prone to selection bias. The strengths and limitations of registry-based populations are discussed in depth in chapter 5.2 'Methodological considerations'.

Ethical considerations

The Norwegian Institute of Public Health (NIPH) is the provider of several of the central health registries in Norway. NIPH collects, stores and maintains the registries, with the goal of facilitating research and health surveillance of the population. Health information stored in the central health registries in Norway is regulated by the Personal Health Data Filing System Act of 2002, which emphasizes the importance of the individual's right to privacy. Access to data is only given if their intendent use is in accordance with the objectives of the respective registry, often defined by authoritative regulations. Approval from the Norwegian Regional Ethical Committee is in most instances required, depending on the level of details and anonymity of the data enquired.

The 11-digit personal identification number (PIN), which is unique for every individual living in Norway, provides a valuable opportunity of linkage of two or more registries, including medical quality registries and national population statistics, e.g. regarding education and income. There are four levels of person identification in the central health registries provided by NIPH:

Anonymous data are data where re-identification of individual persons is made impossible. Such data provide the highest level of protection of privacy, but is often not adequate when studying rare diseases. Anonymous data are hard to validate, and it is not possible to follow individual cases over time, which limits their use.

Pseudonymous data are data where the identity is encrypted, but each individual has been given a person-specific pseudonym, making it possible to follow individuals over time.

De-identified data resembles anonymous data, but the identity may be traced through a serial number given each individual. Researchers do not have access to the serial numbers, and thus in practice the data may be considered as anonymous. The serial number is kept at a trusted third party, usually Statistics Norway, with the purpose of facilitating linkage of data.

Person-identifiable data are data in which each individual's PIN, or name, or both are available to the researcher. Such data are the most extensive for research. Data missing the PIN, or name are characterized as person-identifiable if enough personal information is included to indirectly identify an individual ('*backdoor-identification'*). Written, informed consent with a clear statement of the purpose of the research and each participant's rights must be obtained.

2. Aims of study

Paper I: To determine the incidence and prevalence of symptomatic MG in Norway, with emphasize on age- and gender characteristics and geographic variation.

Paper II: To study comorbidity in MG patients, and thereby assessing the total health burden for patients with MG.

Paper III: To assess the clinical course and prognosis of MG, and thereby identifying factors influencing the outcome.

3. Materials and Methods

3.1 Data sources

The Norwegian Prescription Database (NorPD)

The NorPD was established in 2004 and is maintained by the NIPH. The database contains information on all prescription drugs expedited at every pharmacy in Norway, covering the entire population of 5,000,000. The registration is mandatory, with monthly automatically generated updates from all pharmacies. The objectives of the NorPD are defined in authoritative regulations ('Forskrift for Reseptregisteret'), and data can only be used accordingly; i) to describe patterns of drug use; ii) to form basis for research and review of drug safety and effectiveness; iii) for health care planning and control; iiii) for quality improvement of prescribing practices.

In NorPD, the patient's identity is encrypted, and the unique 11-digit PIN is replaced with an encrypted personal-identifier. This personal-identifier is unique for every patient, which can be used to follow individuals over time. The information available in the NorPD is as follows:

1. *Patient*; Encrypted person-identifier, month/year of birth, month/year of death, gender, place of residence (municipality and county)

2. *Prescriber*; Encrypted person identifier, year of birth, gender, profession, medical specialty

3. *Pharmacy*; Name, licence number, municipality and county

4. *Drug*; Nordic article number (a unique product identifier stating brand name, strength, pharmaceutical form and pack size), number of Defined Daily Doses (DDD), date of expedition, ATC code, price, prescription category, reimbursement code, ICD-10 or ICPC-2 codes (from March 2008, completely implemented from March 2009), free text according to pharmacy label.

NorPD collects information about prescribed and dispensed drugs at pharmacies to individuals living outside institutions. Also included are unlicenced drugs, but not drugs sold over-the-counter unless they are prescribed by a physician. For in hospital patients, and patients living in nursing homes, drug use is collected on an aggregated level, i.e. at the level of the institution or department.[194]

Health care in Norway is free of charge for all Norwegian citizens and covers both specialist and primary care. For chronic diseases, drugs are reimbursed if the diagnosis has been established. For MG, diagnostic workup is performed in specialist health care, and Mestinon is reimbursed for all MG patients with an established diagnosis (§13).

Health Regions of Norway

From 1995 to 2007, the Norwegian Healthcare System was divided into five Health Regions. Calculations of the geographical distribution of symptomatic MG assessed in paper I was based on these five Health Regions, with a population at prevalence day January 1, 2008 of: 916,000 for the Southern Health Region; 1,717,000 for the Eastern Health Region; 982,000 for the Western Health Region; 660,000 for the Central Health Region; 462,000 for the Northern Health Region.[195]

Norwegian Central Population Registry (NCPR)

The NCPR provides the unique 11-digit PIN since 1960 for all individuals living in Norway. The NCPR also register demographic information on the entire population, including date of birth, place of residence and date of death or emigration. The population in Norway on January 1, 2008 was 4,737,000.[195]

Duke MG Patient Database

Paper III is based on data from the Duke MG Patient Database, which is a physician-derived registry, containing clinical information on all MG patients treated , both in- and outpatients, at the Neuromuscular Clinic at the Duke University Medical Center in North Carolina, USA. The registry was established in 1980 and is used for research

as well as for evaluating patient outcomes. Patient information is updated after each visit by trained nurses (Appendix 1). A written consent is obtained from the patients before their clinical information is stored in the registry. The registry is maintained in accordance with the Health Information Portability and Accountability Act, the USA standards to protect personal health information. All research projects based on data from the registry must be approved by the Institutional Review Board of the Duke University.[196]

As of March 2012, 60 new patients with MG, congenital myasthenic syndromes and LEMS were seen at the Neuromuscular Clinic, constituting about 550 clinic visits each year. The registry contains information on 1,545 patients with neuromuscular disease; 1,310 with MG, 20 with congenital myasthenic syndromes and 110 with LEMS.

3.2 Study Population

Paper I and II

Data were obtained from the NorPD. All patients using pyridostigmine (Mestinon, ATC code: N07AA02), and who fulfilled one of the following criteria were included:

1. Minimum two prescriptions of pyridostigmine (Mestinon, ATC code: N07AA2) during the study period
2. A prescription from a neurologist
3. A prescription with a reimbursement code for MG (§13)
4. A prescription with an ICD-10 (G70.0) or ICPC-2 (N99) code for MG (paper II only)

In *paper I*, 723 patients were registered with at least one prescription of Mestinon dispensed during the study period from January 1, 2004 - December 31, 2007. Of

these, 677 (94%) fulfilled the inclusion criteria. 94% of the final study population had a reimbursement code for MG. The remaining 6% did not have an MG specific reimbursement code; three patients received a prescription of Mestinon from a neurologist; 41 patients had two or more prescriptions of Mestinon dispensed during the study period.

In *paper II*, 890 patients were registered with at least one prescription of Mestinon dispensed during the study period from January 1, 2004 – April 30, 2010. Of these 830 (93%) fulfilled the inclusion criteria. 89% had either a reimbursement code for MG, a prescription with an MG specific code, or a prescription from a neurologist. However, the registration of ICD-10/ICPC-2 codes was not fully implemented until March 2009.

Paper III

All patients treated at the Neuromuscular Clinic at Duke University Medical Center from 1980 to 2014 by the same physician, and who had minimum two years of follow-up data were included. 268 patients with at least two years of follow up were included. Clinical information was complete for 262 patients (98%) at two years of follow-up, 213 at five years (80%) and 117 at ten years (44%). Clinical information was complete for all 268 patients at their last clinic visit. Some patients with clinical information missing at two or five years, but who had information registered at a later time point were also included.

3.3 Statistical methods and definitions

Incidence and prevalence

Incidence and prevalence were assessed in paper I. Incidence of symptomatic MG was defined as all patients who fulfilled one of the criteria preset by us for the MG diagnosis during the last year of the study period (2007), and who had no previous pyridostigmine prescriptions dispensed the preceding years (2004-2006). Incidence

was calculated per one million inhabitants per January 1, 2008. Prevalence of symptomatic MG was defined as all patients receiving a prescription with pyridostigmine during the study period (January 1, 2004 - December 31, 2007), and who were alive and living in Norway at the prevalence date, January 1, 2008. Prevalence was calculated per one million inhabitants.

The Anatomical, Therapeutical and Chemical classification system (ATC)

All registered drug in the NorPD is classified according to the World Health Organization's ATC classification system, which are structurally divided into groups at five levels. At the first level, the drugs are divided into 14 main groups. At the second level, the drugs are further divided into pharmacological/therapeutic subgroups. The third and fourth levels are the chemical/pharmacological/therapeutic subgroups. Finally, the fifth level represents the chemical substance. This system makes it possible to compile drug statistics on five different levels.[194]

Textbox 1: Main ATC groups

A: Alimentary tract and metabolism
B: Blood and blood forming organs
C: Cardiovascular system
D: Dermatologicals
G: Genito urinary system and sex hormones
H: Systemic hormonal preparations, exclusive sex hormones and insulins
J: Antiinfectives for systemic use
L: Antineoplastic and immunomodulating agents
M: Musculoskeletal system
N: Nervous system
P: Antiparasitic products, insecticides and repellants
R: Respiratory system
S: Sensory organs
V: Various*

*The ATC group V (Various) was considered too unspecific and therefore not included in the analyses.

Total drug treatment of MG patients was assessed by investigating every prescription dispensed in all main ATC groups during the study period. In paper II, all main ATC groups but one (V: Various*) were assessed (Textbox 1). In addition to the main ATC groups, we also assessed various drugs at different ATC levels: A10 (Drugs used in diabetes), A10A (Insulins and analogues), H03AA (Thyroid hormones), N05A (Antipsychotics), N05B (Anxiolytics), N05C (Hypnotics and sedatives), N06A (Antidepressants), N03 (Antiepileptics), C07 (Beta-blocking agents), C08 (Calcium channel-blockers), C10 (Lipid-modifying agents) and J01G (Aminoglycoside antibacterials).

Defined Daily Dose (DDD)

In paper II, the DDDs were assessed to evaluate immunosuppressive drug use in MG patients. The DDD is defined as the assumed average maintenance dose per day for a drug used on its main indication in adults. The DDD is thus a unit of measurement that enables meaningful comparisons between different drugs. The DDDs are determined on the basis of evaluation of the use of any specific drug internationally, not taking into consideration national therapeutic guidelines which vary between countries.[194]

Standardized Incidence Ratio

The standardized incidence ratio (SIR) was assessed in paper II to evaluate the use of various drugs according to both the main ATC groups, and also for specific drugs. For each subgroup of patients categorised by age and sex, drug statistics for the corresponding age and sex groups in the Norwegian population registered in NorPD from the same period were recorded. Comparisons of age- and sex-specific drug use among MG patients and the Norwegian population were done by comparing the observed number of prescriptions for all main ATC groups in the MG patients divided by the estimated number of prescriptions for the same drug groups dispensed to a similar group, with regard to age and gender, in the general population.

Demographic characteristics and outcome variables

Differences in categorical characteristics of subgroups of MG patients were compared by Pearson's chi-square test. Fisher's exact test was used for cross-tabulations with expected cell count below five. Continuous variables were compared by Student's t-test for independent samples or non-parametric tests when comparing more than two subgroups, or whenever the distribution was not Gaussian.

Statistical analyses in paper I and II were performed using SPSS, version 16-21 (IBM Corp., Armonk, New York, USA). Statistical analyses in paper III were performed using JMP version 11.2 (SAS Institute Inc. Cary, North Carolina, USA). Two-sided *p* values <0.05 were considered statistically significant. 95% CI intervals for SIRs were calculated assuming a Poisson's distribution.

MGFA Clinical Classification and Post-Intervention Status

In paper III, the MGFA Clinical Classification and Post-Intervention Status were used to assess the clinical course and outcome of MG patients. The MGFA Clinical Classification is designed to classify MG patients with similar clinical manifestations and disease severity for comparative analyses of different therapeutic interventions,[22] as this is often difficult due to the fluctuating nature of the disease. This standardization of grading MG severity is universally accepted and offer more precision than the Osserman's classification of 1958, classifying MG only as mild, moderate or severe. The MGFA Clinical Classification defines different levels of disease severity according to which muscles are affected (Textbox 2).

The MGFA Post-Intervention Status (PIS) is designed to assess the clinical state of MG patients at any time after treatment has been given.[22] Determination of the PIS requires that the examination is performed by someone skilled in the evaluation of neuromuscular disease. Isolated weakness of eyelid closure is not an exclusionary criteria from CSR or PR status, as this was not thought to be a sign of active disease by the MGFA Taskforce. Patients receiving AChE-Is, however, are excluded from PR or MM-1 status as these medications mask MG symptoms (Textbox 3).

Kaplan-Meier and Cox regression

In paper III, Kaplan-Meier life tables were used to compare the cumulative chance of achieving an optimal outcome in MG and compared by the log-rank test. Variables with a significant difference in the Kaplan-Meier model were then adjusted for in the Cox regression model for independency and presented as estimated hazard ratios (HR) with 95% CI and corresponding *p* values.

Textbox 2. MGFA Clinical Classification

Class I	Any ocular muscle weakness; may have weakness of eye closure. All other muscle strength is normal.
Class II	Mild weakness affecting muscles other than ocular muscles; may also have ocular muscle weakness of any severity.
	IIa. Predominantly affecting limb, axial muscles, or both. May also have lesser involvement of oropharyngeal muscles.
	IIb. Predominantly affecting oropharyngeal, respiratory muscles, or both. May also have lesser or equal involvement of limb, axial muscles or both.
Class III	Moderate weakness affecting muscles other than ocular muscles. May also have ocular muscle weakness of any severity.
	IIIa. Predominantly affecting limb, axial muscles, or both. May also have lesser involvement of oropharyngeal muscles.
	IIIb. Predominantly affecting oropharyngeal, respiratory muscles, or both. May also have lesser or equal involvement of limb, axial muscles or both.
Class IV	Severe weakness affecting muscles other than ocular muscles. May also have ocular muscle weakness of any severity.
	IVa. Predominantly affecting limb, axial muscles, or both. May also have lesser involvement of oropharyngeal muscles.
	IVb. Predominantly affecting oropharyngeal, respiratory muscles, or both. May also have lesser or equal involvement of limb, axial muscles or both.
Class V	Defined as intubation, with or without mechanical ventilation, except when employed during routine postoperative management. The use of feeding tube without intubation places the patient in class IVb.

Textbox 3. MGFA Post-Intervention Status (modified version).

CSR	The patient has had no symptoms or signs of MG for at least 1 year and has received no therapy for MG during that time. There is no weakness of any muscle on careful examination by someone skilled in the evaluation of neuromuscular disease. Isolated weakness of eyelid closure is accepted.
PR	The same criteria as for CSR except that the patient continues to take some form of therapy for MG. Patients taking cholinesterase inhibitors are excluded from this category because their use suggests the presence of weakness.
MM	No symptoms of functional limitations from MG but has some weakness on examinations of some muscles. This class recognizes that some patients who otherwise meet the definition of CSR or PR do have weakness that is only detectable by careful examination.
MM-0	The patient has received no MG treatment for at least 1 year.
MM-1	The patient continues to receive some form of immunosuppression but no cholinesterase inhibitors or other symptomatic therapy.
MM-2	The patient has recived only low-dose cholinesterase inhibitors (<120 mg pyridostigmine/day) for at least 1 year.
MM-3	The patient has received cholinesterase inhibitors or other symptomatic therapy and some form of immunosuppression during the past year.
	Change in status
Improved	A substantial decrease in pretreatment clinical manifestations or a sustained substantial reduction in MG mediations as defined in the protocol.
Unchanged	No substantial change in pretreatment clinical manifestations or reduction in MG medication as defined in the protocol.
Worse	A substantial increase in pretreatment clinical manifestations or a substantial increase in MG medications as defined in the protocol.
Exacerbation	Patients who have fulfilled criteria of CSR, PR or MM but subsequently developed clinical findings greater than permitted by these criteria.
Died of MG	Patients who died of MG, of complications of MG therapy, or within 30 days after thymectomy.

4. Results

4.1 Paper I

The study included 435 (64%) women, 242 men, with an overall female to male ratio of 1.8. Point prevalence (January 1, 2008) of symptomatic MG was 131 per million; 92 for men, 170 for women. Mean age of prevalent MG patients was 56 years, 60 years for men and 54 years for women. Female to male ratio in the age group <50 years was 3:1 compared to 1.5:1 in the age group ≥50 years. For those <50 years, the point prevalence was 67 per million, and 258 per million for patients ≥50 years. The peak prevalence for both genders was around 70–79 years. The number of female MG patients increased steadily up to 70–79 years, while the number of male patients rose sharply after the age of 50 years.

74 new users of pyridostigmine were registered in 2007 (42 women, 32 men). The incidence rate of symptomatic MG for the year 2007 was 16 per million; 14 for men, 18 for women. Mean age of incident cases was 59 years; 64 and 55 years respectively. Both prevalence and incidence was higher in the age group ≥50 years than <50 years, and highest at 70–79 years. The rates were stable for both men and women until age 60 years, with a small peak for women at age 30–39 years. The highest rate for both genders was at 70–79 years. Incidence for patients <50 years was 7 per million. Patients ≥50 years had an incidence of 34 per million. Prevalence and incidence did not differ in the five geographical health regions in Norway.

4.2 Paper II

In this study, we evaluated the total drug treatment of MG patients, with comparisons to the general population, in the period from January 1, 2004 to April 30, 2010. 87,556 prescription medications were in total dispensed to 830 MG patients. Only 19 individuals (2.3%) received no other medication than Mestinon. The remaining MG patients received all types of medication, and more often than compared to the general

population in nearly all categories of drugs. Most frequent were drug therapies for the Alimentary tract and metabolism, Systemic hormonal preparations and Antineoplastic and immunomodulating agents.

Comedication with insulins was almost three times more frequently prescribed to MG patients ≥50 years, both males and females, compared to the general population. 13% received thyroid hormone therapy, most frequently MG patients <50 years and male MG patients compared to patients ≥50 years and female MG patients, and twice as often as the general population. 29% received treatment with hypnotics and sedatives, and twice as often given as for the general population for the age group <50 years. 21% received antidepressants; twice as often given to male MG patients than to the male population. 20% received anxiolytics, while 7% received antipsychotics, with the same frequency in MG patients as in the general population. MG patients were more often treated with antiepileptic drugs, calcium-channel blockers and lipid-modifying agents, and with the same frequency as in the general population with beta-blocking agents. All four drug groups were given more frequently to MG patients <50 years compared to the general population at the same age.

The DDDs of pyridostigmine were lower for MG patients <50 years compared to those ≥50 years. There was no difference between males and females. Less DDDs of immunomodulating agents were prescribed to MG patients <50 years and females compared to MG patients ≥50 years and males. 406 MG patients (49%) did not use any immunosuppressive drugs during the study period. Patients <50 years were prescribed less DDDs of prednisolone compared to patients ≥50 years. No such age difference was seen for azathioprine. Females were prescribed less DDDs of both prednisolone and azathioprine compared to males.

4.3 Paper III

The study included 197 patients with elevated AChR-antibodies (74%), 13 with MuSK-antibodies (5%) and 58 who were double negative (22%). There were some distinct clinical features related to antibody status; AChR-positives were more often

late onset, male and white, compared to MuSK-positives, who had early onset, were more often female and African-American. Patients without AChR- or MuSK-antibodies were more often early onset, male and white. Thymectomy was performed in 110 patients, and at different rates among the antibody groups ($p=0.02$). Over 50% of MuSK-positives were thymectomized, compared to 26% of the double negatives and 45% of AChR-positives. All 22 patients with a thymoma were AChR-positive. There was no difference among the antibody groups regarding reported thymus histology.

56% of the study population had moderate or severe weakness at maximum, and only 13% had purely ocular manifestations at least two years after onset. Maximum weakness differed among the antibody groups ($p<0.001$), with MuSK-positives being less often purely ocular compared to AChR-positives and double negative patients.

Clinical severity was different among the antibody groups at two ($p=0.02$) and five years of follow-up ($p=0.02$) and at the last clinic visit ($p=0.005$), but not after ten years, probably because too few patients were followed that long. At two years, 30% of the AChR-positives, 26% of the double negatives, and only 15% of the MuSK-positives were in remission. MuSK-positive patients had more severe weakness at initial presentation, but the majority had mild weakness throughout the subsequent follow-up period. The rate of spontaneous remission in the total patient cohort was 1%.

Outcomes did not differ among the different antibody groups at any time point. After two years, 65% had achieved an Optimal outcome; 73% did so after five years; and 75% after ten years of follow-up. Only 3% had a Poor outcome after ten years. The probability of achieving an Optimal outcome did not differ among the antibody groups at any time point. Most consistently, age at onset, thymectomy and disease distribution at maximum severity differed regarding the probability of achieving an Optimal outcome at each time point. However, after adjusting for these variables in the Cox model, only onset after age 50 years independently predicted an Optimal outcome at each time point.

5. Discussion

5. 1 General discussion

Epidemiology

We demonstrate that MG incidence and prevalence is higher than previously expected, especially in the elderly. Our estimated incidence of 16 per million is in line with most previous reports of MG incidence, with the highest reported incidence so far being 24.9 per million.[29] Also, this rate is comparable to MG incidence found in prospective studies and other population-based studies. Furthermore, we found that the increase is particularly profound in the elderly, both men and women, as shown also by others.[40;41] There are several factors that might explain this development: First and foremost, improved diagnostic accuracy and case ascertainment have had a major impact. After the AChR-antibody assay was introduced and commercially available around the mid-1980s, the incidence rates near doubled.[25] Second, the demographic pattern is shifting towards an aging population at higher risk of acquiring the disease, possibly due to age-related alterations of the immune system.[197] At the same time, the birth rates are declining. Third, unknown environmental factors, lifestyle changes or genetics factors may cause increased susceptibility leading to an increased incidence.

Our prevalence rates of 131 per million is comparable with other population-based studies and in line with previous studies on MG prevalence. Over the past six decades, MG prevalence has risen from less than 30 per million[42] to over 300 per million in 2014[43] as a consequence of increasing incidence and longevity of MG patients. In Norway, the reported prevalence has increased from 21 per million in 1951[42] to 90 per million 30 years later[34]. Another three decades later, the prevalence of MG in Norway is around 130-145 per million.[44;45] A similar development in prevalence was found in Italy; from 83 to 130 per million over a period of 20 years.[41] In addition to factors influencing incidence and mortality, these differences in

prevalence also most likely reflect better access to specialized health care and increased awareness of the disease among neurologists and physicians.

In paper I, we found that prevalence and incidence were higher in the age group >50 years than <50 years. MG onset after the age of 50 years used to be considered rare.[46] During the 1990s, epidemiological data suggested that MG is occurring more frequently in older individuals than previously believed. Peak age at onset reflect these differences in incidence between the two genders (Figure 2). In females, the peak occurs in the second and third decade of life, while the peak is in the sixth and seventh decade in males. In some countries, with a particular Scandinavian cluster, there is a bimodal distribution of onset in females with a peak in the sixth and seventh decade as well.[25;41;44;45;100;198] More recently, only one peak of age at onset has been demonstrated in both genders, usually around the seventh and eighth decade.[28-30] In light of these recent findings, it is likely that older individuals, both males and females previously have been grossly underdiagnosed.[46;199]

As Somnier *et al* pointed out in the early 1990s, the subgroups EOMG and LOMG may be different entities of MG altogether.[38] The thymus in EOMG patients is often hyperplastic with lymphocytic infiltrates and germinal centers similar to those found in lymph nodes. All components necessary for developing an immune response are present and can support an intrathymic pathogenesis for the immune response in EOMG. These thymic abnormalities are usually lacking in LOMG. The mechanisms of autosensitisation to AChRs in this subgroup are unclear. Other striking differences between EOMG and LOMG are the HLA-profile, titers of circulating AChR-antibodies, presence of striated muscle antibodies and associated autoimmune comorbidity. Interestingly, several studies, including ours, report a peak age of MG incidence around 70-80 years, with an abrupt fall in incidence in the age group >80 years.[44;45;199;200] Important clinical features in MG such as ptosis, diplopia and facial muscle weakness are more easily overlooked in the elderly as ageing causes sagging of the lower eyelids and weaker tonus of the facial muscles.[46] Another factor is the competing risk for developing other fatal diseases for those patients at risk for

developing MG.[199] In our study, only patients living outside institutions were included. That means that about 19% of the population >80 years are not accounted for. The rate of MG among the population in this age group living in institution is unknown.

Comorbidity

Our findings show that comedication in MG is widespread, requiring more frequent drug treatment for several major disease groups than in the general population. Cardiovascular drug therapy is more often given to MG patients compared to the non-MG population. The possibility for cardiac involvement in MG is well recognised; MG-specific cardiac muscle antibodies and reactive cardiac autoantibodies have been described, and minor cardiac dysfunctions have been demonstrated by functional imaging studies.[176-178] The clinical implications of these findings remain unclear. Nevertheless, our data indicate that there is a clinically relevant association between MG and cardiovascular disease. Death from cardiac diseases, however, is not increased in MG patients.[23;201]

In our study, insulins and thyroid substitution therapy functioned as proxies for autoimmune disease. We found that both these types of comorbidities are elevated in MG. The risk of a second autoimmune disorder is increased in MG patients compared to non-MG, more frequently in EOMG, but also to some degree in LOMG compared to non-MG population.[171] Previous studies have identified autoimmune thyroid disease, SLE, diabetes mellitus and rheumatic arthritis as the most frequent autoimmune disorders associated with MG.[172]

The risk of severe respiratory tract disease is increased in MG,[23] in addition to the increased risk of infections in general due to immunosuppressive treatment.[142] Infections may precipitate MG worsening, and should be treated aggressively, especially of the respiratory tract, as respiratory muscle weakness may lead to myasthenic crisis.[171] This probably explains why antiifectives is more often used in

MG compared to the general population. Death due to respiratory disease in MG is near normal level.[23]

Outcomes

Surprisingly, we found that MG onset after the age of 50 years increased the chances for an Optimal outcome. This is in contrast to previous similar studies where a late onset was associated with a worse prognosis.[21;191] There is a potential referral bias to our clinic towards patients with more severe disease, but this bias is expected to be the same for all age groups. It is probable that elderly patients are more aggressively treated at our clinic: 87% of the patients over 50 years were treated with an immunosuppressive agent, either alone, or in combination with another treatment modality, in contrast to 56% of Norwegian MG patients over 50 years.[44] However, this comparison is complicated by the potential selection bias in patients seen at a specialized University clinic in contrast to the unselected Norwegian cohort which is based on the entire population.

Although the clinical distribution at maximum weakness differed among the three antibody groups, we did not find any difference in the chance of achieving an Optimal outcome for mild/moderate compared to severe disease. MuSK-MG is characterized by rapid deterioration early in the course of disease with generalization usually occurring within the first month. The MuSK-positive patients in our study had more severe disease than AChR-positives and the double negatives. Nevertheless, the majority of these patients achieved an Optimal or Intermediate outcome, a similar long-term prognosis as AChR-positive patients, in accordance with previous studies.[21;202]

Thymectomy was performed both in double negative and MuSK-positive patients. The role of thymectomy in these two groups is still unclear, with conflicting evidence. Only one of our thymectomized MuSK-positive patients was in CSR at last visit; this rate, however, was comparable to thymectomized AChR-positive and double negative patients. Univariate analysis at all time-points clearly indicates that thymectomy is

associated with MG outcome. However, the thymectomized patients represent a selection bias towards more severe disease, including thymoma patients. This could explain why thymectomy does not independently predict outcome after adjusting for age at onset and clinical severity.

5.2 Methodological considerations

5.2.1 Study design

Paper I and paper II are cohort studies based on observational data from a population-based prescription register. NorPD covers the entire population, thus enhances statistical power and minimizes selection bias. The information collected is nearly complete, and is less prone to information bias and other types of systematic errors. Population-based cohort studies also include a wider range of disease severity among the study subjects than other more selected study samples and study designs. Furthermore, Norway has a relatively homogeneous and a stable population, with low rates of emigration. A population-based cohort study design performed in a stable genetic 'pool' is thus suitable for epidemiological research in general and for rare diseases especially.

Although complete, the information in population-based registers is often limited as they may be designed for other purposes, e.g. administrative purposes. Relevant clinical information desired by researchers may be difficult to obtain, or are missing. For NorPD, drug compliance is not known. Also, the indication of drug prescribing was until March 2009 incomplete. There are two main indications for Mestinon in Norway; MG and gastrointestinal dysmotility. MG patients are reimbursed with a specific reimbursement code which functions as a proxy of diagnosis. However, the drug is relatively inexpensive, and reimbursement may be declined by some patients. The efficacy of Mestinon in treating gastrointestinal dysmotility in diagnosis such as chronic intestinal pseudo-obstruction is inconclusive.[203] Mestinon can also be used as supplementary agent when treating orthostatic hypotension. Evidence of the efficacy

of this treatment is limited.[204;205] Information on drug use for patients living in institutions is not available in NorPD. These include many elderly. In 2013, 14% of the Norwegian population over 80 years lived in institutions.[206]

Paper III is based on a physician-derived registry containing clinical patient information. Such patient registries are useful in several ways. First, they contribute to optimizing management of the patient by facilitating evaluation of therapy response. In clinical research, information can be used to assess patient outcome. Public health reporting, geographic surveys, epidemiological research are other ways of utilizing such information. The Duke MG Patient Database is designed to achieve these goals by systematically collecting data and regularly updating the information after each patient visit. Data entry is performed by the physician or other trained personnel (nurses, residents), and the registry is continuously developed in accordance with the latest standards of clinical assessments.[196]

As with all hospital-based materials, data are subject to selection bias, and the disease severity among the study population may not be representative. Referral bias is another potential problem for medical centers with an expertise in specific fields. In countries without universal health care coverage, such as the USA, studying disease severity in specific social classes may not be possible with data from patient registries as entry requires a health insurance, which is often financed through employment.

5.2.2 Internal validity

Internal validity refers to the accuracy or precision of the measured parameters in a study and is dependent on the degree of error in the measurement. Errors in epidemiological research are either random or systematic.

Random errors and precision

Data collection, coding and analysis are subjects of random errors, causing variations around a true value. Random errors are thus a problem of precision. A precise

estimate is an estimate with little random error. Large study samples reduce the effect of random errors, thus enhancing the precision of the study. The precision of an estimate is further indicated by the CI, i.e. the statistical variation, or random error, underlying the estimate. A narrow CI reflects a high precision of the estimate.

NorPD is a nationwide database covering about five million people. Since the establishment in 2004, 96% of the entire Norwegian population has been included in NorPD with at least one prescription drug dispensed from a pharmacy. The one year prevalence of 68-69% has also been stable.[194] Such a large study size reduces the effects of random errors. Furthermore, data collection is generated automatically every month from the pharmacies to NorPD, thus avoiding extra work for the pharmacy. Coding errors or inconsistencies are systematically searched after by the NIPH before transferring data to NorPD. NIPH routinely checks if the data deliveries from each pharmacy seem to be reasonable. Any unusual fluctuations in size of data files from one month to another are identified. Missing data are also checked for, such as empty data files due to technical errors at the pharmacy. Every month, the total number of prescription records and the number of patients and prescribers are checked.

The Duke MG Patient Register is controlled at every single registration by the examining physician, and records are updated by a trained nurse. There is no systematic quality control, but the register is widely used for clinical assessment of patients and for research, and is controlled whenever data is extracted.

Systematic errors and validity

Systematic errors, or bias, occur when the true value is different from the observed value due to any other cause than random variability. A valid estimate is thus an estimate with little systematic error. Systematic errors are not affected by increased study sample.[207] Three main types of systematic errors can affect the *internal validity*: Selection bias, information bias and confounding.

The validity of a diagnostic test or statistical method is described by its sensitivity and specificity. Sensitivity refers to the proportion of actual positives that are correctly identified, i.e. *true positive*. In paper I and II, the sensitivity of using prescription of Mestinon to identify MG patients, reflects the percentage of Mestinon users with an MG diagnosis as defined by us. Specificity refers to the proportion of actual negatives, according to our definition, that are correctly identified, i.e. *true negative*. The validity of a test can also be expressed by its predictive value. The predictive value refers to the probability that a person who is categorized as positive, or negative, has or will develop the disease. In Norway, Mestinon is used by nearly all MG patients with a symptomatic disease,[208] and is in principle reimbursed to MG patients only. However, Mestinon is used by some patients with LEMS, and these would be reimbursed as for MG. Nevertheless, LEMS is extremely rare, with a prevalence of 2-3 per million.[79] 3,4-diaminopyridine is the first choice for symptomatic treatment of patients with LEMS. Pyridostigmine is mostly used when 3,4-diaminopyridine is not available.[79]

In paper II we performed analyses according to the different criteria for inclusion. Using the strictest criteria for MG (reimbursement code for MG only), yielded the lowest number of MG patients. Combining the three criteria highly specific of MG yielded the second highest number of MG patients. Our method of identifying MG patients has a high sensitivity which is lacking from other more selected populations, especially for patients without AChR- or MuSK antibodies.

When comparing data from a national database on AChR-antibody assays with data from NorPD, the estimated prevalence rates are in good concordance. However, the incidence rates were higher with NorPD data than the AChR-antibody assay database, even after a calculated estimate of 15% of MG patients without detectable AChR-antibodies.[27] The comparison was made between MG patients with a symptomatic disease (identified in the NorPD) and patients with a positive AChR-antibody assay. These two groups are not identical as the AChR-antibody assay database only

includes patients with AChR-antibodies, while NorPD includes all MG patients both with and without AChR-antibodies.

The Danish authors, Pedersen *et al,* validated a method for identifying MG patients by linking two automated registers available in Denmark; The Danish National Hospital Register (Patient Register) based on MG specific codes generated from hospital contacts, and the Regional Prescription Register based on prescriptions of pyridostigmine. The MG diagnosis was then verified by reviewing medical records. The results from a national Danish AChR-antibody register were also linked. The authors concluded that subjects identified in the Patient Register were comparable with subjects found in the Prescription Register with regard to age and gender, but that the former were more often seropositive. The proportion of false positives was about 10 %, similar for all three registries. The sensitivity for the Prescription Register only was 88%, with a PPV of 80%.[209] As the Norwegian and Danish healthcare system are comparable, and the structure of both the Patient and Prescription registries in the two countries are essentially similar,[210] we believe that these results are generalizable to Norway, and that using pyridostigmine prescriptions registered in NorPD is a valid method to identify MG patients.

5.2.3 Selection and information bias

Selection bias

Selection bias stems from factors that influence study participation and procedures used to select study subjects. If the exposure-outcome associations in a study are different for those who participate compared to non-participants, the effect estimates in the study will differ from that of the general population.[207] Self-selection bias refers to a situation where background characteristics interfere with an individuals' decision to participate, threatening the validity of the results because the reasons for participation may be associated with the study outcomes.

The compulsory inclusion of all prescriptions expedited in Norway during the study period excludes the possibility of self-selection bias in paper I and II, providing a highly representative population. Data from NorPD are collected systematically and covers the entire population. The risk of selection bias is therefore nonexistent. A diagnostic workup of MG is carried out in specialist health care. In Norway, the citizens have free choice of hospital, but only about 1% of patients are treated by other hospitals than their default hospital. In paper III, data collection is based on patients who are treated at one specific hospital, and the risk of selection bias is therefore higher.

Information bias

Information bias arises due to erroneous measurement of the exposure or outcome variables under study. The term misclassification is often used for information bias that applies to categorical variables, and the error leads to a person being placed in an incorrect category. Misclassification of subjects can be either differential or non-differential. Differential misclassification occurs when incorrect classification of a variable depends on the value of other variables.[207] Such misclassification can either exaggerate or underestimate the effects in a study. Non-differential misclassification arises when incorrect classification of a variable is unrelated to other variables.[207] This tends to dilute the true difference between groups in a study.

Registration errors in large registries such as NorPD are inevitable. Most likely, such errors are non-differential and would result in too low effect-estimates for the MG population. Misclassification due to wrongly assigned diagnosis code (ICD/ICPC) on the prescription is possible, but highly unlikely as Mestinon is mainly indicated for MG only. However, *ex juvantibus* prescribing may occur. Misclassification may also be introduced by the observer (*interviewer bias, follow up bias*), or by the study participants (*recall bias*). In NorPD, these types of biases are nonexistent. In the Duke MG Patient Register, both differential and non-differential misclassifications are possible. First, the information is based on the physician's clinical examinations, which may vary. The MGFA clinical classification and PIS scoring are dependent on

what the patient tells the physician (*recall bias*), and how the physician formulated the questions (*interviewer bias*). In our study, only data from patients treated by one single physician were obtained, minimizing these types of biases as data would be obtained in a consistent manner from a highly trained specialist in MG.

5.2.4. Confounding

Confounding arises when a third factor partly or fully explains the observed association between the exposure and the outcome. Confounding can be regarded as a confusion, or mixing, of effect, and failure to account for such factors can result in spurious associations. Randomization, multivariate adjustment, stratification and matching are common methods used to control for possible confounders.
Confounders in paper II are age and gender; females traditionally are younger at disease onset than males. Age and gender were adjusted for in a linear regression model in paper II. In paper III, age, gender and disease severity are potential confounders and were adjusted for in the Cox regression model.

5.2.5. External validity

External validity is the extent to which the results can be generalized to other settings. NorPD does not contain data on MG patients so that they could be classified into the distinct MG subgroups, such as LOMG and thymoma MG and with varying non-AChR antibody status. Comprehensive subgroup analyses were not possible for paper I and II as this information was not available in the database. However, as paper I aimed to assess incidence and prevalence of symptomatic MG, we believe that the results from this study are applicable to other populations because of the large, unselected cohort. In paper II, we do not know whether specific drug use is associated with specific MG subgroups. Furthermore, there are some genetic heterogeneity in the population with about 15% immigrants and children of immigrant parents, and an estimated Sami population of about 55 000 (approximately 1%).

In paper III, subgroups of MG were assessed. North-Carolina has a population of 9,943,964 inhabitants, consisting of 65% whites, 21% African-Americans, 8% Hispanics and 2% Asians. About 16% do not have a health insurance, which is the same rate as for the whole of the USA.[211]

6. Conclusions

Paper I: Our calculated prevalence and incidence is in agreement with other population-based studies. There were no geographical differences in prevalence and incidence in Norway.

Paper II: MG patients are more often treated with non-MG prescription drugs for nearly all groups of medication compared to the general population. These findings reflect frequent comedication and comorbidity.

Paper III: The prognosis of MG, both short- and long-term, is favorable for the majority of patients, regardless of age, maximum disease severity and antibody status.

MG is one of the best studied and understood autoimmune diseases in humans. The unravelling of the pathophysiology of MG with application for diagnosis and therapy has had major impact on the prognosis of MG, as well as serving as a model for other autoimmune diseases. Although our knowledge of the underlying mechanisms of the disease is well understood, the etiology remains a mystery. Epidemiological studies are one way of studying etiology. Describing disease frequency, analyzing time trends, presenting case characteristics are important pieces in the puzzle in understanding the etiology of a disease. Epidemiological studies ultimately seek to improve prognosis by controlling the spread of the disease and reducing mortality; all of which are elements discussed in this thesis.

A crucial factor in conducting epidemiological research is case ascertainment devoid of selection bias. Population-based epidemiological studies on MG are rare. By using a nationwide, compulsory registry, this research has contributed to further knowledge of the true epidemiology of this rare disease. Assessments of comorbidity and drug treatment have elucidated the total disease burden of MG. By using a longitudinal, comprehensive patient registry to identify prognostic factors, this work should help guide treatment decisions and thereby enhancing a favorable outcome in MG.

Errata

Paper I:

In Methods, prevalence day should be 1 January 2008 (not 1 January 1 2008).

Source of data

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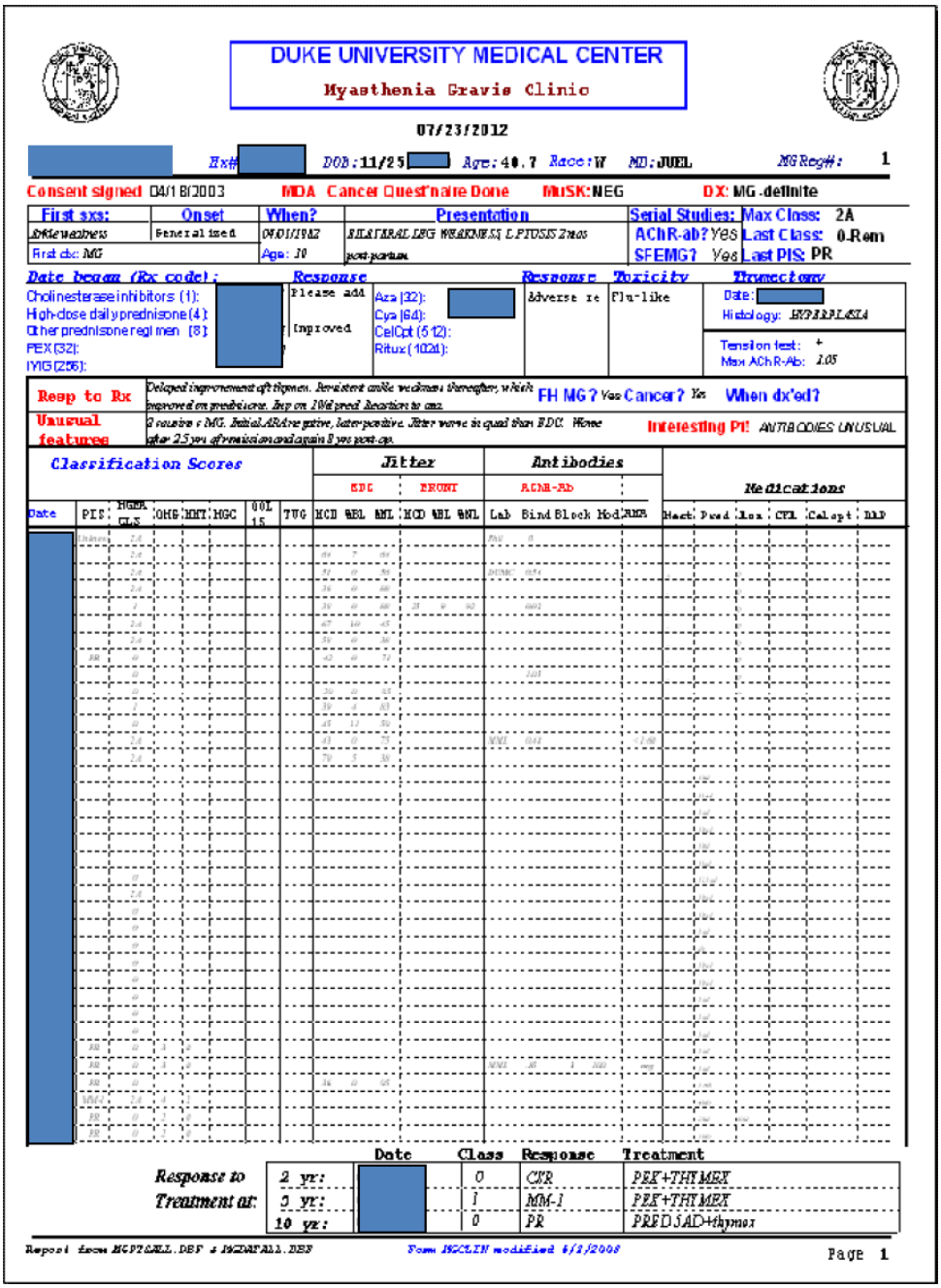
Appendix

Appendix 1.

Example of the standardized report after each clinic visit at the MG clinic at Duke University Medical Center



DUKE UNIVERSITY MEDICAL CENTER

Myasthenia Gravis Clinic

07/23/2012

[redacted] Hx#[redacted] DOB: 11/25[redacted] Age: 40.7 Race: W MD: JUEL MGReg#: 1

Consent signed 04/18/2003 MDA Cancer Questnaire Done MuSK: NEG DX: MG-definite

First sxs:	Onset	When?	Presentation	Serial Studies:	Max Class: 2A
diplopia/ptosis	Generalized	04/01/1982	BILATERAL EOG WEAKNESS; L PTOSIS 2mos	AChR-ab? Yes	Last Class: 0-Rem
First dx: MG		Age: 30	post partum	SFEMG? Yes	Last PIS: PR

Date began (Rx code): Response Response Toxicity Thymectomy

Cholinesterase inhibitors (1):
High-dose daily prednisone (4):
Other prednisone regimen (8):
PEX (32):
IVIG (256):
[redacted]
Please add
Improved
Aza (32):
Cya (64):
CellCpt (512):
Ritux (1024):
[redacted]
Adverse re
Flu-like
Date: [redacted]
Histology: HYPERPLASIA
Tension test: +
Max AChR-Ab: 1.05

Resp to Rx: Delayed improvement after thymus. Persistent ankle weakness thereafter, which improved on prednisone. Dep on 10d/pred. Resection to amc.

FH MG? Yes Cancer? Yes When dx'ed?

Unusual features: 9 relatives c MG. Initial AChR negative, later positive. Jitter worse in quad than EDC. Worse after 2.5 yrs of remission and again 8 yrs post op.

Interesting Pt! ANTIBODIES UNUSUAL

Classification Scores | Jitter (EDC, EXTNT) | Antibodies (AChR-Ab) | Medications

Date	PIS	MGFA CLS	OMG	MMT	MGC	QOL 15	TUG	MCD	%BL	%NL	MCD	%BL	%NL	Lab	Bind	Block	Mod	RIA%	Mest	Pred	Imu	CYA	Calcpt	IVIg
[redacted]																								

		Date	Class	Response	Treatment
Response to Treatment at:	2 yr:	[redacted]	0	CIR	PEX+THYMEX
	5 yr:		1	MM-1	PEX+THYMEX
	10 yr:		0	PR	PRED 5AD+thymus

Report from MGPTALL.DBF + MGMAPALL.DBF Form MGCLIN modified 6/1/2006

Original publications

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Myasthenia gravis requiring pyridostigmine treatment in a national population cohort

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incidence, myasthenia gravis, prevalence, pyridostigmine bromide, symptomatic treatment

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Background: Pyridostigmine is the first drug of choice for patients with myasthenia gravis (MG). The drug is not prescribed regularly to any other patient groups. We aimed to determine the prevalence, incidence and gender-specific characteristics of patients with MG needing drug treatment in a well-defined population cohort.
Methods: Data were retrieved from the Norwegian Prescription Database (NorPD) 2004–2007, containing information on all dispensed drugs in Norway. The study population comprised 677 recipients of pyridostigmine who met the following inclusion criteria (one or more): (i) More than one prescription 1 January 2004–31 December 2007, (ii) prescription from a specialist in neurology, (iii) prescription for MG being specified in NorPD.
Results: A total of 435 (64%) women and 242 men were included; female:male ratio 1.8:1. Point prevalence (1 January 2008) of symptomatic MG was 131 per million; 92 for men, 170 for women. Seventy-four new users of pyridostigmine were registered in 2007 (42 women, 32 men), i.e. the incidence rate for 2007 being 16 per million; 14 for men, 18 for women. Mean age of incident cases was 59 years; 64 and 55 years, respectively. Prevalence and incidence were significantly higher in the age group ≥50 years than <50 years ($P < 0.001$), and highest at 70–79 years. Prevalence and incidence did not differ in the five geographical health regions in Norway.
Conclusions: Reported prevalence and incidence are amongst the highest found in similar studies. This may be explained by optimal case identification, higher incidence of drug requiring MG amongst the elderly, and recurrences of previous MG.

Introduction

Autoimmune myasthenia gravis (MG) is caused by pathogenic antibodies directed at the acetylcholine receptor (AChR) on the post-synaptic muscle membrane endplate, leading to loss of functional AChRs. A small minority of patients with MG have instead antibodies against a kinase, MuSK, in the post-synaptic membrane. More than 90% of MG patients with generalized disease have detectable antibodies in their sera [1]. Clinical manifestations with increased fatigability in skeletal muscles are attributed to the less effective neuromuscular transmission. Symptomatic treatment for patients with MG is managed by inhibiting the action of acetylcholine esterase at the neuromuscular junction. Low-affinity antibodies to the AChR were recently detected in some seronegative patients [2], explaining the similar clinical presentation and effect of anti-acetylcholinesterase agents in seropositive and seronegative MG. Pyridostigmine, an acetylcholine esterase inhibitor, is the recommended drug to all patients with MG as the first line of therapy [3,4].

Increasing MG prevalence and incidence are evident during the past decades [5,6]. Most epidemiological studies are regional- or hospital-based, whilst nationwide population-based cohort studies are rare [7,8]. Our study provides epidemiological data covering an entire country by means of the Norwegian Prescription Database (NorPD). In Norway, pyridostigmine is prescribed regularly only to patients with MG, and only to those requiring symptomatic treatment. Therefore, pyridostigmine prescriptions recorded in NorPD represent a reliable method for epidemiological analysis of both seropositive and seronegative MG that are active, i.e. with ongoing symptoms.

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Our main objectives were to report the prevalence and incidence of active, symptomatic MG, and to present gender- and age-specific characteristics amongst patients with MG requiring drug treatment in Norway.

Methods

Norwegian Prescription Database was established in 2004 and is based on the mandatory registration of all prescription drugs dispensed at pharmacies in Norway. The database covers the whole population of 4.7 million inhabitants (1 January 2008, Statistics Norway-SSB, http://www.ssb.no). All registered drugs are classified according to the Anatomical Therapeutic Chemical (ATC) Classification System. Patient's and prescriber's identity are concealed and replaced with individualized pseudonyms, making it possible to follow each person over time.

Data drawn from NorPD comprised all prescriptions of pyridostigmine dispensed between 1 January 2004 and 31 December 2007. The following variables were examined: Patient's sex, age and county of residence, information about the drug, including brand name, size, number and strength of packages dispensed, date of purchase and ATC code (N07AA02). Whether the prescriber was a specialist in neurology or not was also noted. For chronic conditions, a reimbursement code will be recorded. This code may function as a proxy of diagnosis.

All 723 individuals who had at least one prescription of pyridostigmine dispensed during 2004–2007 were initially included. To ensure the inclusion of patients with established and active MG only, a requirement for final inclusion was more than one prescription in the time period, prescription by a specialist in neurology, or prescription for MG being specified in NorPD. This left 677 patients representing our study population. Of these, 633 (94%) had an MG reimbursement code. For the remaining 44 patients (6%), three received a prescription from a specialist in neurology, whilst 41 had two or more prescriptions of pyridostigmine during the study period.

As prevalence day, 1 January 1 2008 was selected. All population figures were obtained from Statistics Norway. Incidence and prevalence rates were calculated per million inhabitants. The prevalence of MG patients with symptomatic treatment was defined as the number of patients who had received a prescription of pyridostigmine as described above and were alive, living in Norway on prevalence day according to NorPD. Incidence of patients with MG requiring symptomatic treatment was defined as new users of pyridostigmine. Incidence could be determined for 2007 only and was ascertained by identifying all individuals who received pyridostigmine for the first time in 2007, and who had no pyridostigmine dispensed during 2004–2006.

The geographical distribution of symptomatic MG was calculated by examining pyridostigmine prescriptions from pharmacies belonging to the respective health regions according to the regional division of the Norwegian healthcare 1995–2007.

Background variables were compared using cross-tables with Pearson chi square test. Two-sided P values ≤ 0.05 were interpreted as significant. Ninety-five per cent confidence intervals (95% CI) were calculated assuming a Poisson's distribution. The statistical analyses were performed in SPSS version 16.0 (SPSS Inc., Chicago, IL, USA).

Results

Prevalence

On 1 January 2008, a total of 619 patients with MG (216 men, 403 women) were receiving symptomatic treatment, female:male (F:M) ratio 1.8:1. This represents a prevalence of 131 per million (95% CI 123–144), 92 for men (95% CI 83–108), and 170 for women (95% CI 154–187).

Mean age of all patients with MG prevalent was 56 years (95% CI 55–58 years), 60 years for men (95% CI 58–62 years) and 54 years for women (95% CI 52–56 years). Female: male ratio in the age group < 50 years was 3:1 compared to 1.5:1 in the age group ≥ 50 years. For those < 50 years, the point prevalence was 67 per million (95% CI 59–77), whilst for patients ≥ 50 years the point prevalence was 258 per million (95% CI 240–291). When comparing the prevalence for the two age groups < 50 years and ≥ 50 years, a significant difference was found both for the two sexes combined ($P < 0.001$), and for men and women separately ($P < 0.001$).

The peak prevalence for both sexes was found in the age group 70–79 years (Table 1). The number of female pyridostigmine users increased steadily up to 70–79 years, whilst the number of male users rose sharply when reaching the age group 50–59 years (Fig. 1a). The prevalence was lowest in the northern region. When comparing the prevalence in the five geographical health regions in Norway ($P = 0.4$), no significant difference was detected.

Incidence

A total of 74 individuals (32 men, 42 women) obtained their first pyridostigmine prescription in 2007. Therefore, total incidence rate for 2007 was 16 per million (95% CI 12–19), 14 for men (95% CI 9–19) and 18 for

Table 1 Age- and sex-specific prevalence of symptomatic myasthenia gravis

Age group (years)	Women			Men			Total		
	Population	Cases (n = 403)	Rates (95% CI)	Population	Cases (n = 216)	Rates (95% CI)	Population	Cases (n = 619)	Rates (95% CI)
0–9	289748	1/403 (0.3%)	3.5 (0.0–10.2)	303016	1/216 (1%)	3.3 (0.0–9.8)	592764	2/619 (0.3%)	3.4 (0.0–8.1)
10–19	306436	16/403 (4%)	52 (27–78)	323746	5/216 (2%)	15 (2–29)	630182	21/619 (3%)	33 (19–48)
20–29	285700	23/403 (6%)	81 (48–113)	295232	6/216 (3%)	20 (4–37)	580932	29/619 (5%)	50 (32–68)
30–39	335667	54/403 (13%)	161 (118–204)	348269	21/216 (10%)	60 (35–86)	683936	75/619 (12%)	110 (85–135)
40–49	328676	66/403 (16%)	201 (152–249)	346104	20/216 (9%)	58 (35–87)	674780	86/619 (14%)	128 (102–156)
50–59	297061	81/403 (20%)	273 (213–332)	306854	31/216 (14%)	101 (68–140)	603915	112/619 (18%)	186 (153–222)
60–69	234618	69/403 (17%)	294 (229–368)	231236	63/216 (29%)	273 (209–345)	465854	132/619 (21%)	283 (239–336)
70–79	156943	66/403 (16%)	421 (330–536)	129271	49/216 (23%)	379 (293–512)	286214	115/619 (19%)	402 (344–494)
80–89	117548	21/403 (5%)	179 (102–255)	67774	20/216 (9%)	295 (212–496)	185322	41/619 (7%)	221 (172–314)
≥90	25084	6/403 (2%)	239 (48–431)	8188	0/216 (0%)	0–	33272	6/619 (1%)	180 (36–325)
Total	2377481	403/403 (100%)	169 (154–187)	2359690	216/216 (100%)	91 (83–108)	4737171	619/619 (100%)	131 (123–142)

Rates are per million inhabitants. Some numbers were rounded so not all percentages total 100%.

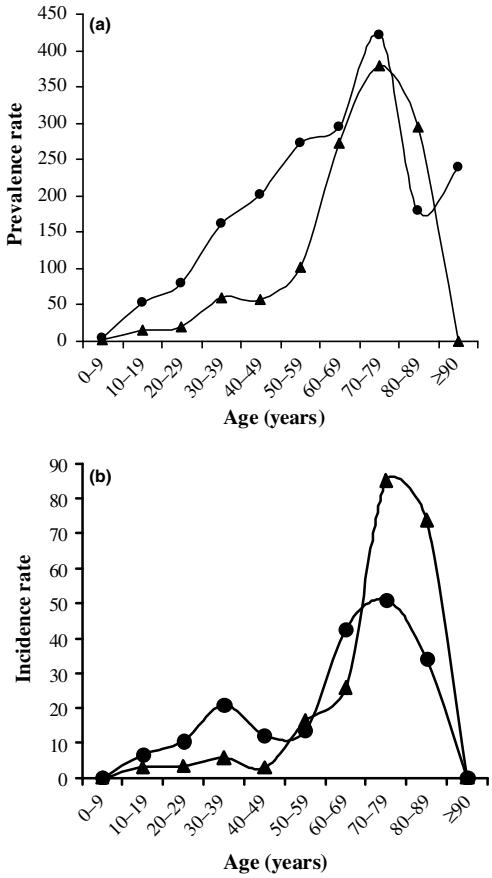

Figure 1 Age and sex specific distribution of symptomatic myasthenia gravis in Norway. (a) prevalence, (b) incidence. Circles = women; triangles = men. Rates are per million inhabitants.

women (95% CI 12–23). Figure 1b shows stable low rates for both men and women until the age of 60, with a small peak for women at the age group 30–39 years. The highest rate of new users of pyridostigmine was for both sexes combined in the age group 70–79 years.

Mean age of incident cases was 59 years (95% CI 55–64 years); 64 years for men (95% CI 58–71), and 55 years for women (95% CI 49–61). Incidence for patients <50 years was 7 per million (95% CI 4–10). Patients ≥50 years had an incidence of 34 (95% CI 25–43). This difference was highly significant ($P < 0.001$). The age- and sex-specific incidence rates of symptomatic MG in Norway are summarized in Table 2. Lowest incidence was found in the northern region. The

Table 2 Age- and sex-specific incidence of symptomatic myasthenia gravis

Age group (years)	Women			Men			Total		
	Population	Cases (n = 42)	Rates (95% CI)	Population	Cases (n = 32)	Rates (95% CI)	Population	Cases (n = 74)	Rates (95% CI)
0–9	289748	0/42 (0%)	0–	303016	0/32 (0%)	0–	592764	0/74 (0%)	0–
10–19	306436	2/42 (5%)	6.5 (0.0–15.6)	323746	1/32 (3%)	3.1 (0.0–9.1)	630182	3/74 (4%)	4.8 (0.0–10)
20–29	285700	3/42 (7%)	11 (0.0–22)	295232	1/32 (3%)	3.4 (0.0–10)	580932	4/74 (5%)	6.9 (0.1–14)
30–39	335667	7/42 (16%)	21 (5.4–36)	348269	2/32 (6%)	5.7 (0.0–14)	683936	9/74 (12%)	13 (4.6–22)
40–49	328676	4/42 (10%)	12 (0.2–24)	346104	1/32 (3%)	2.9 (0.0–8.6)	674780	5/74 (7%)	7.4 (0.9–14)
50–59	297061	4/42 (9%)	14 (0.3–27)	306854	5/32 (16%)	16 (2.0–31)	603915	9/74 (12%)	15 (5.2–25)
60–69	234618	10/42 (24%)	43 (16–69)	231236	6/32 (19%)	26 (5.2–47)	465854	16/74 (22%)	34 (18–51)
70–79	156943	8/42 (19%)	51 (16–86)	129271	11/32 (34%)	85 (35–135)	286214	19/74 (26%)	66 (37–96)
80–89	117548	4/42 (10%)	34 (0.7–67)	67774	5/32 (16%)	74 (9.1–138)	185322	9/74 (12%)	49 (17–80)
≥90	25084	0/42 (0%)	0–	8188	0/32 (0%)	0–	33272	0/74 (0%)	0–
Total	2377481	42/42 (100%)	18 (12–23)	2359690	32/32 (100%)	14 (9–18)	4737171	74/74 (100%)	16 (12–19)

Rates are per million inhabitants.

incidence rates did not differ significantly in the various parts of Norway (P = 0.09).

Discussion

In this large, population-based cohort study on symptomatic MG in Norway, we present for the first time prevalence and incidence of drug requiring MG. Our study had several strengths. This represents the first epidemiological study on both seropositive and seronegative active MG encompassing a whole population. A complete case ascertainment was achieved by obtaining data from a national health registry. This method enhanced power whilst minimizing bias. Furthermore, pyridostigmine being the only non-immunosuppressive drug providing symptomatic relief for both seropositive and seronegative MG is a specific and sensitive indicator for MG requiring drug treatment. Studies on Norwegian MG cohorts report pyridostigmine consumption in nearly all cases [9–11]. Therefore, pyridostigmine use should be considered as a reliable surrogate marker for active MG. Some patients with Lambert-Eaton myasthenic syndrome (LEMS) or congenital myasthenia may use pyridostigmine. Some patients with milder symptoms, especially ocular cases, may not use pyridostigmine. These two sources of error count in opposite directions and should only marginally influence the final results.

The prevalence rate of 131 per million found in this study is comparable to the highest observed in recent studies from other parts of the world, the top ranging from 142 [12] to 175 per million [13]. An incidence rate of 16 per million shown here is amongst the top rates reported, along with 15 [14] and 21 per million [15] as the highest. Compared to previous investigations on MG epidemiology in Norway [16,17], our study suggests an actual increase in MG occurrence, not only because of better case identification [6] and increased recognition of the disease by GPs and neurologists but also because of widespread AChR antibody testing [16]. High prevalence also reflects the reduced mortality [18]. A key factor influencing soaring incidence rates is the ageing population, at higher risk of acquiring the disease [5].

Our data indicate that the increase in new cases of drug requiring MG is chiefly because of the elderly. Fifty-nine per cent of patients required treatment for the first time were ≥60 years and 38% were ≥70 years old. In comparison, 44% of patients had MG onset >65 years [12] and 46% >70 years [15] in two large prospective studies, whereas onset ≥60 years in retrospective studies varies between 36% and 59%. A similar age- and sex-specific incidence was shown in a study on AChR-positive MG in the UK [19]. The relative high

incidence rates compared to prevalence are explained by most patients with MG acquiring the disease in high age. A real change in MG incidence, and especially in the elderly, should be caused by unknown environmental triggers. The immunological dissimilarities in patients with early and late onset MG support that an increase can occur in one and not in the other MG subtype. Patients with late onset MG have lower titre of circulating anti-AChR antibodies; antibodies to striated muscle are more often found in late onset MG. HLA-DR2 is frequently associated with late onset MG, in contrast to early onset MG which is commonly associated with HLA-DR3. Thymic hyperplasia rarely occurs in late onset MG, and autoimmune co-morbidity is more rare [20]. Most studies show similar modes of onset as ours; bimodal for women and only one peak for men [8,21]. This distinction confirms different gene–environment interactions in early and late onset MG [22].

Mean life expectancy for Norwegian women is 5 years longer than that for men, and there are slightly more women ≥50 years in Norway than men. Still, the late onset MG group comprised slightly more men than women. Other investigators also report a small bias towards men in late onset MG [23], establishing them as more susceptible than women.

In the early onset MG group, a female predominance was observed. The sex ratio in the Norwegian population < 50 years is nearly one. No evidence of teratogenic effects of pyridostigmine is documented and in most cases symptomatic treatment is maintained also during pregnancy [11]. Thus, women who temporarily terminate the treatment of MG symptoms whilst pregnant have not caused bias to our incidence estimate, also owing to the 3 years registration before recording incidence. Provoking agents associated with MG onset are seldom identifiable, and pregnancy and delivery only accounted for one per cent in a recent survey [24]. Infections are known to potentially evoke MG symptoms [20]. Autoimmune disorders in general are more common in women [25]. Women experience fatigue and unspecified muscle weakness more often than men, and Norwegian women are twice as likely to be tested for AChR antibodies than men [16].

Myasthenia gravis was evenly geographically distributed in Norway, in contrast to what has been found for multiple sclerosis (MS), with a lower disease frequency in the northern region. Absolute MG prevalence and incidence numbers were lower in the north also for MG, but this did not reach significance. The Sami population lives mainly in the north and has a low MS prevalence [26]. The optimal case identification method assures minimal risk of regional selection bias. Health care in Norway is nearly free of charge and reimbursement regulations cover the drug costs for chronic conditions. Our country also has one of the world's highest rates of practicing physicians per 1000 inhabitants, and neurologists are fairly evenly distributed in all five health regions.

Ninety per cent of all Norwegian patients with MG are currently receiving symptomatic pyridostigmine medication when comparing our data on active MG to Heldal *et al.*'s [16] estimated total MG prevalence of 145 per million in the same region and the same year. Correspondingly, 10% should be in complete remission or in pharmacologic remission with no need of pyridostigmine. Estimates on remission rates from other community-based studies vary from 12% to 20% [27,28]. The low remission rate should be considered in the light of 72% of our patients being late onset MG. Early onset MG is more often associated with complete stable remission [29]. To avoid bias in incidence estimates because of relapsing cases, only new cases identified during the last year of the 4 year period were included. Clinical remission lasting over 6–24 months is considered to be stable [3]. Therefore, incidence overestimation because of relapses is likely to be small.

Norwegian Prescription Database is a nationwide registry and provides a unique opportunity for epidemiological research for diseases requiring specific medication. The diagnosis is not available in NorPD, but the indication for treatment with pyridostigmine covered by reimbursement regulations is exclusive for the MG diagnosis. However, in practice, pyridostigmine prescriptions to patients with LEMS or congenital myasthenic syndromes will be reimbursed as for MG. Also, ex juvantibus prescription and prescribing practice without proper reimbursement coding may theoretically occur, as may prescribing on non-MG indications like gastrointestinal dysmotility in idiopathic pseudo-obstruction. But, because of the rarity of this diagnosis and the inconclusive effect of pyridostigmine in these patients [30], we believe that by our rigid criteria, the odds of including non-MG is small. Conversely, the strict inclusion criteria might have caused an underestimation of active MG. The 46 excluded patients did not differ in drug dispensing from the study population, nor in age and sex. Ten patients were also prescribed corticosteroids, and two with additional immunosuppressive medication. These patients account for $< 10\%$ of pyridostigmine recipients, and a misclassification would not have altered our conclusions significantly.

Autoimmune MG is a potential life threatening but treatable disorder. Diagnosis in the elderly is complicated by higher degree of co-morbidity, and detection of characteristic clinical signs like ptosis and muscle weakness is more easily overlooked than in younger

people [31]. Further epidemiological examinations on seasonal trends in disease onset and exacerbation might help establish a link between genetic susceptibility and putative exogenous factors. The latitudinal range rendering seasonal variety in climate and daylight exposure, a long coastline and a conventional diet supplying marine omega-3 polyunsaturated fatty acids make Norway an ideal country for such research in addition to a relatively homogenous population and high-quality national registries. Our figures reflect a sensitive case finding method, an ageing population and a high predisposition for MG and autoimmune disease.

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CME ARTICLE

Total drug treatment and comorbidity in myasthenia gravis: a population-based cohort study

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Background and purpose: Comorbidity in myasthenia gravis (MG) is important for diagnosis, treatment and prognosis. Disease complexity was assessed by examining total drug treatment, immune therapy and comorbidity in a complete national MG cohort.

Methods: All recipients of the MG-specific drug pyridostigmine 2004–2010 registered in the compulsory Norwegian Prescription Database who met the inclusion criteria were included. The pyridostigmine group was compared with the general Norwegian population.

Results: Myasthenia gravis patients received co-medication more often than the controls for nearly all groups of medication, including insulins (95% confidence interval 2.0–3.7), thyroid therapy (1.7–2.5), antidepressants (1.3–1.7), anti-infectives (1.2–1.4), lipid-modifying agents (1.1–1.4) and immunomodulating agents (6.8–8.8).

Conclusions: Myasthenia gravis patients are more often treated with non-MG prescription drugs than controls, reflecting frequent co-medication and comorbidity.

Introduction

Autoimmune myasthenia gravis (MG) is mainly caused by the destruction of acetylcholine receptors by autoantibodies at the neuromuscular junction. MG is a heterogeneous disease with several subtypes and autoantibodies against skeletal muscles [1]. Life expectancy for MG patients is now near normal [2], but management of a fluctuating disease remains challenging. New therapeutic options are emerging, and MG subtype classification has implications for treatment strategies [3].

The task of controlling symptoms whilst minimizing adverse effects of long-term immunosuppressive treatment is intricate. Furthermore, the clinical implications of heart muscle antibodies, involvement of respiratory function in MG and use of drugs that may worsen neuromuscular blockade have not been widely studied, nor have autoimmune comorbidity and psychiatric disorders been described in unselected MG cohorts. Our study provides a national cohort for evaluating the total drug management of symptomatic MG, offering a new insight into the total disease burden for this group.

The aims of the study were to evaluate drug treatment and thereby also comorbidity in patients with MG. First, an overview is given of the overall national drug consumption amongst MG patients. Secondly, MG autoimmune comorbidity is assessed through co-medication. Thirdly, psychiatric disorders in MG are explored through specific drug treatment. Fourthly, prescription practice is investigated with regard to selected drugs considered as relatively contraindicated in MG. Finally, first- and second-line drug treatment of MG is investigated.

Methods

Registration of all prescription drugs dispensed from Norwegian pharmacies in the Norwegian Prescription Database (NorPD) has been mandatory since 2004, covering the entire Norwegian population (5 096 300). A unique personal identifier enables consecutive monitoring of individuals in the health system over their entire life span. The specific diagnosis or indication

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for the prescription is not registered in NorPD, but the International Classification of Diseases, 10th revision (ICD-10), and/or the International Classification of Primary Care, 2nd edition (ICPC-2), have been recorded since 2008. Medication for chronic diseases such as MG is reimbursed. The reimbursement codes together with the ICD-10 and ICPC-2 codes function as a proxy of diagnosis. The following variables were studied: the patient's year of birth and sex, the prescriber's medical speciality, reimbursement codes, ICD-10/ICPC-2 codes, name of the drug, Anatomical Therapeutic Chemical (ATC) code, date of expedition at the pharmacy, and the defined daily dose (DDD) of the drugs dispensed. In NorPD, the DDD corresponds to the assumed mean maintenance dose of the drug used per day for its main indication in adults [4].

About 890 individuals with at least one prescription of pyridostigmine from 1 January 2004 to 30 April 2010 were identified. Amongst these, 830 (93%) met one or more of the criteria preset by us to confirm a diagnosis of MG and were regarded as having MG: (i) ≥2 prescriptions of pyridostigmine during the study period; (ii) pyridostigmine prescription made by a neurologist; (iii) pyridostigmine prescription with reimbursement code (§13) or ICD-10 code (G70.0)/ICPC-2 code (N99) specific for MG (Fig. 1). Final inclusion for this study was done from the date when one or more of the criteria were fulfilled. Sensitivity analyses with more stringent inclusion criteria to test the robustness of our study population were performed (Table S1).

For each subgroup of patients categorized by age and sex, drug statistics for the corresponding age and sex groups in the Norwegian population registered in NorPD from the same period functioned as controls (Table 1). Total drug treatment of MG patients was assessed by investigating every prescription dispensed in all main ATC groups during the study period. Comparisons of age- and sex-specific drug use amongst MG patients and controls were done by calculating the standardized incidence ratio (SIR), i.e. the observed number of prescriptions for all main ATC groups divided by the estimated number of prescriptions for the same drug groups dispensed to a similar group, with regard to age and sex, in the general population. Patient age was defined as age at 1 July 2004. The SIR was computed, with 95% confidence interval (CI), assuming a Poisson distribution. The ATC group 'Various' was considered non-specific and was excluded from the analyses.

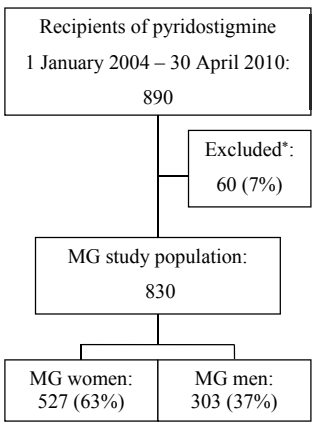

Figure 1 Selection of the MG study cohort. *Recipients of pyridostigmine who did not meet the inclusion criteria during the study period.

Table 1 Demographic characteristics of the study population and population controls, year 2004

	MG patients (n = 830)	Population controls (n = 4 577 457)
Age (mean)[a]	57	39
Sex (n, %)		
Female	527 (64)	2 269 049 (50)
Male	303 (37)	2 308 408 (50)
Age group (n, %)		
0–9	3 (0.4)	598 503 (13)
10–19	29 (4)	591 853 (13)
20–29	36 (4)	570 889 (13)
30–39	105 (13)	698 413 (15)
40–49	97 (12)	639 053 (14)
50–59	148 (18)	595 423 (13)
60–69	159 (19)	374 975 (8)
70–79	175 (21)	299 162 (7)
80–89	67 (8)	180 640 (4)
> 90	11 (1)	28 546 (0.6)

[a]Patient age was calculated from year of birth and defined as age at 1 July 2004.

When exploring comorbidities and contraindicated medications, the following groups of drugs were included: drugs used in diabetes, insulins and analogues, thyroid hormones, antipsychotics, anxiolytics, hypnotics and sedatives, antidepressants, antiepileptics, beta-blocking agents, calcium-channel blockers, lipid-modifying agents and aminoglycoside antibacterials. The following groups of immunomodulating agents were assessed: prednisolone, selective immunosuppressants, tumor necrosis factor alpha inhibitors, interleukin inhibitors, calcineurin inhibitors, and other immunosuppressants.

To detect any differences in prescription of ATC groups related to age and sex, linear regressions were performed, estimating the mean difference and 95% CIs. Two-sided P values ≤ 0.05 were considered

statistically significant. The median DDD prescribed each year was compared for pyridostigmine and for each of the following immunomodulating agents: prednisolone, azathioprine, mycophenolic acid, cyclosporine and methotrexate, as recommended by the European Federation of Neurological Societies guidelines for MG treatment [5]. Non-parametric tests were performed for comparisons regarding amount dispensed between age and sex groups. IBM SPSS Statistics for Windows, version 20.0 (IBM Corp., Armonk, NY, USA), and Microsoft Excel were used in all statistical analyses. Ethics committee approval is not required for studies using anonymous data retrieved from central health registers.

Results

In total, 87 556 prescription medications were dispensed to the 830 MG patients during the registration period (Table 2). The mean number of new ATC groups per year is shown in Table S2. Only 19 individuals (2.3%) received no other medication than pyridostigmine. MG patients more often received nearly all types of medication compared with the control group, most pronounced for the following treatment groups: alimentary tract and metabolism (A); systemic hormonal preparations, excluding sex hormones and insulins (H); antineoplastic and immunomodulating agents (L). Patients <50 years received fewer ATC groups than patients ≥50 years and women received fewer than men, but neither of the differences was significant.

Insulins were almost three times more frequently prescribed to MG patients (95% CI 2.0–3.7, Table 3) compared with controls. This was observed for MG patients ≥50 years (1.9–3.7), for men (1.7–4.3) and for women (1.7–4.0). MG patients <50 years also had increased prescriptions of insulins (SIR = 2.8), but there were too few users to provide sufficient statistical power ($N = 5$). A hundred and ten MG patients (13%) received a prescription of thyroid hormones. Thyroid hormones were prescribed about four times more frequently to MG patients <50 years (2.4–5.5) and male MG patients (2.3–5.0). Patients ≥50 years and female MG patients received thyroid hormones about twice as often compared with controls (1.5–2.2 and 1.4–2.2, respectively).

In all, 29% of MG patients received treatment with hypnotics and sedatives, and such drugs were twice as often given to MG patients than to controls for the age group <50 years (1.2–2.3). 21% received antidepressants, twice as often given to male MG patients than to male controls (1.3–2.2). For the remaining age and sex groups, slightly more MG patients than controls were treated with hypnotics, sedatives and antidepressants. 20% received anxiolytics, whilst 7% received antipsychotics (Table 3). Anxiolytics and antipsychotics were prescribed to MG patients and controls with the same frequency.

Myasthenia gravis patients were twice as often treated with antiepileptic drugs (1.7–2.5). They were also more frequently treated with calcium-channel blockers (1.2–1.7) and lipid-modifying agents (1.1–1.4), but with the same frequency as in the controls with beta-blocking agents (0.9–1.2). All four drug groups were given more frequently to MG patients <50 years compared with controls at the same age (Table 3). However, the number of users of calcium-channel blockers, lipid-modifying agents and beta-blocking agents was too low to provide enough statistical power ($N = 6$, 11, 11 respectively).

The DDDs of pyridostigmine were significantly lower for MG patients <50 years compared with those ≥50 years ($P < 0.001$). There was no difference between men and women ($P = 0.8$). Immunomodulating agents were prescribed less to patients <50 years ($P < 0.001$) and women ($P = 0.001$) compared with patients ≥50 years and men (Table 4); 406 MG patients (49%) had no immunomodulating agents expedited during the study period. The mean number of new groups of immunomodulating agents used per year was not significantly different between the two age and sex groups ($P = 0.2$ and $P = 0.9$, respectively; Fig. 2a and b). Regression analyses with mutual adjustment for age and sex did not alter the differences regarding age and sex.

Significantly fewer DDDs of prednisolone was prescribed to patients <50 years compared with patients ≥50 years ($P < 0.001$). No age difference was seen for azathioprine ($P = 0.1$). Women were prescribed significantly fewer DDDs of prednisolone ($P < 0.001$) and azathioprine ($P = 0.002$) than men. For mycophenolic acid, cyclosporine and methotrexate, the number of users and DDDs prescribed were too small to be included in the calculations.

Discussion

This is the first study to assess the total drug management and comorbidity of MG in a complete national cohort. Our findings show that co-medication in MG is widespread, requiring more frequent drug treatment for several major disease groups than in the general population. Treatment for diabetes, thyroid disease and psychiatric disorders in MG is common, as well as co-medication relatively contraindicated in MG. These findings demonstrate the extensive disease burden of MG and the complexity of the disease.

Table 2 Number of the 830 MG patients receiving prescription medications in the main ATC groups compared with the number in a similar group, with regard to age and sex, in the general national population

	Total (n = 830)			Women (n = 527)			Men (n = 303)			<50 years old (n = 270)			≥50 years old (n = 560)		
ATC group	n (%)	SIR[a] (95% CI)	P value[b]	n (%)	SIR[a] (95% CI)	P value[b]	n (%)	SIR[a] (95% CI)	P value[b]	n (%)	SIR[a] (95% CI)	P value[b]	n (%)	SIR[a] (95% CI)	P value[b]
A – Alimentary tract and metabolism	512 (62)	1.9 (1.7–2.0)	<0.001	318 (60)	1.7 (1.6–1.9)	<0.001	194 (64)	2.1 (1.8–2.4)	<0.001	101 (37)	2.3 (1.9–2.8)	<0.001	411 (73)	1.8 (1.6–2.0)	<0.001
B – Blood and blood forming organs	364 (44)	1.5 (1.3–1.6)	<0.001	206 (39)	1.5 (1.3–1.7)	<0.001	158 (52)	1.5 (1.3–1.8)	<0.001	41 (15)	2.2 (1.6–2.9)	NA	323 (58)	1.4 (1.3–1.6)	<0.001
C – Cardiovascular system	460 (55)	1.4 (1.2–1.5)	<0.001	258 (49)	1.3 (1.1–1.4)	<0.001	202 (67)	1.5 (1.3–1.7)	<0.001	49 (18)	1.7 (1.3–2.3)	NA	411 (73)	1.3 (1.2–1.5)	<0.001
D – Dermatologicals	360 (43)	1.3 (1.2–1.5)	<0.001	237 (45)	1.3 (1.2–1.5)	<0.001	123 (41)	1.4 (1.2–1.6)	<0.001	99 (37)	1.5 (1.2–1.9)	NA	261 (47)	1.3 (1.1–1.4)	<0.001
G – Genito urinary system and sex hormones	308 (37)	1.3 (1.2–1.5)	<0.001	225 (43)	1.2 (1.1–1.4)	0.002	83 (27)	1.5 (1.2–1.9)	<0.001	101 (37)	1.3 (1.0–1.5)	0.02	207 (37)	1.3 (1.2–1.5)	<0.001
H – Systemic hormonal preparations, excluding sex hormones and insulins	456 (55)	3.0 (2.7–3.3)	<0.001	272 (52)	2.5 (2.2–2.8)	<0.001	184 (61)	4.2 (3.7–4.9)	<0.001	105 (39)	3.9 (3.2–4.7)	<0.001	351 (63)	2.8 (2.5–3.1)	<0.001
J – Anti-infectives for systemic use	604 (73)	1.3 (1.2–1.4)	<0.001	391 (74)	1.2 (1.1–1.4)	<0.001	213 (70)	1.4 (1.2–1.6)	<0.001	146 (54)	1.2 (1.0–1.4)	0.02	458 (82)	1.3 (1.2–1.5)	<0.001
L – Antineoplastic and immunomodulating agents	249 (30)	7.7 (6.8–8.8)	<0.001	146 (28)	8.0 (6.8–9.4)	<0.001	103 (34)	7.4 (6.1–9.0)	<0.001	63 (23)	16.4 (12.6–21.0)	NA	186 (33)	6.6 (5.7–7.6)	<0.001
M – Musculoskeletal system	464 (56)	1.2 (1.1–1.3)	<0.001	308 (58)	1.1 (1.0–1.3)	0.01	156 (51)	1.3 (1.1–1.5)	0.005	120 (44)	1.3 (1.0–1.5)	0.01	344 (61)	1.2 (1.0–1.3)	0.006
N – Nervous system	582 (70)	1.4 (1.2–1.5)	<0.001	393 (75)	1.4 (1.2–1.5)	<0.001	189 (62)	1.3 (1.2–1.6)	<0.001	134 (50)	1.5 (1.3–1.8)	<0.001	448 (80)	1.3 (1.2–1.4)	<0.001
P – Antiparasitic products, insecticides and repellents	78 (9)	1.2 (1.0–1.5)	NA	55 (10)	1.2 (0.9–1.5)	NA	23 (8)	1.3 (0.8–2.0)	NA	24 (9)	1.2 (0.8–1.8)	NA	54 (10)	1.2 (0.9–1.6)	NA
R – Respiratory system	488 (59)	1.4 (1.3–1.5)	<0.001	314 (60)	1.3 (1.2–1.4)	<0.001	174 (57)	1.6 (1.3–1.8)	<0.001	127 (47)	1.4 (1.2–1.7)	<0.001	361 (64)	1.4 (1.2–1.5)	<0.001
S – Sensory organs	354 (43)	1.4 (1.2–1.5)	<0.001	241 (46)	1.4 (1.2–1.5)	<0.001	113 (37)	1.4 (1.1–1.7)	0.001	76 (28)	1.4 (1.1–1.7)	NA	278 (50)	1.4 (1.2–1.5)	<0.001

NA, not available or insufficient data available for analysis. [a]SIR, standardized incidence ratio calculated by comparing age- and sex-specific drug use amongst MG patients with the general population (i.e. the control group); [b]the difference between MG patients and the control group.

Table 3 Number of the 830 MG patients receiving various prescription medications compared with the number in a similar group, with regard to age and sex, in the general national population

	Total (n = 830)			Women (n = 527)			Men (n = 303)			<50 years old (n = 270)			≥50 years old (n = 560)		
ATC code – Drug	n (%)	SIR[a] (95% CI)	P value[b]	n (%)	SIR[a] (95% CI)	P value[b]	n (%)	SIR[a] (95% CI)	P value[b]	n (%)	SIR[a] (95% CI)	P value[b]	n (%)	SIR[a] (95% CI)	P value[b]
Autoimmune co-medication															
A10 – Drugs used in diabetes	83 (10)	1.8 (1.4–2.2)	NA	45 (9)	1.8 (1.3–2.4)	NA	38 (13)	1.8 (1.3–2.5)	NA	8 (3)	2.0 (0.8–3.9)	NA	75 (13)	1.8 (1.4–2.2)	NA
A10A – Insulins and analogues	42 (5)	2.7 (2.0–3.7)	NA	22 (4)	2.6 (1.7–4.0)	NA	20 (7)	2.8 (1.7–4.3)	NA	5 (2)	2.8 (0.9–6.6)	NA	37 (7)	2.7 (1.9–3.7)	NA
H03AA – Thyroid hormones	110 (13)	2.1 (1.7–2.5)	<0.001	81 (15)	1.8 (1.4–2.2)	NA	29 (10)	3.5 (2.3–5.0)	NA	25 (9)	3.7 (2.4–5.5)	NA	85 (15)	1.8 (1.5–2.2)	NA
Psychiatric co-medication															
N05A – Antipsychotics	60 (7)	1.1 (0.8–1.4)	NA	47 (9)	1.2 (0.9–1.6)	NA	13 (4)	0.7 (0.4–1.3)	NA	13 (5)	1.3 (0.7–2.2)	NA	47 (8)	1.0 (0.7–1.3)	NA
N05B – Anxiolytics	170 (20)	1.2 (1.0–1.4)	0.03	120 (23)	1.1 (1.0–1.4)	0.13	50 (17)	1.3 (0.9–1.7)	NA	26 (10)	1.2 (0.8–1.8)	NA	144 (26)	1.2 (1.0–1.4)	0.05
N05C – Hypnotics and sedatives	244 (29)	1.3 (1.2–1.5)	<0.001	168 (32)	1.3 (1.1–1.5)	0.001	76 (25)	1.4 (1.1–1.8)	NA	39 (14)	1.7 (1.2–2.3)	NA	205 (37)	1.3 (1.1–1.5)	<0.001
N06A – Antidepressants	175 (21)	1.5 (1.3–1.7)	<0.001	123 (23)	1.4 (1.2–1.7)	<0.001	52 (17)	1.7 (1.3–2.2)	NA	39 (14)	1.5 (1.1–2.1)	NA	136 (24)	1.4 (1.2–1.7)	<0.001
Contraindicated co-medication															
N03 – Antiepileptics	80 (10)	2.1 (1.7–2.6)	NA	57 (11)	2.3 (1.7–2.9)	NA	23 (8)	1.8 (1.2–2.7)	NA	16 (6)	2.2 (1.3–3.6)	NA	64 (11)	2.1 (1.6–2.7)	NA
C07 – Beta-blocking agents	157 (19)	1.0 (0.9–1.2)	0.7	79 (15)	0.9 (0.7–1.1)	NA	78 (26)	1.2 (0.9–1.5)	NA	11 (4)	1.5 (0.8–2.7)	NA	146 (26)	1.0 (0.9–1.2)	0.9
C08 – Calcium-channel blockers	137 (17)	1.4 (1.2–1.7)	<0.001	75 (14)	1.4 (1.1–1.7)	NA	62 (21)	1.5 (1.1–1.9)	NA	6 (2)	2.0 (0.7–4.3)	NA	131 (23)	1.4 (1.2–1.7)	<0.001
C10 – Lipid-modifying agents	194 (23)	1.3 (1.1–1.4)	0.002	99 (19)	1.1 (0.9–1.4)	NA	95 (31)	1.4 (1.1–1.7)	NA	11 (4)	1.8 (0.9–3.2)	NA	183 (33)	1.2 (1.1–1.4)	0.01
J01G – Aminoglycoside antibacterials	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-

NA, not available or insufficient data available for analysis. [a]SIR, standardized incidence ratio calculated by comparing age- and sex-specific drug use amongst MG patients with the general population (i.e. the control group); [b]the difference between MG patients and the control group.

Table 4 Number of MG patients using selected immunomodulating drugs with comparisons of DDDs prescribed, 2004–2010

	Women (n = 527)	Men (n = 303)		<50 years (n = 270)	≥50 years (n = 560)	
ATC group – Drug	n (%)	n (%)	P value[a]	n (%)	n (%)	P value[a]
H02AB06 – Prednisolone	220 (42)	167 (55)	<0.001	102 (38)	285 (51)	0.001
L04AX01 – Azathioprine	109 (21)	92 (30)	0.002	56 (21)	145 (26)	0.12
L04AA06 – Mycophenolic acid	16 (3)	7 (2)	NA	8 (3)	15 (3)	NA
L04AD01 – Cyclosporine	11 (2)	4 (1)	NA	7 (3)	8 (0.1)	NA
L04AX03 – Methotrexate	9 (2)	1 (0.3)	NA	3 (0.1)	7 (0.1)	NA
All immunosuppressants	246 (47)	178 (59)	0.001	113 (42)	311 (56)	<0.001

NA, not available or insufficient data available for analysis. [a]Non-parametric tests were used to calculate the difference in median defined daily dose between sex and age groups in the period 2004–2010.

Increased treatment frequency with drugs for the cardiovascular system in MG patients younger than 50 years was found. Arguably, there is a risk of ascertainment bias as MG patients more regularly visit a physician. However, physical inactivity due to muscle weakness, side effects of steroid treatment such as weight gain and elevated blood glucose levels are factors that may contribute to the increased risk of cardiovascular disease, even in younger individuals. The possibility for cardiac involvement in MG is also well recognized [6], although death caused by cardiac diseases is not increased [2]. The clinical implications remain unclear [7], but our data strongly indicate that there is a clinically relevant association between MG and cardiovascular disease. Early treatment of airway infections in MG patients is recommended [3], and may account for the increased use of anti-infectives. Immunosuppressed patients are also in general more prone to infections [5].

In this study, thyroid hormones were most frequently prescribed to MG patients <50 years and to men compared with controls. A recent systematic review estimated concomitant autoimmune diseases in MG at 13%, with thyroid disease as the most frequent [8]. In prospectively identified MG patients, type 1 and 2 diabetes was found in 10% and 8%, respectively [9]. All antidiabetics in our study were most frequently prescribed to patients ≥50 years. In addition to the general autoimmune disease overlap, reduced physical activity, corticosteroid treatment as well as other comorbid conditions may serve as catalysts for acquired metabolic syndrome and type 2 diabetes.

Use of antidepressants was more frequent amongst MG patients than controls. The frequency of patients receiving antidepressants in our study is in good agreement with previous reports of affective disorders in MG [10]. Drug treatment of anxiety and sleep disturbances was lower in our study compared with previous reports [11,12]. Psychiatric symptoms can mimic MG symptoms, but may also be under-recognized due to overlapping symptoms [13].

Age ≥50 years and male sex were predictors for immunosuppressive treatment in our study. Immunosuppressive drugs and thymectomy represent the main principles in treating moderate to severe MG [3], often lifelong in late-onset and thymoma cases (15% of MG patients). Complete stable remission can be induced in early-onset cases after thymectomy. The benefit of thymectomy for MG symptom relief is questionable for late-onset MG and thymoma MG patients [14]. Only 56% of the patients in our study over 50 years were treated with immunoactive drugs. Some muscle weakness is probably under-recognized in older patients due to the aging process or concomitant illness. One recent hospital-based study reported immunosuppressive therapy in 65% of late-onset cases [15]. In our study early- and late-onset cases were combined in the group above 50 years. A biological explanation implicating differences in disease severity is possible, but inadequate immunosuppression in our patients is also highly probable. Teratogenic and other adverse effects influence immunosuppressive treatment in young females. Such drugs are rarely used in pregnancy [16].

Only 6% of all MG patients in our study had such a severe disease that second-line immunomodulating drugs were required, indicating that prednisolone and azathioprine alone or in combination are sufficient for symptom control in nearly all MG patients. NorPD does not provide information on other treatment modalities, such as thymectomy, plasma exchange and intravenous administration of immunoglobulins. Patients identified with severe MG were predominantly ≥50 years old and females. MuSK-MG is more often seen in females and is associated with more severe disease, but this MG subtype is very rare in Norway [17]. Information on MG subtypes is not available in the NorPD.

The main strength of our study is case ascertainment from one single, unbiased, comprehensive

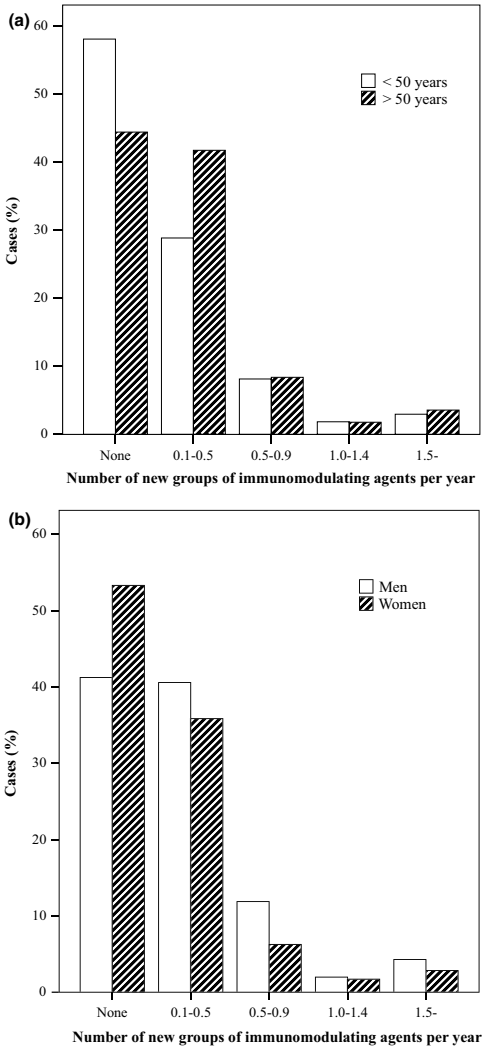

Figure 2 (a) Mean number of new groups of immunomodulating agents used in MG patients (%) below and above 50 years of age after MG diagnosis per year, 2004–2010. Open bars, patients <50 years; hatched bars, patients ≥50 years. (b) Mean number of new groups of immunomodulating agents used in MG men and women (%) after MG diagnosis per year, 2004–2010. Open bars, men; hatched bars, women.

database with a full, national population as controls. 96% of the entire Norwegian population has been included in NorPD since its establishment in 2004 with at least one prescription drug dispensed from a pharmacy. The 1 year prevalence of 68%–69% of the population in NorPD has proven stable [18]. Identifying MG patients by prescriptions of pyridostigmine is considered sensitive with a high positive predictive value for the diagnosis [19–21], and with good agreement of calculated prevalence rates using pyridostigmine prescriptions registered in the NorPD compared with rates calculated from a nationwide acetylcholine receptor antibody database [22]. Amongst 67 patients treated at our department for the past 30 years, only three did not receive pyridostigmine (unpublished data). NorPD did not include indication for prescription until 2008. This represents a potential source of overestimation. The inclusion criteria used in this study secured high sensitivity, although specificity may be lower. However, sensitivity analyses with more stringent criteria did not alter the basic characteristics of the MG cohort. Moreover, nearly 90% of our study population had confirmed at least one MG-reimbursed prescription of pyridostigmine or from a neurologist. Only MG patients with a confirmed diagnosis are given reimbursement. The reimbursement code is therefore highly specific for MG. Pyridostigmine is not prescribed on a regular basis to any other disease groups. The rare disease Lambert–Eaton myasthenic syndrome, with a prevalence of 2–3 per million [23], is treated with pyridostigmine and reimbursement would be given as for MG. Six patients with pyridostigmine were identified with an additional prescription of fludrocortisone, the standard drug for treating orthostatic hypotension, and may marginally bias our findings.

This study reveals the true complexity of MG and contributes to an understanding of the impact of MG on health. Awareness of comorbidities and knowledge of treatment practice should help physicians in choosing the best treatment strategy.

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Disclosure of conflicts of interest

The authors declare no financial or other conflicts of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Characteristics of the study population with different inclusion criteria.
Table S2. Mean (SD) number of new ATC groups for 830 MG patients per year, 2004–2010.

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Supplemental Material Paper II

Table S1. Characteristics of the study population with different inclusion criteria.

Definition	**Number of MG patients**	**Mean age of MG patients** (sd)	**Number of female MG patients** (%)
A	890	57 (19)	565 (63)
B	830	57 (18)	527 (63)
C	782	57 (19)	495 (63)
D	791	57 (19)	500 (63)
E	726	58 (19)	455 (63)
F	660	58 (19)	422 (64)

A = All recipients of pyridostigmine from January 1st 2004 – April 30th 2010.

B = All recipients of pyridostigmine who fulfilled either of the following inclusion criteria: 1) ≥2 pyridostigmine prescription, 2) one prescription of pyridostigmine made by a neurologist, 3) one prescription of pyridostigmine with a reimbursement code specific for MG (§13), 4) one prescription of pyridostigmine with an ICD-10 code (G70.0) or ICPC-2 code (N99) specific for MG.

C = All recipients of pyridostigmine who fulfilled criteria 3 and 4.

D = All recipients of pyridostigmine who fulfilled criteria 2, 3 and 4.

E = All recipients of pyridostigmine who fulfilled criteria 2 and 3.

F = All recipients of pyridostigmine who fulfilled criteria 3 only.

Table S2. Mean (SD) number of new ATC groups for 830 MG patients per year, 2004–2010.

	ATC groups													
	A	*B*	*C*	*D*	*G*	*H*	*J*	*L*	*M*	*N*	*P*	*R*	*S*	**Total**
Sex														
Female	1.1 (2.3)	0.6 (2.4)	1.0 (2.4)	0.5 (0.5)	0.5 (1.3)	0.6 (1.7)	0.8 (0.7)	0.4 (0.6)	0.5 (0.7)	1.5 (3.5)	0.3 (0.3)	0.8 (1.0)	0.5 (0.6)	5.3 (8.4)
Male	0.8 (1.3)	0.6 (1.0)	1.0 (1.3)	0.6 (2.0)	0.4 (0.7)	0.4 (0.6)	0.7 (1.0)	0.4 (0.3)	0.4 (0.3)	2.5 (21.2)	0.3 (0.2)	0.7 (1.6)	0.5 (0.3)	6.1 (21.9)
Age group[a]														
0-9	NA	NA	1.7 (NA)	NA	NA	0.5 (0.5)	0.9 (NA)	0.2 (NA)	NA	2.4 (3.3)	NA	NA	NA	3.7 (2.9)
10-19	0.7 (0.8)	0.8 (0.6)	2.6 (3.9)	0.4 (0.3)	0.8 (1.3)	0.9 (1.6)	0.7 (0.8)	2.0 (2.4)	0.3 (0.1)	1.1 (1.7)	0.3 (0.1)	1.0 (1.6)	0.8 (1.0)	4.2 (5.9)
20-29	1.0 (1.5)	0.5 (0.3)	0.4 (0.2)	0.8 (1.6)	0.4 (0.3)	0.3 (0.3)	0.9 (0.6)	0.3 (0.2)	0.4 (0.3)	0.7 (0.6)	0.3 (0.1)	0.7 (0.8)	0.4 (0.2)	3.4 (3.0)
30-39	0.9 (1.2)	0.6 (0.9)	0.8 (1.2)	0.6 (0.6)	0.6 (1.2)	0.5 (0.6)	0.8 (0.9)	0.4 (0.3)	0.5 (0.8)	1.5 (2.8)	0.3 (0.3)	0.8 (1.0)	0.6 (0.9)	4.5 (5.4)
40-49	0.8 (1.7)	0.5 (0.9)	0.6 (0.7)	0.5 (0.3)	0.6 (1.1)	0.4 (0.6)	0.8 (1.5)	0.3 (0.2)	0.4 (0.3)	1.3 (4.5)	0.2 (0.1)	1.0 (2.4)	0.4 (0.3)	4.5 (9.6)
50-59	1.3 (3.5)	0.4 (0.4)	1.2 (4.1)	0.4 (0.4)	0.3 (0.3)	0.8 (2.9)	0.7 (0.5)	0.3 (0.3)	0.4 (0.3)	4.0 (30.1)	0.2 (0.1)	0.8 (1.1)	0.5 (0.6)	7.7 (31.0)
60-69	0.9 (0.9)	0.6 (1.1)	1.1 (1.5)	0.8 (2.5)	0.4 (0.4)	0.5 (0.7)	0.8 (0.9)	0.5 (0.6)	0.5 (0.5)	1.6 (3.5)	0.4 (0.4)	0.7 (0.9)	0.5 (0.4)	6.1 (6.8)
70-79	1.1 (2.1)	0.8 (3.0)	1.0 (1.1)	0.4 (0.3)	0.6 (2.1)	0.5 (0.6)	0.8 (0.6)	0.3 (0.4)	0.6 (0.9)	1.7 (4.3)	0.2 (0.1)	0.7 (0.9)	0.6 (0.5)	6.3 (10.1)
80-89	0.7 (1.0)	0.4 (0.4)	0.7 (0.8)	0.3 (0.2)	0.3 (0.1)	0.3 (0.2)	0.5 (0.4)	0.2 (0.2)	0.3 (0.2)	0.9 (0.9)	0.2 (NA)	0.6 (0.9)	0.4 (0.3)	3.8 (3.2)
> 90	0.2 (0.1)	0.3 (0.1)	0.4 (0.3)	NA	0.2 (0.0)	0.2 (0.0)	0.2 (0.1)	0.2 (0.0)	0.2 (0.0)	0.4 (0.2)	NA	0.5 (0.3)	0.3 (0.2)	1.4 (1.0)
Total	1.0 (2.0)	0.6 (1.9)	1.0 (2.0)	0.5 (1.2)	0.5 (1.2)	0.5 (1.4)	0.8 (0.9)	0.4 (0.5)	0.5 (0.6)	1.9 (13.1)	0.3 (0.3)	0.8 (1.2)	0.5 (0.5)	5.6 (14.8)

[a]Patient age was calculated from year of birth and defined as age July 1st 2004.