CHAPTER 1

Introduction and aims

based on

MJ Titulaer ¹, JJGM Verschuuren ¹
Lambert-Eaton myasthenic syndrome: tumor versus nontumor forms.
Ann N Y Acad Sci. 2008;1132:129-34

and

MJ Titulaer ¹, PW Wirtz ², AR Wintzen ¹, JJGM Verschuuren ¹
Lambert-Eaton myasthenic syndrome with pure ocular weakness.
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History

In 1672, Thomas Willis, Sedleian Professor of Natural Philosophy at Oxford, described a female patient with variable weakness of the limbs and tongue. She could use her arms freely in the morning, but had “spent all power before noon”. After speaking too much, she became “as mute as a fish”, which recovered over time.

“This has been interpreted as the first written description of myasthenia gravis (MG). He suspected that the weakness was caused by a defect or weakness of the ‘Animal Spirits’, which were transported in the blood into the muscle fibres. Inside the muscles, the Spirits were not able to conjugate with the ‘explosive Copula’ to make the muscle fibres contract. Although this hypothesis is not supported by the present data, Willis did suggest circulating factors being involved in myasthenic syndromes.

Veruntamen qui Spirituum inspexi laborantes, eos quoad possunt in morbus locales urgebant, mane primùm excitati firmiter gradiri, brachia hac illic jactare, aut pondus attenuore valuit, ante meridiem verò, Spirituum qui musculos influerant penus féré absumpsit, vix manum, aut pedem movere possunt. Curo jam nunc feminam prudentam, & probam, quae per pluris annos hujusmodi spuriae paralyti non tantum in membris sed etiam in lingua obvixit, haece per tempus quodam libere, & expeditè fatis loquitur, post fermones tamen longas, & hastia, & laboriosè prolatos, illico sicut piscis obmutescens, amplius ne gläque pro loqui potest, però nec nisi post horam unam, aut alteram vocis usum recuperaet.”

“Nevertheless, those labouring with a want of Spirits, who will exercise local motions, as well as they can, in the morning are able to wal k firmly, to fling about their Arms hither and thither, or to take up any heavy thing; before noon the flock of the Spirits being spent, which had flowed into the Muscles, they are scarce able to move Hand or Foot. At this time I have under my charge a prudent and a honest Woman, who for many years hath been obnoxious to this sort of spurious Palsy, not only in her Members, but also in her tongue; she for some time can speak freely and readily enough, but after she has spoke long, or hastily, or eagerly, she is not able to speak a word, but becomes as mute as a Fish, nor can she recover the use of her voice under an hour or two.”
In the second half of the 19th century, the era of many famous observing neurologists, the clinical picture of the disease called myasthenia gravis (from the Greek words μυς ‘muscle’ and ασθενεια ‘weakness’ and the Latin word gravis, meaning ‘serious’) was delineated, especially by German speaking neurologists. At that time it was called Erb-Goldflam-Oppenheim disease. In 1934, Mary Walker showed “physostigmine” to reverse the symptoms of myasthenia. In the same time, sir Henry Dale, Nobel laureate in 1936, described acetylcholine as the substance transmitting signals from nerve to muscle. He also showed how “physostigmine” allowed acetylcholine to accumulate, reversing symptoms in myasthenia gravis.

Anderson, Churchill-Davidson and Richardson described a 47-year old man with progressive muscle weakness in the legs in 1953. Subsequently, he developed weakness of the arms and neck, suffered from transient diplopia and had problems swallowing. He had overt dysarthria, proximal muscle weakness and diminished tendon reflexes. His symptoms were relieved by edrophonium and neostigmine. At the same time, a small cell lung carcinoma was discovered, The removal of which led to a dramatic symptomatic improvement. The authors proposed “a possible relation between carcinoma of the lung and myasthenia”. This case report is considered the first clinical description of the disease, that is currently known as the Lambert-Eaton myasthenic syndrome (LEMS).

In the following years, several reports were published reporting a possible association between lung carcinoma and neuromuscular disease. Although the symptoms resembled those of MG, several features were unusual. The most prominent early clinical symptom was proximal weakness of the legs as patients could not walk up and down stairs anymore. Three out of six patients had a dry mouth as a sign of autonomic dysfunction. All six had lowered or absent tendon reflexes. A carcinoma of the bronchus was found in four.

Two years later, in 1956, Lambert, Eaton and Rooke reported that those patients with both atypical myasthenia and lung carcinoma had a specific response to repeated nerve stimulation differing from the pattern seen in classical MG. They described six patients with proximal leg weakness, fatigability of weakness, decreased tendon reflexes and a malignant lung tumour. The electromyographic pattern consisted of a low amplitude of the compound muscle action potential (CMAP), a decline during repetitive nerve stimulation (RNS) at low-rates (1-10 Hz), but a progressive increase during high frequency stimulation or immediately following voluntary contraction (up till 10 times the initial amplitude). Today, the diagnosis of LEMS still rests on electromyographic criteria based on these findings.
Biopsies of intercostal muscles from LEMS patients\textsuperscript{10, 11} established the abnormality to be localised in the presynaptic part of the neuromuscular transmission, in contrast to MG in which the problem is located postsynaptically. Lambert and Rooke had already noticed that some patients with “this particular clinical weakness” did not develop a carcinoma.\textsuperscript{9} The co-occurrence of LEMS with other autoimmune diseases\textsuperscript{12} raised the suspicion of an autoimmune aetiology. Injection of immunoglobulins (IgG) from plasma of LEMS patients injected into mice led to both electrophysiological and morphological changes, characteristic for LEMS.\textsuperscript{13-17} Plasma exchange was able to relieve symptoms, producing short-term improvement.\textsuperscript{18} The discovery of antibodies to the P/Q-type voltage-gated calcium channels (VGCC) in 1989\textsuperscript{19-21} sealed the conclusion of LEMS being an autoimmune disorder.

The detection of VGCC antigens expressed by SCLC cell lines further suggests immunization by the tumour as a cause of the disease.\textsuperscript{22, 23}

**Diagnosis of LEMS**

The diagnosis is based on clinical features, antibody testing and electrophysiological studies. The typical clinical triad of LEMS consists of proximal muscle weakness, autonomic features and areflexia.\textsuperscript{24} While proximal muscle weakness is most prominent in the legs, mostly the arms are or will be involved quickly.\textsuperscript{25} The most frequent sign of autonomic dysfunction is a dry mouth; it can become manifest as sexual impotence or constipation as well.\textsuperscript{24, 25} Neurological examination of the patient may show depressed or absent tendon reflexes. A specific characteristic sign in LEMS patients is “post-exercise facilitation”, a short-lasting increase of tendon reflexes and muscle strength after muscle contraction. This phenomenon had already been mentioned in one of the first descriptions of LEMS, in which reflexes were “only obtainable on reinforcement.”\textsuperscript{6}

Antibodies are directed against P/Q-type voltage-gated calcium channels (VGCC) in the presynaptic nerve terminal.\textsuperscript{26} Such VGCC antibodies are found in around 90\% of patients.\textsuperscript{27}

Repetitive nerve stimulation (RNS) is the electrophysiological study of choice to investigate LEMS. Typically, the compound muscle action potential (CMAP) amplitude is low, becoming even lower at low-frequency stimulation (decrement) and increasing following high-frequency stimulation (increment). Active exercise may replace high frequency RNS, as this can be quite painful. As the low CMAP amplitude is abnormal, an incremental response should be regarded
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pathophysiologically as a return to the normal CMAP amplitude. Consequently, it would be more correct to express increment as the percentage blocking by comparing the largest CMAP amplitude, obtained after high frequency stimulation, to the expected normal CMAP value. This is however not a common way of reporting data of increment studies. Most RNS studies recommend stimulating the ulnar nerve, measuring the hypothenar muscles. A decrement of at least 10% and an incremental response over 100% (at least doubling the initial CMAP amplitude) is considered abnormal, although new data suggest 60% increment is specific for LEMS as well.

A diagnosis of LEMS is made by the combination of clinical symptoms and the presence of VGCC-antibodies or electromyographic changes, specific for LEMS.

Differential diagnosis

LEMS can be confused with other myasthenic syndromes. MG with antibodies against the acetylcholine receptor is more frequent and the most common alternative diagnosis. MG is also an autoimmune disorder as well with an impaired function of the neuromuscular transmission, characterized by variable weakness. Clinically, discrimination can be made easily in many patients. In MG 90% of patients start with ocular or bulbar symptoms, while in LEMS 95% of patients start with symptoms of the (lower) limbs. Generally, muscle weakness in MG tends to develop in a craniocaudal direction, and in the opposite direction in LEMS.

Still, debate exists about the frequency of oculobulbar symptoms in LEMS. The prevalence of ocular symptoms in LEMS in the literature shows a wide range (0-65%). However, this is most probably due to observer bias. Wirtz et al studied the initial symptoms at disease onset, whereas Burns described the symptoms present at diagnosis (median 8 months). In 101 Dutch patients with LEMS (52 with SCLC), the frequency of ocular symptoms increased from 5% at onset of disease to 42% within six months (24% had diplopia, 24% had ptosis, 52% had both). For comparison, at disease onset 81% patients had proximal weakness and 47% had autonomic dysfunction, within six months increasing to 96% and 69%, respectively.

Rudnicki described a patient with LEMS and SCLC who presented with isolated ocular weakness. This rare presentation of LEMS might be confused with purely ocular seronegative myasthenia, but a more frequent cause of failure to diagnose LEMS is the absence of ocular weakness. Proximal leg weakness without
Ptosis or diplopia is frequently not recognized as a neuromuscular transmission disorder.

No less than sixty-one percent of patients were misdiagnosed initially: 20% as “generalized MG”, 15% as “myopathy”, 14% as “polyneuropathy”, 6% as “psychosomatic”, 3% as “lumbar spinal stenosis” and 3% as “other”. MG and psychosomatic disorders were commonly diagnosed in young patients without a tumour, whereas in older SCLC-LEMS patients the misdiagnosis was most usually myopathy or polyneuropathy.

It is useful to consider LEMS in patients suspected of isolated ocular seronegative myasthenia, but specifically the combination of proximal leg weakness and autonomic symptoms should trigger the physician to consider LEMS. In our experience with patients with LEMS, diplopia and ptosis suggest a disease of the neuromuscular transmission to most physicians, whereas proximal weakness is often misdiagnosed as a non-myasthenic neuromuscular disorder.

**Tumour**

In about half the patients with LEMS a tumour is found.\textsuperscript{10, 24, 33-35} Although non-small cell lung carcinoma and mixed lung carcinoma have been described, an SCLC is almost invariably found. SCLC is a smoking-associated tumour with neuro-endocrine characteristics. In our Dutch cohort of 53 SCLC-LEMS patients, the diagnosis of SCLC preceded LEMS in 6%. Complaints of the lungs and LEMS concurred in a significant part (25%), but mostly diagnosis of LEMS preceded SCLC in these patients (69%). Careful questioning revealed that in retrospect LEMS related symptoms had already been present in all 53 patients before diagnosis of SCLC. Screening is warranted because early detection of the SCLC will enhance the chance of diagnosing a limited tumour stage.\textsuperscript{36}

There are several reports in the literature describing associations with other tumours.\textsuperscript{37} Although it is difficult to discriminate between causal association and association by chance, there is a possibility that LEMS can be a paraneoplastic phenomenon of lymphoproliferative disorders (LPD) and prostate carcinoma. Thirteen cases of lymphomas and leukaemia have been described.\textsuperscript{38-40} Although a single case seemed to have attained stable remission of both LEMS and LPD, most patients had a devastating clinical picture leading to death, not allowing any definite conclusions regarding chance or causal relationship. Six patients with prostate carcinoma and LEMS are known; they had neuro-endocrine and small cell characteristics of the tumour cells and the symptoms of LEMS correlated with
tumour activity. Remission occurred in response to chemotherapy in a male patient with a retroperitoneal histologically proven small cell tumour extending from the prostate. No lung tumour has been found in the lung in a follow-up of almost three years. In literature a second case has been reported with remission in response to orchidectomy.\textsuperscript{41}

**Prediction of SCLC**

An underlying tumour has an enormous impact on therapy and prognosis, being a major clinical concern for the patient as well as for the doctor. Most SCLC are found within two years of diagnosis of LEMS,\textsuperscript{37} but discovery of a tumour has been described up till more than five years after diagnosis of LEMS.\textsuperscript{42}

Although the characteristics of disease are roughly the same, some factors are found to differ between the two groups. These differences might help to predict which patients are at risk for an associated tumour.

**Age of onset**

LEMS has traditionally been described as a disease of the older, smoking male patients.\textsuperscript{24, 43} Patients with LEMS and an associated SCLC are older than patients without a tumour, median 58 versus 51 years old.\textsuperscript{44}

**Sex**

There is a male predominance in LEMS patients with a SCLC. Percentages vary from 56\%,\textsuperscript{24, 43} 65\%\textsuperscript{44} to 70\%.\textsuperscript{37} For non-tumour LEMS (NT-LEMS) male and females are equally present, 49\%\textsuperscript{44} and 48\%,\textsuperscript{37} although O'Neill presented 18 males (72\%) in a group of 25 NT-LEMS patients.\textsuperscript{24}

In LEMS patients without a tumour, an analogy with patients with myasthenia gravis without a thymoma is suspected. We collected Dutch and English patients to test this hypothesis (chapter 2).

**Hypothesis**

Age of onset exhibits a double peaked curve in non-tumour associated LEMS. At early age the incidence will be higher in female patients, while above 60 years the incidence will be higher in male than in female patients.
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**HLA**
In three separate, although rather small, series of non-tumour LEMS patients a constant association with HLA-B8 in class I and -DQ2 and -DR3 in class II was found.\(^25,45-47\) In previous series\(^44\) 69% of non-tumour patients was HLA-B8 positive. For HLA-DR3 the same frequency was found (71%). HLA-A1 was seen in about half the patients (49%). The frequencies of this 8.1 HLA haplotype were reminiscent of that of MG, as was the preponderance of this haplotype in young female patients. In healthy Dutch controls the frequencies were around 25% for HLA-DR3, 23% for HLA-B8 and 31% for HLA-A1.\(^44\) The combination of these three HLA-types was seen in 41% of non-tumour patients in comparison to 16% in the normal population.\(^44\) As HLA-B8, -DR3 and -DQ2 are in strong linkage disequilibrium, the association could not easily be tracked down to one of them.

No relation has been shown between SCLC related LEMS and HLA. Although a higher frequency of HLA-B8 than expected (50%) was described in 14 English SCLC-LEMS patients,\(^46\) this could not be confirmed in a larger series of Dutch and English patients (20%).\(^44\) In a separate study HLA-B44 has been associated with patients with SCLC without any paraneoplastic syndrome,\(^48\) but this has not been confirmed in LEMS patients with SCLC. We expanded our sample size to study the HLA relationship in more detail (chapter 2).

**Hypothesis**
HLA-B8-DR3 is only more frequent in LEMS patients without SCLC, but not in those with SCLC, in comparison to controls.

**Smoking**
As SCLC is strongly related to smoking, both current smoking as well as former smoking, SCLC-LEMS is associated with smoking as well. In our series all 53 patients had smoked, while 60% in the NT-LEMS group had smoked. At diagnosis of LEMS, 86% was still smoking in the former group, while 28% was in the latter. In the Netherlands 33.3% and 29.6% of the population were smoking in 2001 and 2006, respectively.\(^49\) Although females are catching up, preponderance for men still exists, which explains the sex ratio in SCLC-related LEMS.

**Laboratory investigations**
Weight loss, lactate dehydrogenase (LDH), haemoglobin, leucocytes, non-specific enolase (NSE) and erythrocytes sedimentation rate (ESR) were independent risk factors for prognosis in SCLC.\(^50\) The difference between SCLC-LEMS and non-tumour LEMS patients has not been studied, but for ESR,\(^24\) ESR was significantly higher in SCLC-LEMS than in NT-LEMS, 21 versus 8 mm/h.
Clinical progression

The clinical phenotype of LEMS comprises a trias of proximal weakness, areflexia and autonomic dysfunction. The distribution of symptoms of LEMS is not different for SCLC-LEMS and NT-LEMS patients. Comparing the progression of symptoms there is a profound difference. After onset of the initial symptom, subsequent symptoms evolved in SCLC-LEMS within a shorter timeframe than in NT-LEMS. Wirtz et al. showed in 38 patients that patients with SCLC and LEMS develop on average eight symptoms within the first six months while patients without a tumour develop only two. We described a second Dutch cohort (59 patients) to confirm these results (chapter 3).

Hypothesis

Patients with SCLC-LEMS have a more aggressive onset of disease and show a faster progression towards a severe form of the disease than non-tumour patients.

Nuclear antibodies

In 2005, Graus described a new marker for lung cancer related paraneoplastic syndromes (PNS), AGNA for anti-glial nuclear antibody. This antibody was found by an immunoreaction with the nuclei of the Bergmann glia of rat cerebellum. AGNA was found in 43% of LEMS patients with SCLC, but in no patient with NT-LEMS and in 12% of patients with SCLC without LEMS. Identification of the protein involved in AGNA is described, as well as the sensitivity to discriminate SCLC-LEMS and NT-LEMS (chapter 4).

Hypothesis

The identification of the AGNA-antigen will allow the development of a sensitive test to discriminate SCLC-LEMS from NT-LEMS using recombinant proteins.

After identification of the antigen responsible for AGNA, SOX1, we validated an ELISA to create a reliable, easy test. We tested a cohort of SCLC patients to test sensitivity of SOX1 antibodies in patients without paraneoplastic syndromes (PNS) (chapter 5).

Hypothesis

SOX1 antibodies are more easily detected than AGNA in SCLC patients without PNS.

Prediction model

LEMS with and without a tumour share some similarities, but there are differences that help to predict the presence of a SCLC. Older age, smoking, development of multiple symptoms in the first half year after onset, ESR and
AGNA antibodies\textsuperscript{51} all predict the presence of an associated SCLC. On the other hand young age, never having smoked, slow onset of clinical symptoms and 8.1 HLA haplotype\textsuperscript{25, 45-47} point to an idiopathic autoimmune cause and absence of an underlying tumour. At the moment it is not clear if all these variables are independent, or which are most valuable to predict the presence of a SCLC. We have collected all these parameters in a large group of LEMS patients and created a model by multivariate analysis. This model has been validated in a second cohort of English patients (chapter 6).

A model which is able to distinguish between non-tumour and tumour LEMS in an early stage of the disease will be of great value for clinical practice and further insight into the pathophysiology.

Hypothesis It is possible to predict early in disease course in which patient with LEMS a so far undetected SCLC is present.

Screening for tumours in paraneoplastic syndromes (PNS)

Screening recommendations for SCLC in patients with a paraneoplastic syndrome, like LEMS, currently consist of repeated radiologic imaging, with, among other modalities, computed tomography (CT) of the thorax. Screening strategies recommend X-ray of the thorax, CT-thorax and bronchoscopy. If negative, imaging should be repeated every six months for four years.\textsuperscript{52} These screening protocols are based on expert opinion, and no supporting evidence based on large series with clinical data is available.

We studied the actual screening strategy in 100 Dutch LEMS patients and the yield they did provide (chapter 7).

Hypothesis Screening guidelines for LEMS can be updated, based on level III evidence instead of level IV evidence.

LEMS is often regarded a model disease for paraneoplastic syndromes. We collected all available data on PNS and screening to look if our screening data for LEMS could be extrapolated. We performed a literature search and consulted a panel of internationally acknowledged specialists to create screening guidelines (chapter 8).
Survival

LEMS has been associated with prolonged survival in a cohort retrospectively collected in Oxford (17 months in SCLC-LEMS patients vs 9 months in SCLC patients without LEMS). The data from Dutch LEMS patients with a SCLC (n = 53) were compared to a prospectively collected cohort of 138 consecutive SCLC patients. In these Dutch patients we also observed an extended survival also (15 vs 10 months, Figure). These data suggest the immune response is effective in retarding the growth or possibly even eradicating the tumour. The association between anti-VGCC antibodies, as a marker for the presence of LEMS, and survival could not be confirmed in 200 Danish SCLC patients. However, the mere presence of anti-VGCC antibodies might not be sufficient as in both Dutch and Spanish patients the presence of clinical symptoms of LEMS was a

Figure 1  Survival of LEMS patients with SCLC in comparison to SCLC patients without LEMS.
prerequisite. Only patients with SCLC, VGCC antibodies in combination with clinical manifestations of LEMS or paraneoplastic cerebellar degeneration showed a prolonged survival.\textsuperscript{55} This association has also been investigated for other paraneoplastic antibodies. Anti-Hu antibodies are not related to better survival.\textsuperscript{54-56} CRMP-5 antibodies are not related to severity of disease in SCLC patients.\textsuperscript{57}

This difference might be due to differences in the immune response. Both anti-Hu and CRMP-5 antibodies are directed against nuclear antigens, and merely regarded as a marker of a tumour-evoked autoimmune response, in which cytotoxic T-cells are most likely responsible for the clinical phenotype. VGCC antibodies are aimed at a transmembrane protein, which is freely accessible to serum antibodies. These antibodies have been shown to interfere with the function of the calcium channel and therefore have a close relationship to the course of the clinical disease.

According to this reasoning antibodies to extracellular accessible antigens can, but antibodies binding to intracellular epitopes cannot impair the function of tumour cells (chapter 5).

*Hypothesis* SOX1 antibodies have no impact on survival of SCLC patients with or without LEMS.

In chapter 9, the results and conclusions from this thesis are summarized and discussed.
Hypotheses

• Age of onset exhibits a double peaked curve in non-tumour associated LEMS. At early age the incidence will be higher in female patients, while above 60 years the incidence will be higher in male than in female patients (chapter 2).

• HLA-B8-DR3 is only more frequent in LEMS patients without SCLC, but not in those with SCLC, in comparison to controls (chapter 2).

• Patients with SCLC-LEMS have a more aggressive onset of disease and show a faster progression towards a severe form of the disease than non-tumour patients (chapter 3).

• The identification of the AGNA-antigen will allow the development of a sensitive test to discriminate SCLC-LEMS from NT-LEMS using recombinant proteins (chapter 4).

• SOX1 antibodies are more easily detected than AGNA in SCLC patients without PNS (chapter 5).

• It is possible to predict early in disease course in which patient with LEMS a so far undetected SCLC is present (chapter 6).

• Screening guidelines for LEMS can be updated, based on level III evidence instead of level IV evidence (chapter 7).

• Screening guidelines for paraneoplastic neurological syndromes can be created based on available literature and expert opinion (chapter 8).

• SOX1 antibodies have no impact on survival of SCLC patients with or without LEMS (chapter 5).
References

1. Willis Th: De anima brutorum quae hominis vitalis ac sensitiva est, exercitationes duae. Oxford, 1672


