Clinical features of neuromuscular disorders in patients with N-type voltage-gated calcium channel antibodies

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Abstract

Neuromuscular junction disorders affect the pre- or postsynaptic nerve to muscle transmission due to autoimmune antibodies. Members of the group like myasthenia gravis and Lambert-Eaton syndrome have pathophysiologically distinct characteristics. However, in practice, distinction may be difficult. We present a series of three patients with a myasthenic syndrome, dropped-head syndrome, bulbar and respiratory muscle weakness and positive testing for anti-N-type voltage-gated calcium channel antibodies. In two cases anti-acetylcholin receptor antibodies were elevated, anti-P/Q-type voltage-gated calcium channel antibodies were negative. All patients initially responded to pyridostigmine with a non-response in the course of the disease. While one patient recovered well after treatment with intravenous immunoglobulins, 3,4-diaminopyridine, steroids and later on immunosuppression with mycophenolate mofetil, a second died after restriction of treatment due to unfavorable cancer diagnosis, the third patient declined treatment. Although new antibodies causing neuromuscular disorders were discovered, clinical distinction has not yet been made. Our patients showed features of pre- and postsynaptic myasthenic syndrome as well as severe dropped-head syndrome and bulbar and axial muscle weakness, but only anti-N-type voltage-gated calcium channel antibodies were positive. When administered, one patient benefited from 3,4-diaminopyridine. We suggest that this overlap-syndrome should be considered especially in patients with assumed seronegative myasthenia gravis and lack of improvement under standard therapy.

Key Words: neuromuscular junction disorders, voltage gated calcium channels, myasthenia gravis, 3,4-diaminopyridin, dropped-head syndrome

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Neuromuscular junction disorders are a heterogeneous group of predominantly autoimmune diseases that affect the neuromuscular transmission. Myasthenia gravis (MG) is the most known member of the group with a prevalence of 150-300 per 1,000,000 individuals.[1,2] MG has a postsynaptic defect of neuromuscular transmission as the common feature. The characteristic symptoms are fatigability and focal or generalized muscle weakness that usually effects the ocular, bulbar and proximal extremity muscles.[3-6] Anti-acetylcholine receptor (AChR) antibodies can be detected in 70% of all MG patients.3-6 Autoantibodies against the muscle specific kinase (MuSK) or against the low-density lipoprotein receptor-related protein 4 (LRP4) are less common. Patients with MG but no positive test results for anti-AChR, anti-MuSK or anti-LRP4 antibodies are characterized as having seronegative MG, although a still growing number of autoantibodies are detected in patients with MG, for example anti-Titin, anti-Agrin or anti voltage-gated potassium channel antibodies (Kv1.4).[7] Therapeutic strategies include symptomatic pharmacological treatment (i.e. acetylcholinesterase inhibitor pyridostigmine), immunomodulatory pharmacological treatment (i.e. prednisolone, azathioprine, mycophenolate mofetil), thymectomy, plasma exchange and supportive treatment.[4,8]

Presynaptic affection by autoimmune ion channel blockade defines Lambert-Eaton myasthenic syndrome (LEMS).[9] Antibodies against the P/Q-type voltage-gated calcium channel (VGCC) are detectable in 85% of the patients with LEMS.10 Characteristic symptoms of LEMS are an ascending muscle weakness starting with the lower proximal limb muscles and autonomic dysfunction. Ptosis and ophthalmoplegia are less common compared to MG, and respiratory muscle failure is not typical.[11] First-line treatment of LEMS is 3,4-diaminopyridine,12 which inhibits presynaptic potassium channels and, thus, increases levels of

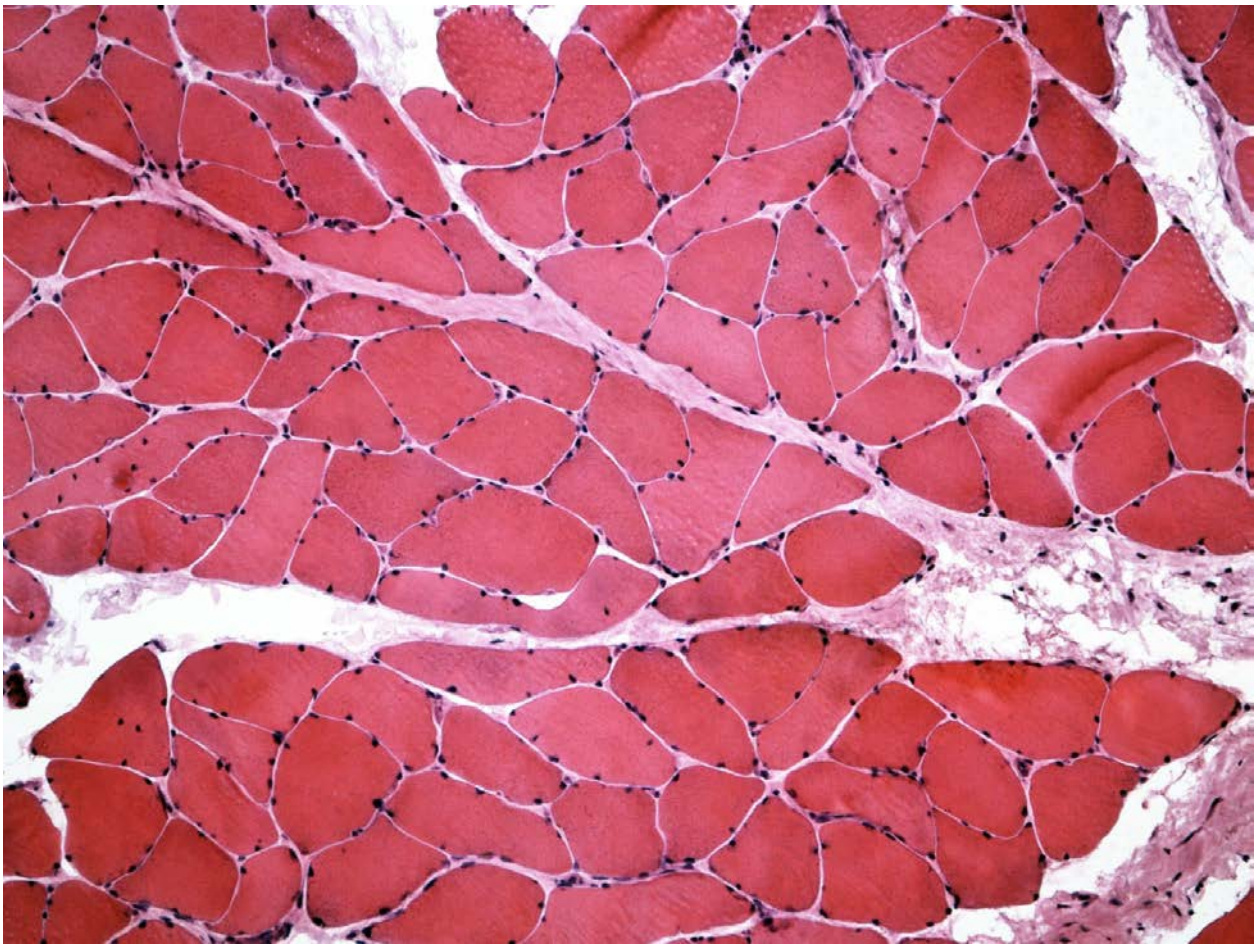

Fig 1. *Muscle biopsy of Patient #1 with unspecific signs of mild to moderate myopathy. No significant inflammatory infiltrates are detectable.*

acetylcholine. Pyridostigmine and intravenous immunoglobulins have less effect.

In the last decades, several autoimmune antibodies were detected in patients with MG and LEMS although clinical distinction has not yet been made.[7,13,14] Recently the existence of a myasthenia gravis Lambert-Eaton overlap syndrome (MLOS) was reported.[15]

We present a case series with myasthenic syndrome with marked dropped head syndrome, dysphagia and dysarthria as a common feature. All patients have electrophysiological features of MG and were tested positive for anti-VGCC N-type but not P/Q-type antibodies.

Case Reports

Patients, Methods, Results

Patient #1

A 71-year old male Caucasian presented in 2013 with myasthenic syndrome and rapidly progressive proximal tetraparesis, dropped head syndrome and dysphagia. Weeks before, a neurological outpatient clinic reported beginning dysphagia and slight proximal paraparesis. Anti-AChR antibodies were elevated (0.53nmol/l, reference value <0.40nmol/l) but no pathological decrement of the orbicular and trapezius muscle was measured and no diurnal or exercise dependent weakness was noted in the repetitive nerve stimulation test. The electromyography (EMG) and nerve conduction studies (NCS) were normal. The patient was admitted to the Department of Gastroenterology for diagnosis and treatment of recurrent diarrhea and thrombocytopenia and a weight loss (20kg). At this point, the patient developed a rapidly progressive severe tetraparesis with affection of the upper limbs more than the lower limbs, with dropped-head syndrome and dysphagia. Deep tendon reflexes were slightly reduced. The origin of the diarrhea could not be clarified. He was transferred to our intensive care unit with aspiration pneumonia and acute respiratory insufficiency due to respiratory muscle weakness. The patient was intubated, later on tracheostomized, and sepsis was treated with empirical antibiotics. A retest of the repetitive nerve stimulation of the n. accessories now showed a pathological decrement (>50% reduction of amplitude) using the 3Hz-stimulation and an increment using a

30Hz (>60% increase of the amplitude). Muscle biopsy found unspecific diffuse atrophy of the muscle fibers but no inflammatory infiltrates (Figure 1). Immunohistochemical workup was negative. The patient was unsuccessfully treated with steroids (500mg prednisolone per day over 5 days).
Anti-VGCC N-type (antibody ratio 41.3, reference ratio <15.0; radioreceptor assay, a commercially used statistically defined endpoint titer determination method for immunoassays was used according to Frey et al.)16 but not P/Q-type were detected. Anti-Titin and anti-MuSK antibodies were negative. The titer of antinuclear antibody (ANA) was elevated (>1:2.000), and differentiation only positive for Anti-Sm-D-antibodies. Further autoantibodies including myositis antibodies and onconeural antibodies were negative. The patient had a history of pulmonary sarcoidosis. Extended tumor screening including whole-body PET/CT imaging, and screening for tumor markers were negative.
The patient received intravenous pyridostigmine with initially little effect, intravenous immunoglobulins (35g per day over 5 days). The intravenous application of immunoglobulins was repeated after 14 days without significant clinical improvement. Then a treatment with 3,4-diaminopyridine (3x15mg per day) was started which resulted in a rapid improvement of symptoms over a few days. While severe tetraparesis, dropped head syndrome, and diarrhea significantly improved, dysphagia first persisted and a percutaneous endoscopic gastrostomy (PEG) probe was placed. Intravenous pyridostigmine was switched to oral pyridostigmine bromide (Mestinon) 30mg three times a day. We started immunosuppressive treatment with mycophenolate mofetil (1500mg per day) accompanied by steroids (70mg prednisone per day) and intensive speech therapy. In the course of the hospitalization, dysphagia improved and the tracheostomy tube could be removed. At discharge the patient was fully mobilized and able to eat and drink without difficulties, anti-VGCC N-type antibodies were negative. No diarrhea was observed. At follow-up a few weeks later the patient was able to play golf again. In the one and two-year follow up, the patient does not complain any neuromuscular symptoms, cancer screening was negative. The repetitive nerve stimulation tests were normal.

Patient #2

An 80-year old male Caucasian, non-smoker, presented in 2013 with myasthenic syndrome accompanied by dropped head syndrome, dysarthria and dysphagia. A few weeks upon arrival at our Department of Neurology, the patient was diagnosed with urothelial squamous cell carcinoma of the urine bladder with lymphatic and possible pulmonary metastasis. He received radical cystoprostatectomy, pelvic lymphadenectomy and an ileal conduit. Chemotherapy had not been started yet. The weakness of the neck muscles had developed over several months in advance. Next to paresis of the neck muscles resulting into dropped head syndrome with lateral shift, physical exam showed long known bilateral ptosis, moderate to severe dysarthria and dysphagia and mild weakness of the upper proximal extremities. Deep tendon reflexes were normal. Blood pressure controls reveal a severe arterial hypotension. Pathological decrement of the n. accessorius was measured (>20% reduction of amplitude) with 3Hz-stimulation pre- and post-exercise. A lowered CMAP of the facial nerve could be detected. Edrophonium chloride test was positive. Computer tomography (CT) of the chest found no thymoma. The patient was started on oral pyridostigmine bromide (Mestinon) and immunosuppression with steroids (prednisone 20mg per day starting dosage). During the next few days, dysarthria and dysphagia had almost completely resolved, dropped head syndrome improved. Electrophysiology showed decline of the pathological decrement. At discharge the patient was on pyridostigmine bromide with a total daily dosage of 345mg and prednisone 40mg. Anti-AChR antibodies, anti-Titin antibodies, anti-MuSK antibodies, and anti-actin (anti-striated muscles) antibodies were negative. Later results were positive for anti-VGCC N-type antibodies (antibody ratio 71.1, reference ratio <15.0, same radioreceptor assay as mentioned above) and negative for anti-VGCC P/Q-type antibodies.
Two months later, the patient presented to our hospital with dyspnea. Dropped head syndrome, dysphagia and dysarthria were almost on the same level as at discharge. The chest CT revealed a bilateral pulmonary embolism and heparin drip was initiated. Due to pulmonary embolism, the severe conditional state of the patient and progressive metastasis chemotherapy was not started. Consecutively, the patient developed pneumonia that was successfully treated with antibiotics. Dysphagia, Dysarthria, dropped head syndrome, and respiratory muscle weakness worsened, although pyridostigmine was increased. Again anti-VGCC N-type antibodies were positive (antibody ratio 53.2, reference ratio <15.0), but anti-AChR antibodies were slightly elevated as well (0.74nmol/l, reference value <0.40nmol/l). Treatment with immunoglobulins and 3,4-diaminopyridine as well as mechanical ventilation were discussed with the patient and his family, but were not started due to the unfavorable prognosis of the cancer diagnosis. A few days later, the patient died due to a respiratory failure.

Patient #3

A 71-year old female presented with myasthenic symptoms since 1970. The patient reported of neuromuscular symptoms including diplopia, bulbar symptoms, a proximal muscle weakness, dropped-head syndrome and dyspnea with no diurnal or exercise dependency and an improvement under pyridostigmin only in the initial phase of the disease. Deep tendon reflexes were diminished. A refractory diarrhea was

Table 1. *Demographics and symptoms of the reported patients.*

No.	Age	Gender	VGCC N-type	ACh-R-AB	Decrement	Increment	Autonomic Symptons	Response to pyridostigmin
# 1	71	m	Y	Y	Y	Y	Y	Initially
# 2	80	m	Y	N	Y	N	Y	Initially
# 3	71	f	Y	Y	Y	N	Y	Initially

reported for several years. A few years before the patient was diagnosed with a mixed connective tissue disease treated with azathioprine, methotrexate and prednisolone. NCS revealed sings of an axonal sensory polyneuropathy. Recent 3Hz-stimulation revealed no pathological decrement, although former testings describe a decrement of the M. trapezius. Anti-AChR antibodies (140.0nmol/l, reference value <0.40nmol/l) and anti-VGCC N-type antibodies were positive (antibody ratio 17.3, reference ratio <15.0). The patient reported worsening of bulbar and ocular symptoms a few weeks before admission to hospital but declined a treatment trial with 3,4-diaminopyridine or a further medication with immunosuppressive drugs and was lost for follow-up.

Discussion

We presented three cases of a progressive myasthenic syndrome and highly positive anti-VGCC N-type antibodies (Table 1). LEMS patients usually present with proximal muscles weakness of lower limb, autonomic dysregulation and hypo-/areflexia. Bulbar and respiratory muscles are typically not affected. In our cases, the patients had initially and predominantly dysarthria, dysphagia, proximal muscle weakness of the upper more than lower limbs and most impressive dropped head syndrome. Deep tendon reflexes were slightly reduced, which is usually more impressive in LEMS. Therefore, the diagnosis of a LEMS is likely due to clinical signs that are consistent with a presynaptic involvement and signs of an autonomic dysfunction. The first and third patient presented with diarrhea, the second patient an arterial hypotension as a sign of autonomic dysregulation, especially while improving with decline of VGCC N-type antibodies in the first case. Respiratory muscles were not spared, which led to respiratory insufficiency. All patients showed at least initially good response to pyridostigmine (Mestinon). While myasthenic symptoms and the pathological decrement were suitable for MG, elevated titers of anti-AChR antibodies were measured, but no diurnal or exercise dependent weakness was found, and effect of pyridostigmine therapy was decreasing. Possibly, anti-VGCC N-type antibodies might be an epiphenomenon of another disease. However, using radio receptor assay for detection, in our opinion antibody levels were too high to be a considerable epiphenomenon or laboratory failure and the clinical improvement of case one under 3,4-diaminopyridine with decreasing anti-VGCC N-type antibodies level was too impressive. We therefore considered a MLOS with post- and presynaptic features. The rapid clinical improvement after beginning with the 3,4-diaminopyridin treatment strongly suggests a presynaptic involvement, nevertheless additional effects of the immunosuppressive treatment could not be excluded.

Underlying cause of anti-VGCC N-type antibodies production in both cases remains unknown. In case one, the antibody work-up implied an autoimmune vasculitis. Positive history of sarcoidosis may support this theory.[17] However, although extensive tumor screening was negative, an occult carcinoma cannot be ruled out. The urothelial cancer found in the second patient is not a typical cause of paraneoplastic syndrome. On the other hand, pulmonary metastasis might have masked an underlying bronchial carcinoma.

The systematic literature search identified only few cases of MG and presynaptic overlap syndrome.[15,18-21] These cases were characterized by ocula-bulbar symptoms, a response to edrophonium, hyporeflexia and an increment of reduced CMAP in NCS. Oh and colleagues presented a case of a young women with myasthenic symptoms as well as proximal muscle weakness, elevated anti-VGCC N-type antibodies and response to intravenous immunoglobulins.[19] Ueda et al. described a case of anti-VGCC P/Q-type antibodies with dropped head syndrome as found in our patients.21 A recent overview of Oh compared 39 as MLOS classified cases with typical signs of MG and LEMS. While in LEMS 98% of the patient has decreased or absent reflexes, in overlap syndromes this was only documented in 74% of the patients.[15] Therefore, normal reflexes do not exclude the diagnosis of a potential MLOS. The detection of anti-VGCC N-type antibodies is often accompanied by a cancer disease, typically a small cell lung cancer (SCLC). While SCLS can be detected in ~50% of the patients with LEMS, in overlap syndromes SCLC was found in 18%.15 In the case series of Oh et al., patients were positive for anti-VGCC N-type and anti-VGCC P/Q-type antibodies. However, LEMS cases with isolated anti-VGCC N-type antibodies are rare.[15] Recently a similar case of dropped head syndrome with positive anti-VGCC N-type antibodies was published, suggesting an anti-VGCC N-type mediated, non-paraneoplastic LEMS.[20] In this patient neither SCLC nor anti-VGCC P/Q-type antibodies could be detected and the patient improved under

immunosuppressive and symptomatic treatment. As far as we know, we present the first series with MLOS characterized by remarkable dropped head syndrome, bulbar symptoms and respiratory muscles weakness, and positive anti-VGCC N-type but not P/Q-type antibodies. Although the role of N-type VGCC in neuromuscular junction disorders remains unclear, detectable antibodies has to be taken in account for the planning of symptomatic treatment with pyridostigmin or 3,4-diaminopyridin. However, the rarity of this disease will limit exact characterization. This phenotype and serological findings should prompt a search for neoplasms beyond thymoma, especially carcinoma like SCLC.22

In conclusion, myasthenic patients with dropped head syndrome, bulbar symptoms, and axial and possible respiratory muscle weakness should carefully be searched for the detection of anti-VGCC N-type antibodies, especially when treated unsatisfactorily with pyridostigmine. If anti-VGCC N-type antibodies are positive, 3,4-diaminopyridine might be a considerable addition to therapy without severe side-effects.

Author's contributions

AT wrote the manuscript and analysed the patient´s history, PM and OK were involved in the patient´s treatment and have corrected manuscript, TH was involved in the patients treatment, analysed the patients' history and supervised the manuscript.

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Conflict of Interest

The authors report no conflicts of interest.

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