Introduction

Myasthenia gravis (MG) is an autoimmune disease of the neuromuscular junction mediated by autoantibodies. Clinically, pathological muscle fatigue occurs mainly in the eye muscles, but other muscle groups can likewise be affected. MG can be limited to the eye muscles (ocular MG) or may extend to additional muscle groups (generalized MG).

In addition to clinical diagnostics with provocation of muscle fatigue and pharmacological testing, neurophysiological and laboratory tests are the most important investigations used to confirm the suspected diagnosis of myasthenia gravis. The identification of new antigens has not only changed antibody diagnostics; rather, MuSK antibody-positive MG has been clinically distinguished as a separate clinical subtype, while others indicate comorbidities such as thymomas.

Autoantibodies and myasthenia gravis

A neuromuscular junction consists of 3 components: (1) the terminal nerve ending where acetylcholine is formed, deposited in vesicles and then released, (2) the synaptic gap (3) the postsynaptic (muscle) membrane containing the acetylcholine receptor and its helping proteins as well as cholinesterase. In myasthenia gravis, autoantibodies can occur that affect various structures of the neuromuscular junction. Some of these autoantibodies determine a separate clinical subtype, while others indicate comorbidities such as thymomas.

Acetylcholine receptor antibodies (anti-AChR-ab)

The acetylcholine receptor antibody was the first pathogenic antibody identified in MG patients, and can be detected in about 80–85 % of the patients. More recently, autoantibodies against muscle-specific kinase (MuSK) and lipoprotein receptor-associated protein 4 (LRP4) have been identified in a subset of MG patients. Additionally, anti-titin autoantibodies can point to an underlying thymoma in younger MG patients. Neurophysiological examination includes a repetitive stimulation to detect a possible decrement as the electrical correlate of pathological muscle fatigability. Single-fiber electromyography can identify neuromuscular transmission disturbances in otherwise unclear cases.
Antibodies against muscle-specific kinase (anti-MuSK-ab)
In 2000 it was first determined that approx. 50 % of patients with seronegative MG (no evidence of AChR-ab despite clinical myasthenia gravis) have autoantibodies against a muscular surface protein that is not identical to the AChR [5]. This antigen was identified as MuSK, a transmembrane protein directly associated with AChR [6]. The binding of antibodies to MuSK leads to a reduced clustering of AChR and thus to a reduced number of AChR at the neuromuscular junction. Interestingly, anti-MuSK antibodies belong to the IgG4 subclass and thus cannot activate a complement [7]. Clinically anti-MuSK-ab-positive MG patients frequently have an involvement of facial, bulbar and axial muscles as well as muscular atrophy [8]. Patients with anti-MuSK antibodies suffer respiratory crises more frequently than patients with anti-AChR antibodies. Thymus histology is as a rule normal; thymomas are almost never observed among anti-MuSK patients [9]. The frequency of anti-MuSK among myasthenia patients appears to be 3–4 % of all cases and 25–30 % of AChR-ab-negative cases of MG.

Antibodies against lipoprotein receptor-associated protein 4 (anti-LRP4)
In 2011 and 2012 two independent working groups first described antibodies against the protein LRP4 in cases of seronegative MG [10, 11]. According to these studies approx. 15–20 % of seronegative MG patients, 7.5 % of AChR-ab-positive MG patients as well as 15 % of MuSK antibody-positive MG patients have anti-LRP4 antibodies. In Germany anti-LRP4-antibody-positive patients appear to be a rarity, and it is estimated that they make up make up less than 1 % of all MG cases. In mice, passive transfer of the antibody leads to myasthenic symptoms. Whether only LRP4-ab-positive patients are less severely affected by myasthenia is a matter of controversy due to the low number of case histories. However, patients with anti-LRP4 and an additional antibody were more severely affected [10–12].

Titin antibodies
In patients less than 50 years of age, titin antibodies are indicative of the presence of a thymoma [13]. There is no clear selectivity here; thus a negative finding of titin antibodies does not exclude the possibility of a thymoma in patients under 50. Therefore, thymoma diagnosis by means of thoracic CT or MRI is a necessary part of a standard initial investigation of myasthenia gravis. In patients older than 50 years of age, such antibodies are more common even without presence of thymoma; the frequency of titin antibodies in late-onset MG increases with age [14].

Antibodies against agrin and other proteins
Agrin antibodies have been demonstrated in some myasthenia gravis patients who generally also had antibodies against ACHR, MuSK or LRP4. The significance of these antibodies is currently unclear. In addition, antibodies against the intracellular protein cortactin have been detected; their relevance is likewise unexplained [1, 15].

Detection methods for myasthenia-associated antibodies
The radioimmunoassay (RIA) is the standard method of detecting acetylcholine receptor antibodies. With pertinent clinical symp-
only at high stimulation frequencies of 30–50 Hz [16]. Since this test is very painful, nowadays a test with two individual supramaximal stimuli is preferred before and after a 10–20 s muscle contraction. An increment greater than 100 % is demonstration of presynaptic neuromuscular transmission dysfunction; incremental values between 60–100 % are already highly suspicious, however. It should be noted that in LEMS a significantly reduced amplitude of the starting MSAP can normally be observed [16] ▶ Fig. 2.

Single-fiber electromyography

When a motor axon becomes depolarized, the stimulus is distended distally and excites the individual muscle fibers almost simultaneously within the motor units. The variation in the excitement interval from one muscle fiber to another is called jitter and is an expression of the variability of neuromuscular transmission. If there is a functional limitation of the neuromuscular junction, the jitter will be prolonged. A single-fiber EMG needle has a smaller receiving radius than a concentric needle electrode. Using this special needle it is possible to derive potential pairs of 2 fibers of the same motor unit and thus determine the jitter. The muscles most frequently used are the extensor digitorum muscle on the forearm or the frontalis muscle, since they can be constantly innervated over a longer period and since these muscles are less subject to age-related change (overview in [18]). In purely ocular forms, the SFEMG can also be performed by the orbicularis oculi muscle, but which places higher demands on the examiner and the patient. A normal SFEMG in a paretic muscle practically rules out myasthenia gravis [16]. Currently SFEMG is rarely employed due to the time factor and experience required of the examiner.

Pharmacological tests

The Tensilon test, once regularly performed, is still occasionally used today. This test uses the briefly active cholinesterase inhibitor edrophonium (Tensilon, Camsylon) injected intravenously. The patient should be connected to an ECG monitor; initially 2 mg of Tensilon are administered as a test dose, if bradycardia does not occur, the remaining 8 mg are subsequently injected. During the test, atropine should always be available as an antidote. Muscle force generally improves after 30–45 s and continues for about 4–5 min. The test can be combined with repetitive stimulation; after administration of Tensilon the decrement should decrease. The clinical interpretation of the test should take into account that Tensilon yields a false negative in approx. 25 % of myasthenia cases, and can produce false positive results in some muscular diseases or spinal muscular atrophy.

Another option is the provisional administration of pyridostigmine bromide in a dosage of 3–4 × 10 mg to 4 × 60 mg over several days.
The diagnosis of myasthenia gravis is based on clinical progression, diagnosis of autoantibodies and, as needed, electrophysiological examinations. In the case of a negative finding of acetylcholine receptor antibodies, anti-MuSK, anti-LRP4 and anti-titin antibodies should be determined. An electrophysiological examination is dispensable if the clinical symptoms are unambiguous and there is a positive autoantibody test.

Conflict of Interest

The author declares no conflict of interest.

References