Myasthenia gravis: subgroup classification and therapeutic strategies

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Myasthenia gravis is an autoimmune disease that is characterised by muscle weakness and fatigue, is B-cell mediated, and is associated with antibodies directed against the acetylcholine receptor, muscle-specific kinase (MUSK), lipoprotein-related protein 4 (LRP4), or agrin in the postsynaptic membrane at the neuromuscular junction. Patients with myasthenia gravis should be classified into subgroups to help with therapeutic decisions and prognosis. Subgroups based on serum antibodies and clinical features include early-onset, late-onset, thymoma, MUSK, LRP4, antibody-negative, and ocular forms of myasthenia gravis. Agrin-associated myasthenia gravis might emerge as a new entity. The prognosis is good with optimum symptomatic, immunosuppressive, and supportive treatment. Pyridostigmine is the preferred symptomatic treatment, and for patients who do not adequately respond to symptomatic therapy, corticosteroids, azathioprine, and thymectomy are first-line immunosuppressive treatments. Additional immunomodulatory drugs are emerging, but therapeutic decisions are hampered by the scarcity of controlled studies. Long-term drug treatment is essential for most patients and must be tailored to the particular form of myasthenia gravis.

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Introduction

Dysfunction at the neuromuscular junction underlies several disorders that are characterised by skeletal muscle weakness usually involving some but not all muscle groups. Genetic forms of these disorders are termed congenital myasthenic syndromes. Some toxins, like botulinum toxin and curare, can cause neuromuscular dysfunction; acquired antibody-mediated forms include autoimmune and neonatal myasthenia gravis, Lambert–Eaton myasthenic syndrome, and neuromyotonia.

Myasthenia gravis forms the largest disease group of neuromuscular junction disorders and is caused by pathogenic autoantibodies to components of the postsynaptic muscle endplate (figure 1).[1–4] Fluctuations in severity of muscle weakness are typical. Some, but not all, muscles are affected and not necessarily symmetrically. Increased weakness with continued muscle activity represents a diagnostic clue for myasthenia gravis, but these clinical features can vary. Patients with myasthenia gravis should be classified into subgroups, with implications for diagnosis, optimum therapy, and prognosis. In myasthenia gravis guidelines and consensus reports, subgrouping is recommended,[1–5] but exact definitions vary and new subgroups are emerging as a result of increased knowledge. As this subgrouping takes into account myasthenia gravis autoantibodies, epidemiology, clinical presentation, and comorbidities, the subgroups are discussed after these sections in this Review. For a few patients, subgrouping is not possible owing to insufficient precise information, including suboptimum autoantibody testing and pathological changes of the thymus below the detection threshold of imaging.

Autoantibodies against the acetylcholine receptor (AChR), muscle-specific kinase (MUSK), and lipoprotein-related protein 4 (LRP4) are well established as sensitive and specific diagnostic markers and pathogenic factors, and these autoantibodies are instrumental for subgrouping patients with myasthenia gravis. A prerequisite for optimum diagnosis and treatment, therefore, is access to autoantibody testing.[1–5]

With modern immunosuppressive, symptomatic, and supportive treatments, the prognosis for patients with myasthenia gravis is good. Most patients with mild-to-moderate symptoms will obtain full remission or substantial improvement. Full remission is rare in severe cases, some variation over time is common, and steady progression is unusual. Daily life functions of individuals with myasthenia gravis are not, or only modestly, affected and life expectancy is not reduced.[6] Long-term drug treatment is necessary for nearly all patients with myasthenia gravis.[2,7] In 10–15% of these patients, full control of the disease is not possible or is only at the cost of severe side-effects of immunosuppressive therapy.[8]

Treatment protocols at leading centres are not based purely on results from well controlled studies or guidelines based on such studies, because well controlled studies are sparse for this disease, and do not take into account the variation in therapeutic response among the diagnostic subgroups. Myasthenia gravis is a rare disease, and most patients do well on existing treatments, both aspects that are a challenge for new trials. We will combine information from controlled studies, consensus reports, and expert views with insights from theoretical and experimental studies relevant for myasthenia gravis subgroups, with the aim of assessing the evidence base for the use of treatments, including interventions directed at the pathophysiological process.

Autoantibodies in myasthenia gravis

AChR antibodies are highly specific for myasthenia gravis, and their presence combined with muscle weakness confirms the disease. Further diagnostic investigation is necessary only to define the subgroup and disease severity. The value of repeated AChR antibody testing in patients with this disorder is debated,

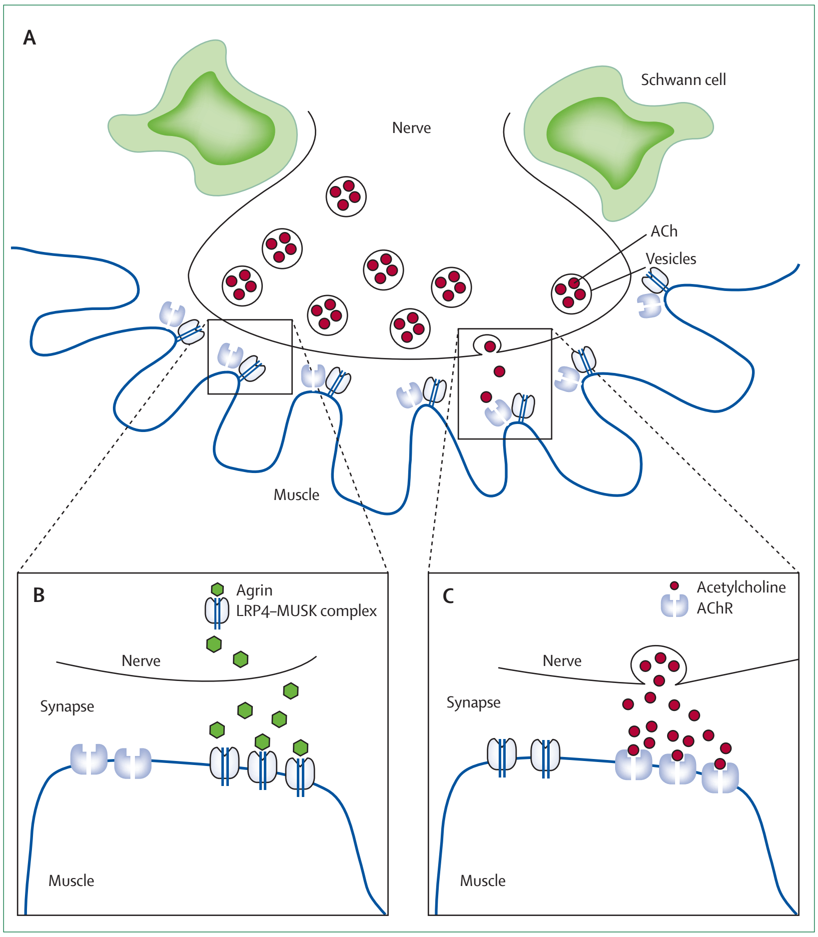

Figure 1: **The neuromuscular junction**
(A) The AChR and MUSK are expressed at the top of the junctional folds. (B) Trophic signal: binding of agrin to the LRP4–MUSK complex activates aggregation of AChRs and promotes transition from the plaque to pretzel form of the neuromuscular junction. (C) Activation signal: binding of acetylcholine to the AChR induces a brief opening of the central ion channel causing membrane depolarisation, which in turn elicits a muscle action potential that leads to contraction of the muscle fibre. AChR=acetylcholine receptor. MUSK=muscle-specific kinase. LRP4=lipoprotein-related protein 4.

but changes in antibody concentration might predict disease severity in patients given immunosuppressive drugs and therefore can support therapeutic decisions. No correlation has been shown between AChR antibody concentration and disease severity. AChR antibodies are directly pathogenic through crosslinking of AChRs leading to accelerated degradation of these receptors, through complement binding and activation, and by inducing AChR conformational changes or blocking acetylcholine binding.[1–4] Radioimmunoprecipitation is the standard commercial test and gives a quantitative AChR antibody measure. Cell-based assays can have an even higher sensitivity than radioimmunoprecipitation, but are not yet commercially available and standardised.[9] Tests avoiding radioactive ligands are also in use such as ELISA and fluorescence tests based on immunoprecipitation,[10] but they tend to be less sensitive than assays with radioactive ligands.

Standard tests for MUSK antibodies use radioimmunoprecipitation or an ELISA. Cell-based assays used for research can increase sensitivity. MUSK antibodies are directly pathogenic in experimental animal models,[11–13] even if the predominant IgG4 antibodies do not bind complement. Any value of repeated tests in the follow-up of patients has not been established because prospective, high-quality studies have not been done.

LRP4 antibodies bind to the membrane protein in vivo, block the agrin–LRP4 interaction and thereby also inhibit AChR clustering in the membrane. Interference with the LRP4–MUSK interaction might also be a relevant disease mechanism for this subgroup. Mice immunised with LRP4 develop typical myasthenia gravis.[14] Thus, LRP4 antibodies are directly pathogenic through interference with AChR function.

Agrin antibodies have been detected in a few patients with myasthenia gravis and AChR, MUSK, or LRP4 antibodies.[15,16] Agrin is essential for AChR function, but whether these antibodies contribute to the muscle weakness in this disease is still unclear. Similarly, cortactin autoantibodies have been reported in patients with myasthenia gravis, both with and without other neuromuscular autoantibodies.[17]

Titin and ryanodine receptor antibodies occur in some patients with AChR-associated myasthenia gravis. Titin maintains the flexibility of the cell structure, whereas the ryanodine receptor is a sarcoplasmic reticulum calcium channel that mediates contraction of the muscle cell. Titin and ryanodine receptor antibodies probably do not enter the muscle cell in vivo and might not mediate any muscle weakness, but rather could be disease markers.[18] These antibodies are present with a high frequency in thymoma-associated myasthenia gravis, with an intermediate frequency in late-onset myasthenia gravis, and very rarely in early-onset and ocular myasthenia gravis; they are not detected by standard testing in MUSK, LRP4, or antibody-negative myasthenia gravis.[7,19] Titin and ryanodine receptor antibodies can be used to diagnose a thymoma in patients younger than 50 years.[19] These antibodies have been proposed as markers for severe myasthenia gravis with a need for long-term immunosuppression and no response to thymectomy. Commercial tests with ELISA are available for titin but not for ryanodine receptor antibodies.

Epidemiology

Autoimmune myasthenia gravis has a reported worldwide prevalence of 40–180 per million people, and an annual incidence of 4–12 per million people.[20–23] Recently collected figures of prevalence and incidence tend to be higher than older ones, especially for late-onset myasthenia gravis, partly explained by increased case finding and more widespread autoantibody testing. Population demographics with an increased number of elderly people and reduced myasthenia gravis mortality affect incidence and prevalence. AChR-associated myasthenia gravis has a bimodal age pattern of incidence, with a peak in young adults aged about 30 years and then a steady increase in incidence with increasing age older than 50 years.[20,21] The incidence peak in young adults is mainly because of the high frequency in women, typical for many autoimmune disorders, although late-onset myasthenia gravis is slightly more frequent in men. No evidence suggests that the occurrence of this disease is increasing as a result of a change in external causative factors such as infections or diet.[24]

Overall, myasthenia gravis incidence and prevalence shows little geographical variation; however, this distribution is not the case for all subgroups of the disease. Juvenile myasthenia gravis, a subtype of early-onset disease, has a high frequency in east Asia, in which up to 50% of all cases have onset before age 15 years, many of them with ocular symptoms only.[22,25] Myasthenia gravis incidence in children (aged <15 years) in a mixed population from Canada was 1–2 per million per year, and highest in those of Asian ethnicity, especially for the ocular subgroup. LRP4 antibodies were recorded in 19% of patients without AChR antibodies,[5] and MUSK antibodies in a third of patients without AChR antibodies.[3,4,26] Epidemiological data suggest that LRP4-associated myasthenia gravis is half as frequent as the MUSK form of the disease. MUSK-associated myasthenia gravis incidence is estimated at 0·3 patients per million per year, with a prevalence of 2·9 per million people, and is more common in southern than northern Europe.[27] Genetic predisposition and external factors linked to infections or diet are potential explanations for some geographical variation in this disease and its subtypes.

Clinical presentation

Muscle weakness is a major symptom and sign in myasthenia gravis. The combination of weakness localisation, variation in weakness over time, and exercise-induced weakness usually gives strong clues to the diagnosis of the disease for all subgroups. In older individuals with eye muscle weakness and bulbar symptoms, cerebrovascular disease of the brainstem is sometimes suspected. In younger individuals, unspecific fatigue disorders can be part of the differential diagnoses.[1,3,7]

Weakness in myasthenia gravis arises in the extraocular, bulbar, limb, and axial muscles (figure 2). 60% of patients present with ptosis or diplopia, or both, and in 20% of patients, the disease is restricted to ocular myasthenia gravis.[1–4] Weakness of external eye muscles is nearly always asymmetrical (figure 3), whereas limb weakness is symmetrical and more proximal than distal (figure 2).[28] The variability in symptoms in skeletal muscles is surprising because they all express the autoimmune target protein. This variation results from many subtle factors affecting neuromuscular transmission, muscle cell depolarisation or contraction, resistance to an immunological attack, and regenerative capacity of muscle structures.[2,17]

Subtype	AChR myasthenia gravis	MUSK myasthenia gravis	LRP4 myasthenia gravis	Seronegative myasthenia gravis	LEMS
Relative prevalence	80%	4%	2%	5%	4%

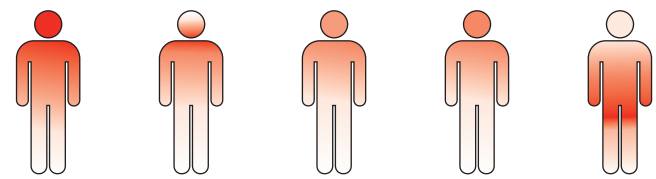

Figure 2: **Distribution of weakness and relative prevalence of subtypes of myasthenia gravis**
AChR=acetylcholine receptor. MUSK=muscle-specific kinase. LRP4=lipoprotein-related protein 4. LEMS=Lambert–Eaton myasthenic syndrome.

Comorbidities

Patients with early-onset and ocular subgroups of myasthenia gravis have increased frequency of organ-specific and general autoimmune disorders, especially thyroiditis.[29] Patients with thymoma-associated myasthenia gravis are at an increased risk of developing haematological autoimmune disorders. Thymectomies have not been shown to increase the risk of infections, autoimmune disease, or cancer. Myasthenia gravis muscle weakness might increase the risk of respiratory infections and osteoporosis, becoming overweight, and developing other complications. A widespread autoimmune inflammatory myopathy can occur in myasthenia gravis.[30] AChR antibodies and myasthenia gravis-like features have been described occasionally in patients with amyotrophic lateral sclerosis.[31]

Several studies[32–34] have investigated the cancer risk in patients with myasthenia gravis and its subgroups. Methodological challenges due to myasthenia gravis patient selection, sensitivity in cancer detection, follow-up time, and types of control groups have led to varying conclusions. Thymomas in general seem to confer a moderately increased risk for other cancer types,[32] whereas myasthenia gravis and its immunoactive treatment, according to a Danish population-based study[33] with a long-term follow-up and relevant controls, was not associated with a significantly increased risk, perhaps with the exception of non-melanoma skin cancer.[34]

AChR, MUSK, and LRP4 antibodies do not cross-react with the heart muscle. In population studies,[6] no increased mortality or morbidity related to cardiac factors have been established. However, cardio-physiological function can be marginally affected by these antibodies.[35] Many case reports of severe cardiomyositis and heart conduction abnormalities in thymoma-associated myasthenia gravis and late-onset myasthenia gravis have been noted, most probably induced by heart muscle autoimmunity.[36,37] Heart function monitoring is recommended during severe myasthenia gravis exacerbations, especially in patients with various antimuscle antibodies.[38]

Figure 3: **Two patients with AChR-associated myasthenia gravis**
Female patient with ophthalmoplegia (note adduction of right eye) and ptosis of the left eye (A). Male patient with opthalmoplegia (note the upward position of the left eye) and ptosis of the right eye before treatment (B) and 1 year after immunosuppressive treatment (C).

Myasthenia gravis subgroups

Early-onset myasthenia gravis with AChR antibodies

Patients with early-onset myasthenia gravis have, by definition, onset of their first symptom before age 50 years (table 1).[1,7,39] Serum AChR antibodies are detected by standard diagnostic testing. Patients with a thymoma detected on imaging or during surgery are excluded from this myasthenia gravis subgroup. Thymic follicular hyperplasia occurs often but is not a prerequisite, and this group responds to thymectomy. Female cases outnumber male cases by three to one.[20,22] Early-onset myasthenia gravis has an association with *HLA-DR3*, *HLA-B8*, and other autoimmune risk genes (table 1),[40,41] and all autoimmune disorders are more widely reported in relatives of patients in this myasthenia gravis subgroup.[42] These findings suggest subgroup differences in the pathogenesis of myasthenia gravis.

Late-onset myasthenia gravis with AChR antibodies

Patients with late-onset myasthenia gravis are defined as having their first onset of symptoms after age 50 years. In this group, serum AChR antibodies are present, thymoma is not evident on imaging or during surgery, and thymic hyperplasia occurs only rarely; these patients most often will not respond to thymectomy. The disease is slightly more frequently reported in males than females, and weak HLA associations occur with *HLA-DR2*, *HLA-B7*, and *HLA-DRB1*15:01*.[43]

Thymoma-associated myasthenia gravis

Thymoma-associated myasthenia gravis is a paraneoplastic disease. Myasthenia gravis is by far the most widely reported autoimmune disease associated with a thymoma, although pure red aplasia and neuromyotonia are also associated with thymoma; this association does not occur in other autoimmune disorders. A thymoma is recorded in 10–15% of all patients with myasthenia gravis. Nearly all have detectable AChR antibodies and generalised disease. About 30% of patients with a thymoma develop myasthenia gravis, and even more have AChR antibodies without myasthenia gravis.[44]

MUSK-associated myasthenia gravis

MUSK is a protein expressed in the postsynaptic muscle membrane that is functionally linked to AChR and necessary to maintain AChR function. Overall, 1–4% of patients with myasthenia gravis have serum MUSK antibodies, but more cases will probably be identified with increasingly sensitive test assays. MUSK and AChR antibodies rarely coexist in the same patient. MUSK-associated myasthenia gravis is usually reported in adults, and rarely in the very old or in children.[45] No thymus pathological changes are reported and patients usually have no response to thymectomy. IgG4 antibodies have an important role in the pathogenesis, and there is an HLA association with *HLA-DQ5*,[46–48] unlike in other myasthenia gravis subgroups.

MUSK-associated myasthenia gravis shows predominant involvement of cranial and bulbar muscles. About a third of the patients present with ptosis and diplopia.[27] In more than 40% of patients with MUSK-associated myasthenia gravis, bulbar weakness is a first symptom, with facial, pharyngeal, and tongue weakness, often associated with neck and respiratory involvement. Limb weakness is not common, and ocular muscles are often unaffected.[27] Little variation in muscle strength is reported during the day, and muscle atrophy might occur.

LRP4-associated myasthenia gravis

LRP4 is expressed in the postsynaptic muscle membrane; it is a receptor for nerve-derived agrin and an activator of MUSK, and is necessary to maintain AChR function. LRP4 antibodies have been detected in 2–27% of patients with myasthenia gravis without AChR and MUSK antibodies, with a female preponderance.[49,50] Most of these patients present with ocular or generalised mild myasthenia gravis, and about 20% of patients have only ocular weakness for more than 2 years. Respiratory insufficiency occurs very rarely, except in a subgroup with additional MUSK antibodies. In two-thirds of patients with LRP4-associated myasthenia gravis, the thymus is atrophic and normal for age, but hyperplasia has been reported.[5] Commercial tests are not yet available for LRP4 antibody testing, meaning that this group can be identified only by a few institutions.

Antibody-negative generalised myasthenia gravis

Myasthenia gravis without detectable AChR, MUSK, or LRP4 antibodies represents a heterogeneous group pathogenically. Some patients have low-affinity antibodies or low concentration of antibodies to AChR, MUSK, or LRP4 antigen targets, identified by cell-based methods only, that are not detectable in routine assays.[51,52] Low-affinity antibodies are pathogenic in vivo, and the disease in patients with such antibodies is probably similar to that in the myasthenia gravis subgroup with detectable antibodies. Low-affinity antibodies seem to account for 20–50% of patients in the antibody-negative generalised myasthenia gravis subgroup.[51,52] Antibodies to agrin and cortactin often occur in combination with other autoantibodies.[15,17,52] Their functional relationship to other targeted proteins is not clear. Some patients with myasthenia gravis probably have pathogenic antibodies against yet-undefined antigens in the postsynaptic membrane. The diagnosis is more challenging in patients in whom no specific autoantibodies are detected. In such patients, non-myasthenia gravis myasthenic syndromes and other muscle and non-muscle disorders should also be considered.[3]

Ocular myasthenia gravis

In some patients with myasthenia gravis, the weakness is restricted to the ocular muscles. Patients with purely ocular weakness are at risk of developing generalised myasthenia gravis, especially early in the disease. 90% of those who have had the ocular form for more than 2 years will remain in this subgroup.[53] Half of patients with ocular myasthenia gravis have detectable AChR antibodies, whereas MUSK antibodies very rarely occur.[53]

	Myasthenia gravis subgroup	Age at onset	Sex	HLA associations	Thymus pathological changes
Active immune response					
AChR	Early onset	<50 years	More female than male	*DR3-B8-A1*	Hyperplasia
AChR	Late onset	>50 years	More male than female	Diverse	Normal or hyperplasia
AChR	Thymoma	Variable	..	..	Lymphoepithelioma
MUSK	MUSK-myasthenia gravis	Variable	Substantially more female than male	*DR14, DR16, DQ5*	Normal
LRP4	LRP4-myasthenia gravis	Variable	..	..	Normal
Unknown	SNMG	Variable	..	..	Normal or hyperplasia
Passive transfer of antibodies					
AChR, or MUSK, or LEMS	Neonatal myasthenia gravis	Neonate	Equal proportion of female to male	..	None

AChR=acetylcholine receptor. MUSK=muscle-specific kinase. LRP4=lipoprotein-related protein 4. SNMG=seronegative myasthenia gravis. LEMS=Lambert-Eaton myasthenic syndrome.

Table 1: **Myasthenia gravis antibody and subgroup characteristics**

Thymus pathological changes

Thymoma, but no other thymic tumours, is associated with myasthenia gravis. Thymic hyperplasia is reported in most patients with early-onset myasthenia gravis and in some patients with late-onset, ocular, and antibody-negative disease. CT scanning or MRI of the mediastinum should be undertaken in all patients with myasthenia gravis to assess for a thymoma.[1–4,7] Both sensitivity and specificity are challenges for imaging.

Experimental and clinical evidence strongly suggests that early-onset and thymoma-associated myasthenia gravis are initiated within the thymus.[44] Myoid muscle-like cells and professional antigen-presenting cells are elements of the thymus and are active in early-onset myasthenia gravis, whereas thymoma cells contain muscle-specific antigens and have antigen-presenting properties.[54] AChR expression can be activated in thymic epithelial cells through cytokine and receptor signalling, potentially triggered by a virus;[3,55] however, no specific virus has been identified so far. MicroRNAs can mediate immunoregulatory processes, be induced by environmental events, and seem to be abnormally expressed in myasthenia gravis.[56] Autoreactive T cells, specific for AChR, escape the normal intrathymic surveillance and are exported to the periphery where they stimulate B cells to produce antibodies. Differences in autoantibody pattern, HLA associations, thymic pathological changes, cytokine intrathymic pattern, and T-cell subsets and clones all point to differences in induction mechanisms for early-onset, late-onset, and thymoma-associated myasthenia gravis.[44]

Neurophysiological testing

Neurophysiological tests are unnecessary in patients with typical myasthenia gravis symptoms because diagnosis can be confirmed by specific antibody tests; these tests are also not helpful for myasthenia gravis subgroup classification. However, they are important for correct diagnosis in patients with myasthenia gravis without detectable autoantibodies.

Repetitive nerve stimulation and single-fibre electromyography for an increased jitter are useful tests for patients with myasthenia gravis. Single-fibre testing is the most sensitive, whereas decrement at repetitive stimulation is the most specific.[1] Both sensitivity and specificity rely on investigation quality. Even after combined neurophysiological and antibody testing, myasthenia gravis can be difficult to rule out. Most patients for whom some doubt about diagnosis remains after testing, from our experience, do not have autoimmune myasthenia gravis.

	Drug	Control or comparator	Number of participants	Duration	Primary outcome measure	ClinicalTrials.gov number	Result
Corticosteroids							
Mount (1964)[58]	Corticotropin	Placebo	43	12 weeks	Eye movements	..	No significant difference
Howard et al (1976)[59]	Alternate-day prednisone	Placebo	13	24 weeks	Clinical score	..	No significant difference
Lindberg et al (1998)[60]	Pulse methylprednisone	Placebo	19	2 weeks	Muscle fatigue test	..	p<0·01
Benatar et al (2015)[61]	Prednisolone	Placebo	11	16 weeks	Treatment failure	NCT00995722	Completed
Assistance Publique—Hôpitaux de Paris (2009–2015)	Slow decrease of prednisolone plus azathioprine	Rapid decrease of prednisolone plus azathioprine	118	60 weeks	Minimal manifestation	NCT00987116	Ongoing
Azathioprine							
Bromberg et al (1997)[62]	Azathioprine	Prednisone	10	52 weeks	Observational	..	Descriptive
Palace et al (1998)[63]	Prednisolone and azathioprine	Prednisolone and placebo	34	156 weeks	Prednisone dose	..	p=0·02
Ciclosporin							
Tindall et al (1987)[64]	Ciclosporin	Placebo	20	52 weeks	QMGS, AChR titre	..	Only QMGS significant
Tindal et al (1993)[65]	Ciclosporin	Placebo	39	26 weeks	QMGS, AChR titre	..	p=0·004
Tacrolimus (FK506)							
Nagane et al (2005)[66]	FK506	Placebo	34	52 weeks	Prednisone dose	..	p<0·05
Yoshikawa et al (2011)[67]	Tacrolimus	Placebo	80	28 weeks	Prednisone dose	NCT00309088	No significant difference
Astellas Pharma Inc (2011–14)	Tacrolimus	Placebo	83	24 weeks	QMGS	NCT01325571	Ongoing
Mycophenolate							
Meriggioli et al (2003)[68]	Mycophenolate mofetil	Placebo	14	20 weeks	QMGS	..	No significant difference, except SFEMG (p=0.03)
Hoffmann-La Roche (2004–07)	Mycophenolate mofetil	Placebo	136	36 weeks	Responder status	NCT00683969	Completed
Hoffmann-La Roche (2004–07)	Mycophenolate mofetil	Placebo	136	12–52 weeks	Adverse events	NCT00408213	Completed
FDA Office of Orphan Products Development/Duke University, NC, USA (2008)	Mycophenolate mofetil	Placebo	80	12 weeks	QMGS	NCT00285350	No significant difference
Sanders et al (2008)[69]	Mycophenolate mofetil	Placebo	176	36 weeks	Myasthenia gravis composite	..	No significant difference
Qualitix Clinical Research Co Ltd (2009–11)	Mycophenolate mofetil	Azathioprine	40	52 weeks	Remission	NCT00997412	Completed
Methotrexate							
Pasnoor et al (2013)[70]	Methotrexate	Placebo	50	36 weeks	Prednisone dose	NCT00814138	Ongoing
Immunoglobulin or plasma exchange							
Gajdos et al (1997)[71]	Plasma exchange *vs* intravenous immunoglobulin	..	87	15 days	Myasthenic muscular score	..	No significant difference
Wolfe et al (2002)[72]	Intravenous immunoglobulin	Placebo	15	6 weeks	QMGS	..	No significant difference
Gajdos et al (2005)[73]	Intravenous immunoglobulin (two doses)	Placebo	173	2 weeks	Myasthenic muscular score	..	No significant difference
Zinman et al (2007)[74]	Intravenous immunoglobulin	Placebo	50	4 weeks	QMGS	NCT00306033	Intravenous immunoglobulin effective (p<0.047)
Barth et al (2011)[75]	Intravenous immunoglobulin	Plasma exchange	84	2 weeks	QMGS	NCT01179893	Equally effective
Benesis Corporation (2007–10)	Intravenous immunoglobulin (GB-0998)	Plasma exchange	46	4 weeks	QMGS	NCT00515450	Completed

(Table 2 continues on next page)

	Drug	Control or comparator	Number of participants	Duration	Primary outcome measure	ClinicalTrials.gov number	Result
(Conintued from previous page)							
Rituximab							
Yale University, CT, USA (2014–17)	Rituximab	Placebo	50	52 weeks	Prednisone dose reduction	NCT02110706	Ongoing
Eculizumab							
Howard et al (2013)[76]	Eculizumab	Placebo	14	18 weeks	QMGS, adverse events	NCT00727194	p=0·0144
Alexion Pharmaceuticals (2013–16)	Eculizumab	Placebo	92	26 weeks	Myasthenia gravis ADL score	NCT01997229	Ongoing
Belimumab							
GlaxoSmithKline (2013–14)	Belimumab	Placebo	42	24 weeks	QMGS	NCT01480596	Ongoing
Leflunomide							
First Affiliated Hospital, Sun Yat-Sen University, China (2012–15)	Leflunomide	Azathioprine	158	48 weeks	Clinical response	NCT01727193	Ongoing
Tirasemtiv (CK-2017357)							
Sanders et al (2015)[77]	CK-2017357	Placebo	32	2 days	QMGS, VC, MMT	NCT01268280	Completed
Thymectomy							
University of Alabama at Birmingham, AL, USA/ National Institute of Neurological Disorders and Stroke, USA (2008)	Thymectomy plus prednisolone	Prednisolone	150	3 years	AU QMGS	NCT00294658	Ongoing

QMGS=quantitative myasthenia gravis score. AChR=acetylcholine receptor. SFEMG=Single-fibre electromyography. ADL=activities of daily living. VC=vital capacity. MMT=manual muscle test. AU=area under the curve.

***Table 2:* Randomised trials of treatments for autoimmune myasthenia gravis**

Treatment of myasthenia gravis

Symptomatic drug treatment

Drugs that increase the amount of acetylcholine at neuromuscular endplates after motor nerve stimulation improve muscle weakness in all myasthenia gravis subgroups; pyridostigmine is the preferred drug for symptomatic treatment.[7] Other acetylcholinesterase inhibitors, such as neostigmine and ambenonium chloride, have different durations of action and can differ regarding side-effects. The improvement reported in patients with these drugs is so specific that it is used as a diagnostic clue in patients who are antibody negative. Reduction of acetylcholine breakdown by acetylcholinesterase inhibition is the most effective symptomatic treatment in myasthenia gravis, and is better than increasing acetylcholine release presynaptically, although a mild beneficial effect of ephedrine or 3, 4-diaminopyridine might be seen. The observational effects are so clear that randomised studies have not been undertaken and are difficult to justify.[57] In MUSK-associated myasthenia gravis, acetylcholinesterase inhibitors are less effective and induce frequent side-effects.[27] The optimum dose is a balance between increased muscle strength and side-effects due to cholinergic stimulation in the autonomic nervous system. Glycopyrronium bromide, atropine sulfate, and loperamide can be used to treat muscarinergic side-effects. Long-term treatment with acetylcholinesterase inhibitors is safe and habituation or cumulative side-effects have not been reported. Some patients with no or only very mild symptoms choose to continue to take an acetylcholinesterase inhibitor. This continuation might be out of habit or concern of disease, or because the inhibitors induce a substantial subjective improvement in these patients.

Immunosuppressive drug treatment

For patients with myasthenia gravis in all subgroups who do not have a fully satisfactory functional result with symptomatic treatment alone, immunosuppressive drugs should be initiated (tables 2,3, and figure 4). Both treatment effects and side-effects are dose dependent. Finding the optimum drug dose for each patient is as important as selecting the optimum drug. To maximise effect and minimise side-effects, a combination of immunosuppressive drugs is preferable for most patients. Placebo-controlled studies and those comparing alternative treatments are rare. Recommendations are generally based on the sum of many studies with weak evidence, or on guidelines, clinical experience, and consensus reports.[78] Formal standards for patient assessment can be helpful to assess treatment response.[79]

Prednisone and prednisolone improve muscle strength in all myasthenia gravis subgroups. Prednisone and prednisolone are used in the same manner and are equally effective. Prednisone is activated by the liver into prednisolone. The beneficial effect manifests after 2–6 weeks, faster than for most other treatments. In a few

	Number of participants	Duration	Study design	ClinicalTrials.gov number	Study period
Bortezomib	18	6 months	Open	NCT02102594	2014–16
GM-CSF	12	120 days	Open	NCT01555580	2012–13
Plasmapheresis	10	14 weeks	Observational	NCT01927692	2013–14
Rituximab	10	12 months	Open	NCT00619671	2004–09
Rituximab	30	12 months	Open	NCT00774462	2008–11
Stem-cell therapy	10	5 years	Open, phase 1	NCT00424489	2002–16
Subcutaneous intravenous immunoglobulin	25	12 weeks	Open, phase 2	NCT02100969	2014–17
Subcutaneous intravenous immunoglobulin	10	6 months	Open	NCT01828294	2011–15
Tacrolimus	11	28 weeks	Open	NCT00309101	2006–09

GM-CSF=granulocyte-macrophage colony stimulating factor.

Table 3: **Ongoing non-randomised trials of treatments for autoimmune myasthenia gravis registered in ClinicalTrials.gov**

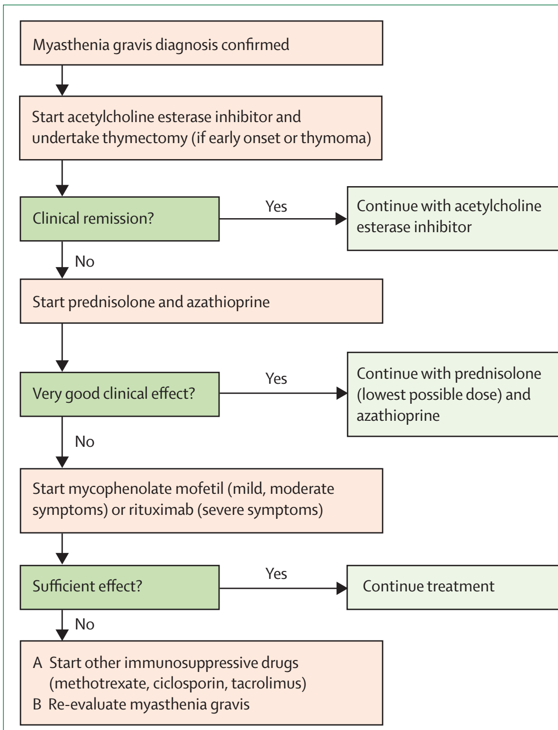

Figure 4: **Treatment of generalised myasthenia gravis**

patients, initial deterioration of generalised myasthenia gravis has been reported lasting for up to 3 weeks.[7,54,80] The starting dose is most often 0·75–1·0 mg/kg per day[1] for prednisone and prednisolone and is gradually increased; alternate-day dosing is thought to reduce side-effects and is recommended by some treatment guidelines.[2,3,81] After optimum improvement has been induced, the drug dose should be gradually reduced, and continued at the lowest dose necessary to obtain maximum effect. Prednisone or prednisolone should not be given as an alternate-day treatment to patients with diabetes because fluctuations in glucose concentrations result from this treatment approach. If muscle strength differs for off-treatment days and on-treatment days, a low dose (5–10 mg) of prednisone or prednisolone can be added on off-days. For ocular myasthenia gravis, observational studies[53,82] suggest that prednisolone treatment reduces the risk of developing generalised myasthenia gravis, although this observation has not been confirmed. For patients who take long-term corticosteroids, specific precautions should be taken to reduce the risks of glucose intolerance, gaining excess bodyweight, hypertension, and development of osteoporosis. A UK registry-based study[83] did not report an increased fracture risk in patients with myasthenia gravis.

Azathioprine is an effective drug for all myasthenia gravis subgroups, with 2–3 mg/kg being the most effective dose in combination with prednisolone.[62,63,84] This combination is often recommended as a first-choice treatment for patients with generalised myasthenia gravis who need immunosuppression, and is more beneficial than corticosteroids alone with fewer side-effects. The azathioprine effect is delayed and from clinical experience is usually seen after 6–15 months, and might further increase during the subsequent 1–2 years.[63] This makes the combination with prednisolone convenient, and prednisolone can be reduced when the azathioprine effect has been established. Regular follow-up is necessary because of the risk of leucopenia and hepatotoxic effects, especially during the first months of treatment. Low thiopurine methyltransferase activity increases the risk for azathioprine toxic effects, and can be tested before the start of treatment. Long-term treatment is also safe and effective in young individuals.[85] Azathioprine and corticosteroids in combination are effective in almost all patients with myasthenia gravis. Patients with ocular myasthenia gravis often respond well to a small dose (10–30 mg on alternate days) of corticosteroids alone.

Mycophenolate mofetil is a prodrug that after conversion blocks purine synthesis and interferes with B-cell and T-cell proliferation. Most guidelines recommend the drug for mild and moderate myasthenia gravis if the initial immunosuppressive therapy fails,[2–4] often together with prednisolone. This recommendation is based on retrospective studies[1–3,8] and clinical experience. Mycophenolate mofetil is not recommended as first-line treatment. In two prospective and controlled trials,[69,86] mycophenolate mofetil did not show additional benefit when given as initial treatment combined with prednisone. The studies had short durations of only 12 weeks and 9 months. There were no stopping rules for the use of corticosteroids and the lowest prednisone dose was 7·5 mg per day, which might have obscured an effect of mycophenolate mofetil. Little is known about myasthenia gravis subgroup responses for this drug.[87]

Side-effects are rare, with mild headache, nausea, and diarrhoea the most commonly reported.

Rituximab has emerged as a potentially effective drug in myasthenia gravis.[81,88,89] It is a chimeric IgG1 monoclonal antibody that depletes all types of B lymphocytes through specific binding to the transmembrane CD20 antigen. This drug should, in our opinion, be considered in moderate and especially severe myasthenia gravis that does not respond sufficiently to first-line immunosuppressive treatment. However, controlled studies have not been done, and rituximab is not regarded as a fully established treatment. About two-thirds of patients with severe myasthenia gravis and insufficient response to prednisolone and azathioprine have a substantial improvement on this treatment.[81,88–91] Open and uncontrolled studies[90–92] show that patients with MUSK-associated myasthenia gravis in particular have a favourable response, which is especially important as this myasthenia gravis subgroup often has a lower response to the first-line symptomatic and immuno-suppressive treatment. In most reports, the induction treatment recommended for rheumatological diseases has been used, which is two doses of rituximab 1000 mg, and then another two doses of 1000 mg after 2 weeks.[81,88–91] Lower doses have been suggested for myasthenia gravis.[88] Most centres would give additional rituximab doses only to patients with deterioration after a substantial and long-lasting response, and then in the lowest effective dose.[92] Rituximab is most often combined with prednisolone and the combination with prednisolone and azathioprine is also regarded as safe. Severe side-effects have been reported as rare events with rituximab for other autoimmune disorders, including JC-virus-related pro-gressive multifocal leukoencephalopathy, and have restricted the use of rituximab in myasthenia gravis. Even in the absence of controlled prospective studies and with high drug costs, rituximab has, in our opinion, a place as an early treatment for an increasing number of patients with MUSK and AChR-associated myasthenia gravis.

Prospective and controlled studies have shown that ciclosporin and methotrexate are effective as secondary drugs for myasthenia gravis.[65,70,93] The effect occurs in all myasthenia gravis subgroups. Although comparative studies have not been undertaken, ciclosporin and methotrexate are thought to be as effective as azathioprine.[1–4,7] Patients should be monitored for potential side-effects, especially nephrotoxic effects and hypertension.

Tacrolimus has similarities to ciclosporin. A small (34 patients) randomised but unblinded study[66] showed that prednisone could be given at a reduced dose after 52 weeks when combined with tacrolimus. However, a large double-blind study[67] comprising of 80 patients did not confirm this finding. The length of this study was only 28 weeks and the therapeutic effect of prednisone alone was better than expected.[94] A new trial comparing tacrolimus with placebo for patients with an insufficient response to glucocorticoids is in progress (NCT01325571). Tacrolimus has an additional effect on ryanodine receptor-mediated calcium release from the sarcoplasmic reticulum, which theoretically could lead to improvements in muscle strength in patients with myasthenia gravis.

Thymectomy

Many studies have reported a substantial effect of thymectomy in myasthenia gravis. These studies have included control groups, but prospective and randomised studies have not been done.[1–3,7,95,96] For early-onset myasthenia gravis, we recommend a thymectomy early after symptom onset. All thymus tissue needs to be removed. Video-assisted thoracoscopic and robotic-assisted methods are well established, used by an increasing number of centres, and are usually preferred by patients.[97] Thymectomy can be safe for juvenile myasthenia gravis, down to an age of about 5 years.[98] Improvement in response to thymectomy occurs gradually after some months, and according to follow-up studies, continues for up to 2 years postoperatively.[95] No other autoimmune disorders have been shown to improve after thymectomy. Thymectomy should be undertaken as an oncological intervention when a thymoma is detected or is strongly suspected to avoid local compression and spread to the thoracic cavity. Any positive effect on myasthenia gravis is more unpredictable for the thymoma than for the early-onset subgroup.

Use of thymectomy in late-onset myasthenia gravis is debated. For patients with late-onset disease with an atrophic thymus or onset at age 60–65 years or older, thymectomy is not recommended because no convincing data support surgery for this group. However, some guidelines[7] recommend treating young patients (up to age 60–65 years) with late-onset disease who have an enlarged thymus on imaging and no antibodies to muscle titin or the ryanodine receptor, similar to patients with early-onset myasthenia gravis. For younger patients with late-onset myasthenia gravis, the thymus is most probably involved in the pathogenesis and the response to thymectomy would be expected to be similar to that for early-onset disease.

Thymectomy is not recommended for patients with MUSK, LRP4, or ocular forms of myasthenia gravis as no therapeutic effect has been shown. For patients with generalised myasthenia gravis and low-affinity AChR antibodies, thymus hyperplasia is usually impossible to establish by imaging. Such patients would be expected to respond to thymectomy but cannot be distinguished from other patients with myasthenia gravis who are found to be antibody negative.

Thymectomy should be done early, but is never an emergency; patients should be in a stable condition. Intravenous immunoglobulin or plasma exchange immediately before surgery will improve the myasthenia gravis symptoms, reduce the risk of complications, and contribute to a faster recovery.

Supportive treatment

Physical activity and low intensity and medium intensity training provide short-term and long-term benefits for patients with myasthenia gravis. Weakness increases with repetitive muscle use, but patients with myasthenia gravis can still find activities for which they can adjust intensity and duration to increase their long-term physical ability. Rest after such exercise is needed. No controlled studies of myasthenia gravis training programmes have been published.

Bodyweight control is important, as for other disorders with muscle weakness. Such control is especially relevant in patients with involvement of respiratory muscles. Infections in patients with myasthenia gravis should be treated early and vigorously because they can lead to myasthenia gravis exacerbation and add to respiratory impairment.[1–4,7]

Drugs that interfere negatively with neuromuscular transmission should be avoided. D-penicillamine and telithromycin should not be given to patients with myasthenia gravis, and fluoroquinolones, aminoglycosides, macrolides, and neuromuscular blocking drugs will often cause worsening of the disease. Neuromuscular blockade should be used with care during anaesthesia. Sedatives that could suppress respiration should be avoided in the treatment of patients with severe myasthenia gravis. If a patient deteriorates when given a new drug, this drug should be withdrawn. However, most patients with myasthenia gravis with mild-to-moderate disease, or in stable remission, tolerate drugs that have a relative warning, and most drugs can be used with caution.

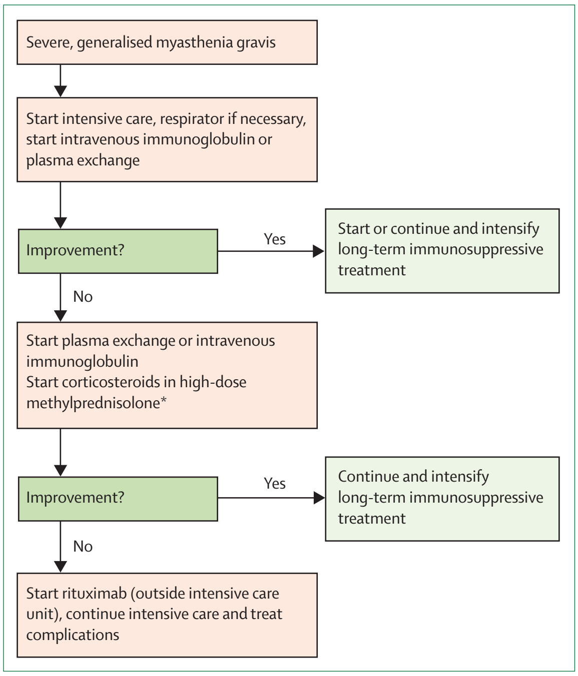

Figure 5: **Treatment of severe myasthenia gravis exacerbations**
*1000 mg a day for 3 days.

Treatment of myasthenia gravis crisis

Crisis is defined as a need for intubation for respiratory support caused by muscle weakness related to the disease. Treatment includes intensive care with respiratory support, treatment of infections, and monitoring of vital functions and mobilisation (figure 5). Intravenous immunoglobulin and plasma exchange are specific immunosuppressive treatments with a rapid effect occurring after 2–5 days, and either one should be given to patients with severe myasthenia gravis exacerbations and always for crisis.[99–103] These two treatment alternatives are equally effective, and can be given in sequence if necessary, as patients can respond to one but not to the other. Standard protocols include treatment for 3–6 consecutive days. Intravenous immunoglobulin is often slightly more convenient and with a lower risk of severe side-effects, whereas plasma exchange might have a slightly faster effect. Catheter placement procedures for plasma exchange can be complex because access to large veins is necessary. The treatment effect is usually restricted to 2–3 months, owing to continuing antibody synthesis. Plasma exchange and intravenous immunoglobulin can be repeated when the effect tapers off. To secure long-term improvement, this treatment is usually combined with standard immunosuppressive drugs, in higher doses than before the crisis or with add-on drugs. In patients with an acute exacerbation that does not respond to intravenous immunoglobulin or plasma exchange, corticosteroids in high doses can be tried. Myasthenia gravis crisis is a reversible condition. Sometimes the treatment response is delayed, but intensive care and vigorous immunosuppression should be continued for as long as necessary, sometimes for several weeks.

Treatment of myasthenia gravis in pregnancy

Pregnancy does not affect myasthenia gravis in any consistent way, with no increased risk of severe deterioration or myasthenia gravis crisis.[85,104,105] During the first weeks and few months post partum, the risk of symptom worsening is moderately increased, mainly because of stress and new demands.

Pyridostigmine and corticosteroids are regarded as safe treatments for pregnant women.[85] These drugs do not increase the risk of fetal malformations or delayed fetal development. Plasma exchange and intravenous immunoglobulin can be used safely for exacerbations in pregnancy, and also as preparation for women giving birth. Evidence for potential teratogenic effects of other immunosuppressive drugs is sparse. However, caution is recommended for use of these drugs, and the manufacturers of immunosuppressive drugs generally advise against their use in pregnancy. Azathioprine has been widely used for many years by young women with AChR, MUSK, or LRP4 forms of myasthenia gravis. The general view is that this drug has very low, if any, increased teratogenic risk.[85] Lactation should be encouraged in patients with myasthenia gravis, also for women on immunosuppressive drugs,[105] but the passage of some medications into breastmilk should be taken into account. Mycophenolate mofetil and methotrexate have teratogenic potential. Methotrexate might also reduce female fertility. These two drugs should only rarely be used in young women, and not in pregnancy.

Most female patients with myasthenia gravis give birth in an uncomplicated way. Apart from the risk of neonatal myasthenia gravis, no precautions are usually needed. Caesarean section is not recommended as a routine for these women, but should be considered in prolonged births for women with moderate or severe generalised myasthenia gravis because of muscle fatigue.

Treatment of neonatal myasthenia gravis

Neonatal myasthenia gravis occurs in 10–15% of babies of mothers with the disease. The cause of this transient muscular weakness in these babies is transfer of the mother's AChR or MUSK antibodies of the IgG class across the placenta. This weakness usually lasts for only days or a few weeks and is typically mild but can interfere with feeding and respiration. Mothers with myasthenia gravis should always give birth at hospitals experienced in respiratory support treatment for newborn babies. The fact that neonatal myasthenia gravis does not occur in all babies and that occurrence in babies is not correlated with maternal disease severity or AChR antibody concentration might be explained by variation in AChR epitopes, epitope-binding affinity, and non-AChR factors.[105]

Transplacental AChR antibodies can, in rare cases, produce arthrogryposis due to severe intrauterine movement inhibition. Such skeletal malformations were reported in three of 127 babies in an unselected national cohort.[106] Arthrogryposis, AChR-antibody induced stillbirths, and repeated spontaneous abortions can be avoided by intravenous immunoglobulin infusions or plasma exchange before and during pregnancy. This treatment should be given in female patients with myasthenia gravis who have already experienced such a pregnancy outcome.

Conclusions and future directions

Most patients with myasthenia gravis do well and have well controlled disease. However, most need long-term and often life-long drug treatment with acetylcholinesterase inhibitors and usually low-dose immunosuppression. Pathogenic autoantibodies are well characterised and myasthenia gravis subgroups are defined accordingly. However, treatment is far from antibody specific and is not even specific to the disease subgroup. Many new and more traditional drugs that have not been tested properly in myasthenia gravis have modes of action that are expected to suppress autoantibody production directly or indirectly, and therefore might benefit patients with myasthenia gravis. For patients with severe symptoms that do not respond sufficiently to standard treatment, with a diagnosis confirmed by the presence of autoantibodies and no comorbidity as the symptom cause, such drugs could be tried, off-label, and with strict monitoring. These include monoclonal antibody drugs with a proven effect for other autoimmune disorders. Complement inhibition is one of several potential strategies,[76] with a focus on several factors in the complement system. Eculizumab, belimumab, leflunomide, and etanercept are drugs that might have the potential to become new myasthenia gravis treatment options,[76,107–109] although some immunoactive drugs can precipitate or worsen myasthenia gravis.[110] Tirasemtiv (CK-2017357) selectively sensitises fast skeletal muscle to calcium by binding to its troponin complex and amplifies the muscle response when neural input is diminished secondary to neuromuscular disease.[111] A dose-related, short-term improvement was reported in a phase 2a randomised placebo-controlled trial.[77] Any functionally relevant long-term benefit to patients is still to be proven. Several non-antibody factors linked to the immune system and skeletal muscle affect the individual's muscle strength and immune responses, and thereby each patient's myasthenia gravis manifestations.

The high number of factors associated with muscle function in myasthenia gravis should drive future research towards an individually adapted treatment approach based on biomarker (autoantibody) assessment and monitoring. The aim should be to suppress the anti-AChR, anti-MUSK, or anti-LRP4 immune response without affecting other immune reactions. An alternative approach could be treatment that promotes tolerance to the antigens (AChR, MUSK, and LRP4) that induce myasthenia gravis.[112] Patients with myasthenia gravis without detectable antibodies probably have pathogenic antibodies against undefined antigens in the neuromuscular junction; many proteins affect AChR function, synthesis, and maintenance that could potentially underlie antibody-negative disease. Autoimmune myasthenia gravis with a T-cell-mediated and non-antibody mechanism affecting neuromuscular transmission could theoretically exist.

When the causes of myasthenia gravis can be identified, they might be possible to avoid or prevent, potentially, for example, by vaccination. Until antigen-specific treatment is available, however, research efforts should target new

Search strategy and selection criteria

We searched MEDLINE and the Cochrane Library with the terms "myasthenia gravis", "myasthenic syndromes", and "myasthenia" from January 1995, to April, 2015. Guideline and review papers were assessed in detail, and controlled studies sought for in particular. Papers were selected by title and abstract. Only papers in English were included. Randomised trials on established and emerging therapies for myasthenia gravis are often scarce, so our recommendations are based on the best available evidence or clinical experience, where stated.

immunosuppressive drugs and drug combinations for the myasthenia gravis subgroups. Prospective and controlled studies should be encouraged and supported. Severe myasthenia gravis is a reversible disorder that should be treated with intensity and optimism.

Contributors

NEG planned the Review and wrote the first draft. JJV edited and rewrote the first draft. Both authors searched primary sources for information, produced tables and figures, and finalised the text.

Declaration of interests

NEG has received speaker's honorarium from Octapharma, Baxter, and Merck Serono. JJV is a partner in an FP7 European grant that is associated with Curavac. The Department of Neurology at Leiden University Medical Center has received fees from BioMarin for JJV's consultancy work and royalties from antibody tests. JJV received research grants from Prinses Beatrix Spierfonds and National Institutes of Health.

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