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# Autoimmunity at the neuromuscular synapse: pathophysiology and disease course

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# PART I

# Lambert-Eaton myasthenic syndrome

# CHAPTER 1

# Introduction and aims

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Based on:
Lambert Eaton-myasthenic syndrome.
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## Introduction

Lambert-Eaton myasthenic syndrome (LEMS) is a rare autoimmune disease characterised by proximal muscle weakness, loss of tendon reflexes and autonomic dysfunction.[1] Autoantibodies against P/Q-type voltage-gated calcium channels (P/Q-type VGCC, also classified as $Ca_v2.1$), located presynaptically on the neuromuscular junction are presumed to be pathogenic.[2;3] Because of the rare nature of the disease, an initial misdiagnosis is common, if recognized however multiple effective treatment options are available.[4] Once a LEMS diagnosis is considered, a diagnosis be confirmed relatively easy using serology (VGCC antibodies) and repetitive nerve stimulation.[4] Another important implication after diagnosis is the presence of small cell lung cancer (SCLC) in about 50-60% of patients.[1;5;6] Diagnosis of LEMS usually precedes diagnosis of SCLC by several months, thus prompting vigorous tumour screening.[7;8]

The first clinical description of this syndrome characterized a male patient with progressive muscle weakness and small cell lung cancer, showing marked improvement after removal of the tumour.[9] Lambert, Eaton and Rooke subsequently reported the first case series with typical clinical manifestations and specific electrophysiological abnormalities in these patients.[10] Further reports showed a strong association of LEMS with small cell lung cancer in a majority of patients.[1;11] This association was elucidated by the presence of VGCC antigens in SCLC cell lines and inhibition of $Ca^{2+}$ flux in these cells upon exposure to IgG from LEMS patients.[12;13] These findings suggest immunization by the tumour as the cause of the disease in patients with both SCLC and LEMS.

## Epidemiology

LEMS is a rare disorder that can occur at all ages and affects both men and women. A bimodal distribution of age at onset in non-tumour associated LEMS can be observed, as is the case in myasthenia gravis. A first peak in the prevalence is observed around 35 years of mostly female patients and a second, higher peak is seen around an age of 60 years. The youngest patients with a SCLC are around 35, and their frequency increases with age. Almost all are smoking, and predominantly male patients.[14]

In an epidemiological study from the Netherlands, a yearly incidence of 0.75 per million and a prevalence of 3.42 per million is reported, which seems to increase due to improved recognition of this rare disease.[4;15;16] Large studies in SCLC patients suggest that 1-3% of these patients have LEMS, which would result in a higher incidence and suggest that careful evaluation of SCLC patients might reveal more cases of this treatable disease.[17;18]

Pediatric cases of LEMS have also been described, starting at an age of 9 years.[19;20] These patients seem to have comparable symptoms and signs, also presenting with proximal leg weakness and frequently showing typical electrophysiological abnormalities and VGCC antibodies.

Immunogenetic factors could have an important role in development of LEMS in patients without an associated tumour. An increased frequency of other autoimmune diseases is found in these patients and their families.[1;21] Production of certain cytokines in family members of these patients has been reported to differ from the healthy population as well, which might confer susceptibility for the disease.[22] A significant association has been described with the HLA B8-DR3 haplotype, especially in young female patients.[23;24] This HLA 8.1 ancestral haplotype is present in about 10-20% of the healthy population in Western countries and is associated with 17 autoimmune diseases, also including myasthenia gravis.[25;26] Of note, the frequency of HLA B8-DR3 in female patients with early onset of both MG and LEMS are comparable, suggesting a common genetic pathway for predisposition to these related autoimmune diseases.[14]

## Pathophysiology

About 90% of LEMS patients have autoantibodies against presynaptic P/Q-type (Cav2.1) voltage gated calcium channels, which are presumed to be pathogenic.[5;27;28] The target of these IgG antibodies, the P/Q-type VGCC, is present at the neuromuscular junction and autonomic nervous system as well as in SCLC tumour cells.[12;29] At the neuromuscular junction, this protein complex is necessary for $Ca^{2+}$-dependent neurotransmitter release (Figure 1).

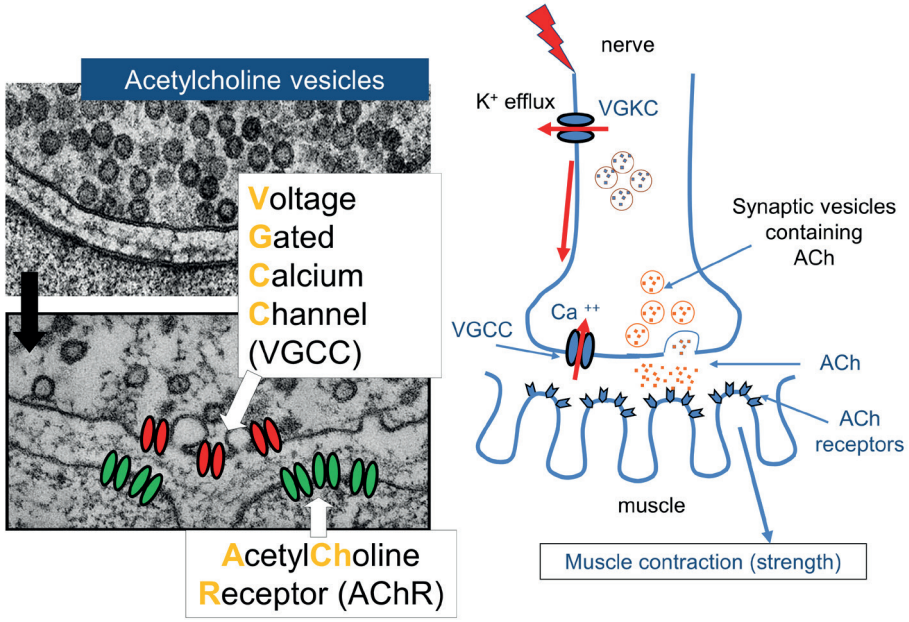


**Figure 1. Pathophysiology of the Lambert-Eaton myasthenic syndrome.**

Depolarisation of the presynaptic nerve terminal results in fusion of synaptic vesicles with the membrane, followed by release of the neurotransmitter acetylcholine (ACh) from these vesicles. ACh consecutively binds to acetylcholine receptors, ultimately leading to contraction of the muscle fibre. In LEMS, antibodies to P/Q-type voltage gated calcium channels cause internalisation of these channels and therefore block calcium influx, leading to a decrease in release of ACh vesicles, which results in a decrease of ACh supply to the postsynaptic membrane. Voltage-gated potassium channels (VGKC) can be blocked by treatment with 3,4-diaminopyridine, which prolongs depolarisation presynaptically. This prolonged depolarisation increases Calcium influx, even though less functional calcium channels are available, resulting in improved ACh release and ultimately improving neuromuscular transmission and muscle strength.

Proof of an autoimmune origin of LEMS can be derived from presence of these antibodies, improvement upon antibody removal; and several models for passive and active transfer of disease. Passive transfer of LEMS IgG in mice results in decreased quantal content and other electrophysiological abnormalities compatible with a presynaptic neuromuscular transmission defect.[30;31] Animal models have shown active immunization with α1 subunits of P/Q-type VGCC can result in muscle weakness and electrophysiological features characteristic of LEMS.[32] Transfer of antibodies from affected mother to neonate has also been described and can result in transient neonatal weakness.[33;34] Removal of antibodies, most directly and effectively by plasma exchange can dramatically increase LEMS symptoms, which also supports a humoral factor to cause the disease.[35] Additionally, effect of immunosuppressive treatment such as prednisone and IVIG has been described in both seropositive and seronegative LEMS patients.[36;37]

Biopsies of intercostal muscles established the localisation of abnormalities in LEMS to be presynaptic, resulting in a decrease of acetylcholine (ACh) quanta (release packages) after a nerve impulse.[38;39] Freeze-fractured presynaptic membranes, as imaged by electron microscopy, from mice treated with purified LEMS IgG showed a depletion and aggregation of active zone particles, which are presumed to represent VGCCs.[3] VGCCs at the active zone of the neuromuscular junction are necessary for $Ca^{2+}$ influx after a nerve impulse, which facilitates release of the neurotransmitter ACh from synaptic vesicles. These active zone particles are normally arranged in double parallel rows 16-21 nm apart, close enough for both Fab arms of an antibody to bind two antigens at once, i.e. cross-linking. Passive transfer experiments have shown both LEMS IgG and divalent antibody fragments, but not monovalent (Fab) fragments, can affect neuromuscular transmission.[40] Taking these findings together, it is likely that IgG antibodies from LEMS patients cross-link P/Q-type VGCCs, leading to internalisation of these VGCCs necessary for ACh release. Only very recently, the pathogenic relevance of P/Q-type VGCCs has been shown in a more direct approach. LEMS IgG was shown to decrease action potential-evoked synaptic vesicle exocytosis in cultured neurons, measured by a fluorescent dye in synaptic vesicles.[2] No such effect was shown in knockout mice neurons lacking P/Q-type VGCCs, even in presence of patient antibodies against N-type VGCCs.

A direct blocking effect, or competitive binding of autoantibodies is less likely, since conductance of single channels remained intact following exposure to LEMS.[41] The

effects of LEMS IgG also seems to be independent of complement activation, because passive transfer experiments induce the same electrophysiological abnormalities in C5-deficient mice and after C3 depletion by cobra venom factor.[30;42] Apart from an apparent dysfunction in regulatory T cells, the role of the cellular immune response is mostly unknown.[43] A major role seems unlikely, since passive transfer of IgG is enough to induce disease in several models.

The several types of VGCCs present in neurons, skeletal and cardiac muscle have a common basic structure, consisting of multiple subunits, of which multiple isoforms exist.[28;44] The α1 subunit isoform determines the subtype of a calcium channel and contains the ion conducting pore and voltage sensor. Autoantibodies from LEMS patients are likely to target the α1A subunit, which confers the P/Q-type VGCC phenotype.[45] Linker regions between domains of this subunit are exposed extracellularly and are proposed to be the main immunogenic target.[46;47] Both immunoblots and cytological studies have shown that patient antibodies can also target other VGCC subunits, including other α1 isoforms and the intracellular β subunit.[48-50] The relevance of antibodies to these subunits for the pathophysiology or disease course remains unclear and might solely result from epitope spreading later in the disease course.

Patient antibodies have also been shown able to block Ca2+ influx through voltage-gated calcium channels in SCLC cell lines.[13] Presence of VGCCs in these tumour cells are likely to trigger or be part of an anti-tumour immune response, which ultimately leads to VGCC autoantibodies and muscle weakness since the exact same antigen is present in both tumour and motor nerve terminal. Cross-linking is relevant in the tumour as well, since divalent but not monovalent antibody fragments can also affect Ca2+ flux in SCLC cells.[40] These antibodies are also likely to cause autonomic dysfunction, since passive transfer of LEMS IgG was shown to impair neurotransmitter release from sympathetic and parasympathetic neurons.[29]

In LEMS patients without associated tumour, the trigger for the autoimmune response remains unclear. The HLA association and increased frequency of other autoimmune diseases in patients and their families suggest these patients are predisposed to development of autoimmune diseases in general.

Overall, the immunopathogenesis of LEMS has several overlapping features with myasthenia gravis.[51] In both autoimmune diseases, IgG autoantibodies are responsible for cross-linking and internalisation of cell surface protein complexes. Both can also occur in the same immunogenetic background, i.e. association with HLA 8.1 haplotype, and as a paraneoplastic phenomenon.[25]

The antigenic target in 10% of LEMS patients who are seronegative mostly remains unclear. Several other antibodies have been detected in both seropositive and seronegative LEMS patients, which do not necessarily have to be of pathogenic relevance, but could also result from epitope spreading during the course of the disease.[51] Autoantibodies to another presynaptic protein, synaptotagmin I, have been detected in some patients.[52] This protein is present in both SCLC cells as well as at presynaptic active zones, where it is implicated

in fast ACh release. Immunization of rats resulted in electrophysiological abnormalities comparable to those seen in LEMS models. Another candidate antigen against which antibodies are detected is the M1-type muscarinic acetylcholine receptor. This receptor is also present extracellular at the motor nerve terminal and could play a role as part a compensatory mechanism for the impaired $Ca^{2+}$ entry.[53] ERC1 (also known as ELKS) is another presynaptic active zone protein that has been reported as an antigen, which is however located intracellularly and therefore unlikely to be of pathogenic relevance.[51]



## Tumour Association

An associated tumour is found in about 50-60% of patients with LEMS. By far the most frequently associated tumour is small cell lung cancer.[1;5;6] SCLC accounts for approximately 15% of pulmonary tumours and belongs to the group of neuroendocrine lung tumours. It is an aggressive disease with a median survival of only 10 months.[54] SCLC is a very immunogenic tumour, resulting in an association with several paraneoplastic neurological autoimmune syndromes in SCLC patients.[55] Many of the SCLC-associated auto-antigens are expressed in the nervous system, constituting both intracellular and cell surface targets for an immune response.[56] A SCLC cell line derived from a LEMS patient has shown that several proteins involved in exocytosis in motor nerve terminals, including P/Q-type VGCCs, can be present in these tumour cells.[12] Exocytosis of transmitters such as serotonin in SCLC cells partly depends on VGCCs and can actually be influenced by co-incubation with LEMS serum.[57]

Besides SCLC, association with multiple other tumours have also been reported, although the association is less clear since some of these tumours could have arisen by chance. A neuroendocrine pathology or temporal relation of tumour activity with LEMS symptoms can strengthen the likelihood of association in individual cases. For non-small cell lung cancer, the pathological differentiation with SCLC can be difficult in some cases, with an overlap in expression of cell surface markers and other characteristics.[58] As a marked example of this overlap, VGCC expression has been reported in lung adenocarcinoma in two cases, suggesting NSCLC to be a likely trigger for the paraneoplastic syndrome in some patients.[59;60] Prostate carcinoma can also show neuroendocrine characteristics, making a direct association more likely.[61] Lymphoproliferative disorders are also reported to be associated, although most of the cases lack a temporal relation or supporting neuroendocrine pathology, therefore a causal relation is less certain.[62 4]

### Tumour screening

Diagnosis of LEMS usually precedes diagnosis of an associated SCLC (in 94% of cases).[8] The presence of an underlying SCLC causing LEMS is of uttermost importance and therefore discriminating SCLC-related LEMS from LEMS without tumour as well. In patients with associated SCLC, specific symptoms are no different from patients without associated tumour. However, a more progressive course of the disease has been described in patients with SCLC, in whom the same symptoms develop earlier in the course of the disease.[6;63] Smoking and weight loss are also related to SCLC-related LEMS. Antibodies against SOX, member of a family of developmental transcription proteins present in both the

developing nervous system and SCLC, are also highly specific for SCLC-related LEMS.[64-66] Combining all individual risk factors, the DELTA-P score was developed and validated to determine the risk of SCLC in an individual patient. It combines bulbar symptoms, erectile dysfunction, age at onset, smoking at onset of symptoms, weight loss and Karnofsky performance score, all within 3 months of disease onset with very high sensitivity and specificity.[67]

Regardless of the DELTA-P score, all patients should undergo tumour screening after LEMS diagnosis. Chest CT scans have been shown to be clearly superior for tumour detection compared to chest X-rays.[8] If negative, FDG-PET scan also has additional value for screening as is the case in other paraneoplastic neurological syndromes.[68] In case of an associated SCLC, the tumour is detected in 91% of patients within 3 months and in 96% within a year.[8] A time interval longer than 2 years has been mentioned in the literature, but is unlikely to occur with the current quality of screening modalities. In the authors' opinion, screening using chest CT and/or FDG-PET should be repeated at least once after 6 months in low risk patients (DELTA-P score of 0 or 1), and repeated every 6 months until 2 years after LEMS diagnosis in all others. High-risk patients (DELTA-P 3-6) should have repeated screening within 3 months after diagnosis as well, since the tumour is most likely to be detected in this period.[4]

**Tumour survival**

A profound improved survival has been observed for SCLC patients with LEMS, which may be due to the fact that the antibodies target an extracellular accessible antigen. Three studies report a significantly prolonged median survival of 17, 20 or 24 months in SCLC-LEMS patients, compared to 10 months in SCLC patients without this paraneoplastic disease.[17;18;69] Three year survival was improved from 2% to 33% in one study (Figure 2).[14] A paraneoplastic neurological syndrome known to be associated with tumours may lead to early screening and tumour detection while in fact it does not change the normal course of the cancer (lead-time bias). Alternatively, the paraneoplastic syndrome could be part of an ongoing anti-tumour immune response that is truly successful in retarding tumour growth. Supporting this concept are cases with anti-Hu syndrome, another paraneoplastic neurological disorder associated with SCLC, in whom spontaneous remission of proven lung tumours have been described.[70;71] However, a biochemical explanation for this survival advantage and relevance of either cellular of humoral immunity in this anti-tumour immune immune response remain to be elucidated.

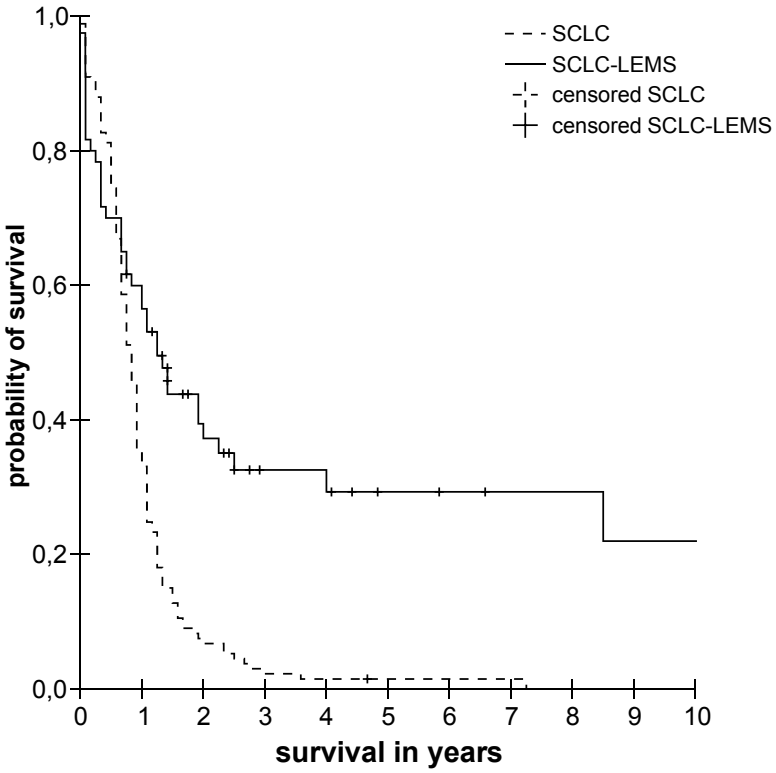


**Figure 2. Tumour survival**
Survival curve for LEMS patients with SCLC compared to SCLC patients without LEMS Censored cases of surviving patients or patients lost to follow-up are indicated by crosses. Based on reference Wirtz et al., 2005, reprinted with permission from Titulaer et al., 2008.[14;17]

## Clinical manifestations

The typical clinical triad of symptoms and signs consists of proximal muscle weakness, low or absent tendon reflexes and autonomic dysfunction.[1;4] Muscle weakness almost invariably starts in the upper legs, both severity and distribution of affected muscle groups frequently spread over the first months to years of the disease. As in myasthenia gravis (MG), weakness usually progresses to other muscle groups. These include the arms, feet and oculobulbar muscles causing ptosis, diplopia and dysarthria. However, in LEMS weakness generally spreads in caudocranial direction, while in myasthenia gravis it usually spreads in the opposite direction.[72]

Regardless of the specific muscle groups affected, fluctuations occur both over the day and between days to weeks. Many patients report an increase in weakness in the course of the day, after prolonged exercise and sometimes also worsening of symptoms in hot

weather or a hot bath.[1] Upon examination, augmentation of strength can be shown during the first few seconds of muscle contraction. After prolonged contraction, fatigue starts and weakness increases again. Also, discrepancies can be present between the reported functional impairment and limited objective weakness, which can contribute to misdiagnosis.

Frequency of symptoms at onset and during the disease course are presented in Table 1.

| Frequency of symptoms | At onset (< 3 months) | During disease course |
|---|---|---|
| **Limb Weakness** | | 100 |
| proximal leg | 93 | 100 |
| distal leg | 32 | 46 |
| proximal arm | 55 | 78 - 82 |
| distal arm | 29 | 54 |
| muscle pain or stiffness | 5 | 12 - 36 |
| **Bulbar weakness** | 39 | 70 |
| dysarthria | 31 | 24 - 64 |
| swallowing | 24 | 24 - 46 |
| chewing | 20 | 16 - 32 |
| neck | 22 | 14 - 39 |
| **Ocular weakness** | 35 | 57 |
| ptosis | 24 | 28 - 46 |
| diplopia | 26 | 5 - 50 |
| **Autonomic** | | 80 |
| dry mouth | 56 | 31 - 78 |
| dry eyes | 19 | 29 - 36 |
| constipation | 14 | 11 - 30 |
| male impotence | 56 | 4 - 65 |
| miction difficulties | 13 | 29 |
| blurred vision | | 3 - 10 |
| impaired sweating | 5 | 4 - 7 |
| **Respiratory failure** | rare cases | 5 - 11 |
| **Cerebellar ataxia** | 6 | 9 |

**Table 1. Frequency (in %) of symptoms at onset and during the entire disease course.** Estimated frequencies and variation are based on a single large study for symptoms at onset; and for multiple large case series and reviews for the entire disease course.[1;5-7;67;78]

## Limb weakness

Muscle weakness usually starts in the proximal leg muscles, resulting in difficulty climbing stairs or walking uphill as a presenting symptom in many patients.[1;6] As in myasthenia gravis, muscle weakness can be fluctuating, usually worsening as the day progresses. Weakness in proximal arm muscles usually follows in the first year after onset, distal muscles are less frequently involved.[6] Limb weakness is usually symmetrical, although minor asymmetry is possible. In our experience, the most frequently involved muscle

groups include the iliopsoas, quadriceps, leg abductors and gluteus muscles in the legs and deltoid muscles in the arms, although this has not been formally studied.

Although a sensation of fatigue in the affected limb muscles is common, muscle ache or stiffness is usually not a frequent or predominant symptom.



**Oculobulbar muscle weakness**

Both the extra-ocular muscles (resulting in ptosis, diplopia) and bulbar muscles (speech, swallowing, chewing) can be involved in LEMS, as is the case in MG. Ptosis can be more symmetrical as compared to MG (personal observations). Oculobulbar muscles are usually affected to a milder degree than in MG and usually appear later in the disease course, but otherwise follow a comparable pattern of symptoms.[1;72] Prominent bulbar weakness however should alert the clinician for the possibility of respiratory muscle weakness.

**Respiratory muscle weakness**

As in myasthenia gravis, respiratory muscles can be affected, ranging from mild diaphragmatic weakness detected by respiratory pressure measurements to respiratory failure requiring ventilation.[73] In several of the ventilated patients, respiratory muscle weakness was provoked by muscle relaxant drug or general anesthesia.[1;74] In rare patients, spontaneous respiratory failure can be the presenting symptom, usually following a short history of undiagnosed generalized weakness.[75;76] Respiratory weakness can respond well to both symptomatic treatment and immunosuppression.[75-77]

**Autonomic dysfunction**

The most common autonomic symptoms are impotence (for men) and dry mouth, occurring in up to 60 and 80% of patients respectively.[1;78] Other frequent symptoms include dry eyes, constipation and micturition problems. Even in cases in whom symptoms are not directly reported, autonomic function testing can show abnormalities in almost all patients.[79] Orthostatic hypotension can occur but is not as frequent or debilitating as in other neurological diseases with autonomic dysfunction.

**Low or absent tendon reflexes**

LEMS patients frequently show decreased or absent tendon reflexes, initially often limited to the lower limbs.[1;5] After voluntary contraction, tendon reflexes can reappear for a short while. This phenomenon is called facilitation and is present in a minority of patients, but still useful since it is pathognomonic for LEMS.[80] Facilitation is also relevant for the neurological examination, in which tendon reflexes should be tested after a short period of rest to prevent masking of lowered tendon reflexes. Although less frequently studied, decreased reflexes seem to be present in the autonomic nervous system as well, as recorded by abnormal pupillary responses to light in an minority of patients.[81;82]

**Other symptoms**

Cerebellar ataxia can also be seen in a small portion of LEMS patients, especially in cases with associated small cell lung cancer.[1;6] Antibodies to P/Q-type VGCC can however also be present in SCLC patients with paraneoplastic cerebellar degeneration but without LEMS (Mason 1997 Brain); and can cause ataxia in an mouse model.[83;84] Therefore, it

remains unclear whether this is a LEMS-specific symptom or an overlap with another paraneoplastic neurological disease.

Weight loss can also be an early symptom, but is mostly present in tumour cases and therefore less likely to result from LEMS itself.[4]

**Disease course**
Most patients report a fluctuating but progressive course of the disease over the first few months or years of the disease, up to diagnosis and treatment. Upon increasing severity, distribution of affected muscle groups can spread to more distal muscles, oculobulbar and respiratory muscles. Severity of weakness can fluctuate, as in patients with myasthenia gravis.[1;4] As in other autoimmune diseases, infections can temporarily aggravate symptoms, usually for a few days to weeks. After start of treatment, the disease is usually more stable.

In a long term follow-up study of LEMS patients without a tumour, a variable prognosis was reported.[85] Sustained clinical remission was achieved in 43%, mostly with immunosuppression. About a quarter of patients remained (at least partly) wheelchair-dependent at follow-up, despite adequate treatment including immunosuppression. In patients with associated SCLC, survival is dependent on the tumour, although prompt tumour treatment can greatly improve LEMS symptoms.[86]

## Electrophysiology

**Repetitive nerve stimulation**
Repetitive nerve stimulation (RNS) in LEMS patients typically shows a triad of abnormalities (Figure 3)[87-89]:

- Low initial CMAP amplitude
- Abnormal decrement (>10% ) upon low rate RNS
- Abnormal increment (>60%) upon high rate RNS or directly after voluntary contraction.

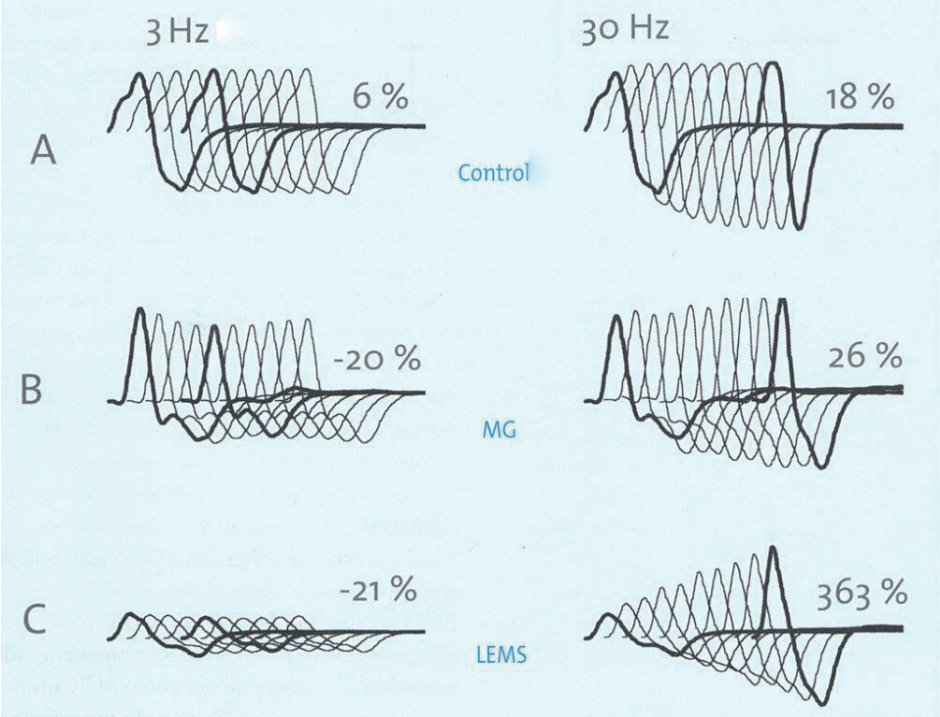


**Figure 3. Repetitive nerve stimulation**

Results for repetitive nerve stimulation studies in the hypothenar muscle are presented at low rate (3 Hz) and high rate (30 Hz) stimulation. Representative CMAP amplitudes after trains of 10 stimuli are shown for a healthy control, a patient with MG with acetylcholine receptor antibodies and in a patient with LEMS. Percentages represent changes from baseline CMAP amplitude. Abnormal decrement (> 10%) is present in both MG and LEMS. Significant increment (> 60%) after high rate repetitive nerve stimulation and after voluntary contraction can only be found in patients with LEMS, in MG and healthy subjects at maximum a low percentage (<60%) of increment can be detected. (Courtesy to prof. Gert van Dijk for providing these figures).

The underlying mechanism for this triad of abnormalities can be deduced from the pathophysiology. The low initial CMAP amplitude, in response to a single supramaximal nerve stimulus, represents the basic presynaptic defect of ACh release, resulting from decreased $Ca^{2+}$ influx through VGCCs. At low rate repetitive nerve stimulation, the CMAP amplitude decreases (decrement) as ACh release is reduced further because of depletion of immediately available presynaptic ACh vesicles, resulting in a decrease of responding muscle fibres. High rate stimulation and voluntary contraction increase $Ca^{2+}$ at the motor nerve terminal, which enables increased ACh release, resulting in an increase of the CMAP amplitude (increment).

Decrement can be found in 94-98% of patients and can usually be detected in multiple muscles.[88;89] Since even clinically not affected muscles can show decrement, the choice of muscle is not as critical as in MG and testing of hypothenar muscles, requiring the least skill, is often sufficient. Increment after high rate RNS (30-50 Hz) or 10 to 30 seconds of maximal voluntary contraction is a slightly less frequent finding (85-96%) in LEMS patients, but highly specific if performed adequately.[1;88;89] Abnormal increment has historically been defined as an increase of 100% from baseline.[90] The authors support the more recently suggested change to a cut-off of 60% increment, as this increases sensitivity without losing much on specificity, in both a previous study and our own observations.[88] The increase of the CMAP amplitude is in fact a recovery of the decreased CMAP amplitude. The increment is commonly expressed in percentages of the initial CMAP, whereby a threefold increase from 2 to 6 mV is reported as "200% increment". However, the CMAP can never exceed the normal values for the muscle that is being tested. Thus, an increment of 200% implies that the original CMAP was only one-third or lower of the normal value for that muscle.

The curve for the consecutive CMAP amplitudes upon low rate RNS also differs from the curve in MG and can therefore be helpful in the distinction between the two diseases.[91;92] In MG, the curve usually drops to a minimum around the 5th stimulus, while the amplitude continues to drop up to the end of the (usual) train of 10 stimuli in LEMS (Figure 3).[93] After the initial facilitation of CMAP amplitude after exercise, a worsening decremental response can occur a few minutes later, which is known as post-exercise exhaustion.[94] This phenomenon is of limited diagnostic importance in LEMS, but might explain part of the electropathophysiology. Since patients can also report an initial short increase in strength, followed by increasing weakness in minutes, post-exercise exhaustion might come closest to explaining the easy fatigability in LEMS patients.

**Single fibre electromyography**

Single fibre electromyography (SFEMG) shows increased jitter values and blocking in LEMS, at a rate usually exceeding abnormalities found in MG.[95;96] Various jitter parameters have also been shown to improve with clinical improvement.[95] However, few studies have characterised specificity of SFMEG and the ability to distinguish between LEMS and other myasthenic syndromes, which is theoretically possible but remains difficult.[96] For these reasons, as well as the special training required for adequate SFEMG and the high sensitivity of abnormal decrement, RNS remains the electrophysiological investigation of choice.[87]

## Serology

Antibodies to P/Q-type VGCC are detected in 85-90% of patients, this percentage is even higher in LEMS patients with associated SCLC.[5;27;28] The most frequently used diagnostic assay is based on immunoprecipitation of VGCC antibodies in patients' sera with solubilised VGCCs, extracted from mammalian brain tissue and complexed with $^{125}$I-labelled ω-conotoxin MVIIC, a toxin from cone snails specific for this type of VGCC.[27;28]

Although the results are reasonably specific for LEMS when using a relevant patient selection with clinical suspicion of LEMS, these antibodies can also be found in lower titres in up to 5-8% of SCLC patients, mostly without corresponding symptoms.[17;97;98] These antibodies can also be present in serum and CSF of patients with paraneoplastic cerebellar degeneration, usually also related with SCLC.[83] The overlap in associated antibodies is likely to be related to the presence of the same P/Q-type VGCC subtype in the neuromuscular junction as well as the cerebellum.



N-type VGCC have also been reported in 30-40% of LEMS patients, mostly in patients who also have P/Q-type VGCC.[99;100] N-type channels do not normally play a role in neuromuscular junction, but are relevant for the autonomic nervous system also affected in LEMS.[78] A specific pathogenic relation between these antibodies and autonomic symptoms has however not been described as of yet.

In rare LEMS patients, antibodies to acetylcholine receptors can be found. These antibodies can occur in typical LEMS patients (low reflexes, autonomic dysfunction and increment), possibly occurring as a result of epitope spreading.[101] Presence of these antibodies or conflicting electrophysiology results sometimes leads to a diagnosis of MG-LEMS overlap syndrome, although in the view of the authors and others, frequently a preferential diagnosis can be made based on initial and predominant symptoms.[101;102]

## Diagnosis

A diagnosis of Lambert-Eaton myasthenic syndrome is based on:[4]

- Typical clinical features of fatigable muscle weakness starting predominantly in the legs, low or absent reflexes and autonomic dysfunction; and
- At least 1 abnormal additional investigation:
- Serology: presence of VGCC antibodies
- Electrophysiology: presence of at least 10% decrement upon low rate repetitive nerve stimulation and increment of 60% at high rate stimulation or directly after 10-30 seconds of voluntary contraction.

Because of the rare nature of the disease, about 50 - 60% of patients are initially misdiagnosed.[4] MG is the most common initial diagnosis in these patients. Clinically, the initial complaints can help to distinguish the various myasthenic syndromes.[72] In LEMS, muscle weakness usually starts in the legs and spreads in cranial direction, while MG usually manifests in the extra-ocular or bulbar muscles and spreads in the opposite direction. Absence of tendon reflexes and autonomic dysfunction are also incompatible with a diagnosis of MG. Both RNS and serology can easily distinguish the two myasthenic syndromes in most cases, as described above. Therefore, VGCC antibodies and increment testing should be considered in all patients with perceived limb girdle MG.

Alternative causes of proximal muscle weakness include inflammatory myopathies, such as inclusion body myositis and polymyositis. Especially in patients with lung cancer, muscle weakness can also result from tumour-associated cachectic myopathy, which can result in marked muscle atrophy.[103] Muscle atrophy is reported to be present in the majority of lung cancer patients, although usually asymptomatic. The apparent effect of the lung cancer on muscle atrophy is usually more evenly distributed between proximal and distal muscle groups. Other misdiagnoses include genetic and toxic neuromuscular junction disorders.[104] Congenital myasthenic syndromes usually present at an earlier age, with negative serology and no autonomic dysfunction. Botulism can present with a comparable distribution of weakness, autonomic dysfunction and low tendon reflexes as well, but should mainly be suspected in patients with an acute onset, gastrointestinal symptoms and upon clustering of cases.[105;106] In cases with only mild or no objective weakness upon investigation, functional or psychogenic disorders may also be suspected at first.

Although an overlap can occur with other SCLC-associated paraneoplastic disorders such as sensory neuropathy with Hu antibodies, or paraneoplastic cerebellar degeneration, this rarely results in diagnostic difficulty.[83;107]

## Treatment

Several treatment options are available for LEMS in spite of the rare nature of the disease, probably as a result of the detailed understanding of the pathophysiology. If treated, almost all symptoms can be reversible and controllable. Treatment options can be divided in three groups: tumour treatment, symptomatic drugs and immunosuppression.[37;108;109]

### Symptomatic treatment

Multiple treatment options are available that either directly increase neurotransmitter release from the nerve terminal, or prolong the action of acetylcholine in the neuromuscular synapse. These include cholinesterase inhibitors, 3,4-diaminopyridine (3,4-DAP, also known as amifampridine when in a phosphate salt preparation), 4-aminopyridine (4-AP) and guanidine.[37]

For first line treatment in all LEMS patients 3,4-diaminopyridine is preferred above 4-aminopyridine, because of a more favourable side-effect profile . Both aminopyridines (4-AP and 3,4-DAP) block presynaptic potassium channels, prolonging the duration of nerve action potentials, which ultimately results in an increase in transmitter release. Four randomised controlled trials (RCTs) comparing different dosing schedules (10 mg i.v. to 100 mg orally /day) of 3,4-DAP to placebo have been performed and analysed in a recent Cochrane review.[108;110-112] All four trials reported a significant improvement in muscle strength as well as CMAP amplitudes.[108] Two trials measuring QMG score as a standardised measurement for myasthenic weakness report a significant mean improvement of 2.4 points (scale 0 – 39).[111;113]

Side effects of 3,4-DAP are generally mild, with most patients reporting distal or perioral paresthesia and less common mild gastrointestinal symptoms.[114] Epileptic seizures have been reported in one patient using 100mg daily in one of the trials, which did not recur at a lower dose.[110] Other patients with seizures were either treated with doses > 100mg daily, had brain metastases or other seizure-inducing medication.[111;115] Cardiac arrhythmia has also been reported as a rare side effect, mainly consisting of palpitations or premature ventricular contractions, but seem uncommon in clinical practice at a normal dose (personal observations).[116] Also, in a large observational study of mostly MS patients using 3,4-DAP no serious cardiac disorders with a likely or possible link have been recorded.[117]

4-aminopyridine is likely to have comparable effects as 3,4-DAP, but is limited in its use because of higher frequency and severity of side effects.

Cholinesterase inhibitors alone usually do not or only minimally improve symptoms.[37] Several patients report an additional effect on muscle strength or fatigability when combined with 3,4-DAP (personal observations).[116] However, this was not confirmed in a randomised crossover study, which reported no significant effect on isometric muscle strength or CMAP amplitudes within 3 hours after intravenous administration.[112]

Guanidine has been used in the past, but has mostly been replaced by 3,4-diaminopyridine. No RCTs are available, a few case series reported a moderate effect.[89;118] Treatment with guanidine is mostly limited because of the side effects. Common side effects include mild gastrointestinal symptoms and paresthesias, more serious side effects are bone marrow suppression and renal failure.[37;119;120]

**Tumour treatment**

The presence of a tumour has important consequences for the treatment, as tumour treatment can decrease or even abolish symptoms. In the largest case series of patients with both SCLC and LEMS, 7 out of 11 patients treated for the tumour underwent sustained improvement and an additional 3 patients temporary improvement.[86] Although patients were also treated with various drugs, a temporal relation was present in most patients with tumour treatment and less obvious with medication.[86;121] The neurological condition of these patients may therefore never be the reason to refrain from aggressive treatment of the SCLC.

**Immunosuppression**

A marked clinical improvement has been reported in several studies after treatment with plasmapheresis or intravenous immunoglobulin (IVIg).[35;37;89] One RCT compared the effects of IVIg with placebo for 8 weeks following infusion and reported significant increase in limb strength, as well as a decrease in antibody titer.[36] No RCTs have been performed studying the effect of corticosteroids or azathioprine.[108] Most studies however report a modest effect of prednisone up to 60mg/day, with the effect starting to show in weeks to months.[37;122;123] Azathioprine is frequently started alongside corticosteroids to decrease the dose needed for the latter, as in MG, although a steroid-sparing effect has not been formally studied.

**Recommendations**

First line treatment in all LEMS patients is 3,4-DAP in a starting dose of about 10-15 mg daily.[37;114;116] By starting with one dose a day, the patient can experience the effect of the drug, the duration of the effect, as well as the side effects. Dosing can be increased based on efficacy and side effects, but should not exceed 100mg a day because of a risk of seizures and arrhythmia at higher doses.[114] Most patients need 3 to 5 doses a day. This also depends on the formulation of the drug that is locally available. Patients should be explained that this is a symptomatic treatment that does not affect the underlying immune response of the disease. They should be encouraged to develop their own schedules and adapt the frequency of the intake of the drug according to their daily activities. If immunosuppressive treatment is started and successful, it sometimes is possible to decrease or even stop the intake of 3,4-DAP. In patients with associated SCLC, prompt tumour treatment, which has a powerful immunosuppressive effect, can result in sustained improvement of symptoms and should be the mainstay of LEMS treatment aside from the symptomatic treatment.[86]

If no tumour is detected, use of immunosuppression should depend on severity of the disease. Long term treatment with prednisone and azathioprine should be considered for these patients, similar as in autoimmune myasthenia gravis. For acute treatment in case of exacerbation, both intravenous immunoglobulins and plasmapheresis seem to be effective, although these emergency treatments are rarely needed.

Some concern exists whether immunosuppression is desirable in case of concomitant SCLC, when diminishing the anti-tumour immune response as well could adversely affect tumour outcome. No evidence for this adverse effect has been reported as of yet; and the only study comparing SCLC-LEMS patients with and without immunosuppression suggests the median survival in these treated patients is at least as good.[86] In the authors' opinion, immunosuppressants should not be withheld for this reason in case of moderate or severe disability due to LEMS, if tumour or symptomatic treatment are not sufficient to reach an acceptable level of daily activities.

Autonomic symptoms can also respond to symptomatic or immunosuppressive treatment.[110;124] Other relevant treatment options include artificial tears for dry eyes and laxatives in case of constipation, which are easily overlooked but can be very helpful in some patients.

Patients should also be advised to mention their diagnosis in case of other treatment. L-type calcium-channel blockers have been reported to worsen muscle weakness in multiple LEMS patients.[125] Drugs that can worsen myasthenia gravis, such as beta blockers and aminoglycosides, are also likely to worsen symptoms in LEMS.[126] When undergoing anesthesia, neuromuscular blocking agents should be avoided, which can lead to prolonged muscle weakness.[9;127] The most relevant perioperative complication in a recent study of surgical patients with LEMS were respiratory complications (mostly prolonged mechanical ventilation), although most of the reported patients were not yet treated for LEMS at the time of surgery.[128]

Despite the rare nature of the disease, multiple additional treatment options are currently being studied. Calcium agonists seem to have a strong additional effect to 3,4-DAP in cell lines and passive transfer mouse models.[129] Proteasome inhibitors are also under study, with the hypothesis that these drugs could both modulate the immune response as well as induce apoptosis in human tumour cells.[130]



## Summary

Lambert-Eaton myasthenic syndrome is a rare autoimmune disease, which is probably under recognized. However, when considered by the clinician based on typical clinical manifestations (proximal muscle weakness, loss of tendon reflexes and autonomic dysfunction), a diagnosis is usually made relatively easy (see panel 1).[1;4] Pathogenic antibodies to P/Q-type VGCC antibodies can be found in about 90% of patients and the presence of decrement and increment upon repetitive nerve stimulation is also a highly sensitive diagnostic test. Rapid diagnosis is also important because of the association with SCLC in 50-60% of patients, which should lead to vigorous tumour screening after diagnosis. Treatment of the tumour as well as symptomatic treatment (3,4-DAP) and immunosuppression can effectively control symptoms in most patients.

Either an anti-tumour immune response or an immunogenetic predisposition seem to able to trigger an autoimmune response leading to LEMS, with the same clinical result. Still much can be learned on autoimmunity and paraneoplastic disease mechanisms from this rare but well-defined clinical entity. Understanding the triggers for eliciting the potent immune response against the VGCCs and discovering how we can manipulate the underlying mechanisms could greatly advance our knowledge about autoimmunity and tumour immunology in general.

## Panel: 1 - Diagnosis

Clinical

- proximal muscle weakness
- low or absent tendon reflexes
- autonomic dysfunction

Serology

- presence of P/Q-type VGCC antibodies

Electrophysiology

- low CMAP amplitude
- decrement at low rate repetitive nerve stimulation
- increment after voluntary exercise or high-rate repetitive nerve stimulation

*Required for diagnosis are typical clinical features (at least proximal muscle weakness), combined with serological and / or electrophysiological abnormalities.*

## Aims and outline of this thesis

This thesis addresses several pathophysiological and clinical aspects of both the Lambert-Eaton myasthenic syndrome and myasthenia gravis.

In chapter 2, the search for new LEMS associated SCLC markers is described, leading to the discovery of a new antigen that is associated with LEMS.



In chapter 3, a literature review of screening methods for SCLC in LEMS is presented, as well as the role of associated SOX1 antibodies in SCLC-LEMS patients.

In chapter 4 the long-term follow-up, functional impairment and quality of life in LEMS patients is described. It also reports the survival in LEMS patients, which we hypothesise to be close to normal in patients without associated tumour. An improved tumour survival has been described in SCLC-LEMS patients, which might also relate to a lead-time bias, i.e. earlier tumour detection due to neuromuscular weakness triggering intensive screening. We hypothesize that this tumour survival benefit still holds even after correction for SCLC tumour stage, and might be related to a relevant anti-tumour immune effect.

In chapter 5, the focus is on improving the diagnosis of LEMS. The hypothesis is that lowering the cut-off value for increment from 100% to 60% will increase sensitivity for diagnosis of this rare disease, without a negative effect on the specificity.

Chapter 6 addresses the relation between initial compound muscle action potential (CMAP) amplitude and disease severity in MG. A low CMAP amplitude is a well-known electrophysiological hallmark for LEMS. We hypothesize that the initial CMAP amplitude is also lower in severe MG, which might help our understanding of the mechanism of muscle weakness in these patients.

In chapter 7, the use of ephedrine as a symptomatic treatment in MG patients is described. We hypothesize that this drug might cause a relevant reduction in symptoms, which could help to postpone or delay the need for immunosuppressive treatment and therefore diminish related side effects.

Chapter 8 and 9 provide a summary and discussion of this thesis as well as future perspectives.

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