Myasthenia Gravis: Diagnosis

Matthew N. Meriggioli, M.D.,[1] and Donald B. Sanders, M.D.[2]

ABSTRACT

The clinical history and neurological examination provide the most important data on which the diagnosis of autoimmune myasthenia gravis (MG) is based. MG produces symptomatic weakness that predominates in certain muscle groups and typically fluctuates in response to effort and rest. The diagnosis of MG therefore depends on the recognition of this distinctive pattern of fatigable weakness. Laboratory confirmation of the clinical diagnosis may be obtained using pharmacological, electrophysiological, and serological (immunological) tests. This article reviews the tests used to confirm the diagnosis of MG.

KEYWORDS: Acetylcholine receptor antibody, repetitive nerve stimulation, single-fiber electromyography, edrophonium chloride

Objectives: On completion of this article, the reader will be able to describe the pharmacological, electrophysiological, and serological tests used to confirm the diagnosis of myasthenia gravis.

Accreditation: The Indiana University School of Medicine is accredited by the Accreditation Council for Continuing Medical Education to provide continuing medical education for physicians.

Credit: The Indiana University School of Medicine designates this educational activity for a maximum of 1 Category 1 credit toward the AMA Physicians Recognition Award. Each physician should claim only those hours of credit that he/she actually spent in the educational activity.

Disclosure: Statements have been obtained regarding the authors' relationships with financial supporters of this activity, use of trade names, investigational products, and unlabeled uses that are discussed in the article. Dr. Meriggioli has nothing to disclose. Dr. Sanders discloses consultation fees from Athena Diagnostics and research support from Roche Pharmaceutical Co.

Pharmacological, electrophysiological, and serological tests are commonly used to confirm the clinical diagnosis of autoimmune myasthenia gravis (MG). A basic knowledge of the physiology of neuromuscular transmission is required to understand the principles upon which the pharmacological and electrophysiological tests are based.

PHYSIOLOGY OF NEUROMUSCULAR TRANSMISSION

Depolarization of the nerve terminal results in the opening of voltage-gated calcium channels. The subsequent influx of calcium into the nerve terminal causes synaptic vesicles containing acetylcholine (ACh) to fuse with the plasma membrane and release their contents into the synaptic space by exocytosis. Acetylcholine diffuses across the synaptic space and interacts with a specific ACh receptor (AChR) protein on the external surface of the muscle plasma membrane of the motor end plate. The combination of ACh with its receptor increases the conductance of the postjunctional membrane to cations, resulting in a transient depolarization of the end-plate region. This transient depolarization is called the end-plate potential (EPP). If the EPP is of sufficient magnitude, a muscle fiber action potential is generated. The difference between the EPP amplitude and the depolarization required for generation of a muscle action potential is referred to as the "safety factor of neuromuscular transmission."

Myasthenia Gravis; Editor in Chief, Karen L. Roos, M.D.; Guest Editor, Robert M. Pascuzzi, M.D. *Seminars in Neurology*, Volume 24, Number 1, 2004. Address for correspondence and reprint requests: Matthew N. Meriggioli, M.D., Department of Neurological Sciences, Rush University, 1725 W. Harrison Street, Suite 1106, Chicago, IL 60612. [1]Director, Section of Neuromuscular Disease, Department of Neurological Sciences, Rush University Medical Center, Chicago, Illinois; [2]Department of Medicine, Division of Neurology, Duke University Medical Center, Durham, North Carolina. Tel: +1(212) 584-4662. 0271-8235,p;2004,24,01,031,039,ftx,en;sin00294x.

The EPP is transient because the action of ACh is ended by its hydrolysis to choline and acetate by the enzyme acetylcholinesterase (AChase), which is present in high concentrations at the postsynaptic membrane. After the end-plate region is depolarized, adjacent regions of the muscle cell membrane are depolarized by electrotonic conduction. When these regions reach threshold, action potentials are generated that are propagated along the muscle fiber at high velocities, initiating the chain of events that lead to muscle contraction.

Muscle-specific tyrosine receptor kinase (MuSK) is an enzyme on the receptor region of the muscle membrane that is essential in aggregating AChRs in the developing muscle. Muscle-specific tyrosine receptor kinase is activated by nerve-released agrin. Its role in mature muscle is not yet clear, but antibodies to MuSK have been demonstrated in ~40% of patients with generalized acquired MG who do not have antibodies to the AChR.

PHARMACOLOGICAL TESTING

Clinical observation of the response to administration of pharmacological agents that affect neuromuscular transmission forms the basis for several diagnostic tests. These include tests that demonstrate improved strength induced by agents that enhance neuromuscular transmission (e.g., edrophonium, pyridostigmine) or an exaggerated response to agents that block the neuromuscular junction (e.g., curare). With the development of sensitive electrophysiological tests, the latter are no longer routinely used.

Edrophonium Chloride (Tensilon) Test

The use of edrophonium chloride (Tensilon) as a diagnostic test for MG was described in 1952.[1] Its rapid onset (30 seconds) and short duration of effect (~5 minutes) make it an ideal agent for this purpose. By inhibiting the normal action of AChase, edrophonium chloride and other cholinesterase inhibitors allow ACh molecules to diffuse more widely throughout the synaptic cleft and to interact with AChRs sequentially, resulting in a larger and longer EPP.[2]

The dose of edrophonium that will produce improvement varies among patients and cannot be predicted in advance. A dose that is too high may exacerbate weakness in patients with abnormal neuromuscular transmission. For this reason, edrophonium is administered in incremental doses, beginning with a test dose of 2 mg, followed by doses of 3 and 5 mg. Atropine (0.6 mg intramuscularly or intravenously) should be available to counter possible muscarinic side effects, although the dose of edrophonium typically needed to diagnose MG is rarely more than 5 mg and unlikely to cause significant problems. Bronchial asthma and cardiac dysrhythmias are relative contraindications for edrophonium testing. Resolution of eyelid ptosis or improvement in strength of at least one paretic extraocular muscle are the most reliable end points. Although widely used and generally accepted, the edrophonium test is neither absolutely sensitive nor specific for MG.[3–6] It is limited by the dependence on patient effort to give maximal exertion both before and after the drug is administered. For this reason, diagnostic sensitivity and specificity are acceptable only in patients with clear-cut weakness in muscles that can be assessed in a serial and objective manner (i.e., ptosis or ophthalmoparesis).

Other Cholinesterase Inhibitors

Some patients with MG do not improve after administration of edrophonium, but respond to neostigmine methylsulfate or Prostigmin.[7,8] The onset of action after an intramuscular dose is ~5 to 15 minutes, and the clinical effects last from 2.5 to 4 hours. This longer duration of action compared with edrophonium is potentially useful in the evaluation of children. However, the reliability of the selected end point is often questionable in the interpretation of diagnostic trials using longer-acting cholinesterase inhibitors, and this severely limits their diagnostic value. Similarly, administration of oral pyridostigmine (Mestinon) as a therapeutic trial may demonstrate a subjective beneficial effect on muscle strength and fatigability that is not apparent after a single dose of edrophonium. Once again, caution is recommended in interpreting these trials as they rely largely on the patient's subjective observations.

ELECTROPHYSIOLOGICAL TESTING

Electrophysiological studies are performed in patients with suspected MG to confirm a defect in neuromuscular transmission and also to exclude other diseases of the motor unit that may confound or contribute to the clinical findings. The two principal electrophysiological tests used to confirm a defect in neuromuscular transmission are repetitive nerve stimulation (RNS) studies and single-fiber electromyography (SFEMG).

Repetitive Nerve Stimulation

RNS is the most commonly used electrophysiological test of neuromuscular transmission. In this technique, a peripheral nerve is stimulated supramaximally and the compound muscle action potential (CMAP) is recorded. The CMAP is recorded with a surface electrode placed over the motor point of the muscle and a reference recording electrode placed on a distal point where minimal electrical activity is recorded, usually a tendon or bony prominence. Repetitive nerve stimulation serves to "stress" motor end plates with marginal safety factors by

depleting the store of readily releasable synaptic vesicles. In clinical practice, a train of 5 to 10 stimuli is delivered at a rate of 2 to 3 Hz. This rate is effective for this purpose because it normally results in a sequential decrease in the amount of ACh released from the nerve terminal. This phenomenon becomes crucial in myasthenic end plates when the EPP drops below threshold. As the EPP drops below threshold for muscle fiber activation in an increasing number of end plates, the number of muscle fibers contributing to the CMAP declines and the resulting CMAP is reduced in amplitude and area (decremental response) (Fig. 1B).

Fast rates of stimulation (>10 Hz) in patients with neuromuscular junction disease may produce an increase in CMAP amplitude due to buildup of calcium in the nerve terminal and the resulting facilitated release of ACh (posttetanic facilitation). This occurs only in diseased end plates as normally all EPPs result in muscle fiber action potential generation, and the facilitated release of added ACh is of no consequence. In normal or abnormal end plates, a rapid rate of stimulation may result in an increased CMAP amplitude by increasing synchronization of the action potential velocities in the tested muscle fibers[9] or by hyperpolarization of the muscle fiber membrane from increased Na^+-K^+ pumping.[10] This produces a CMAP of increased amplitude but decreased duration (the negative peak area is essentially unchanged). This phenomenon is called "pseudofacilitation" and is not indicative of neuromuscular junction disease but may mask a decrement.

In repetitive nerve stimulation testing, the decrement is defined as the percentage of change between the amplitude or area of the fourth, fifth, or lowest potential (usually the fourth) compared with the first potential. The area is a more accurate measure of the number of muscle fibers contributing to the CMAP and is usually not affected by pseudofacilitation. However, area measurements are affected by the G2 electrode, repolarization, and muscle fiber fatigue and are markedly dependent on filter settings. In most cases, the results from area and amplitude measurements are concordant. Any discrepancy should lead to a search for technical problems.

A decrement greater than 10% is considered abnormal. However, the criteria for abnormality will vary to some degree between laboratories. The following technical considerations are useful to maximize diagnostic sensitivity and prevent artifactual changes:

1. *Reproducibility.* The same decrement should be acquired on repeat testing after a period of rest. The muscle should be rested for at least 30 seconds

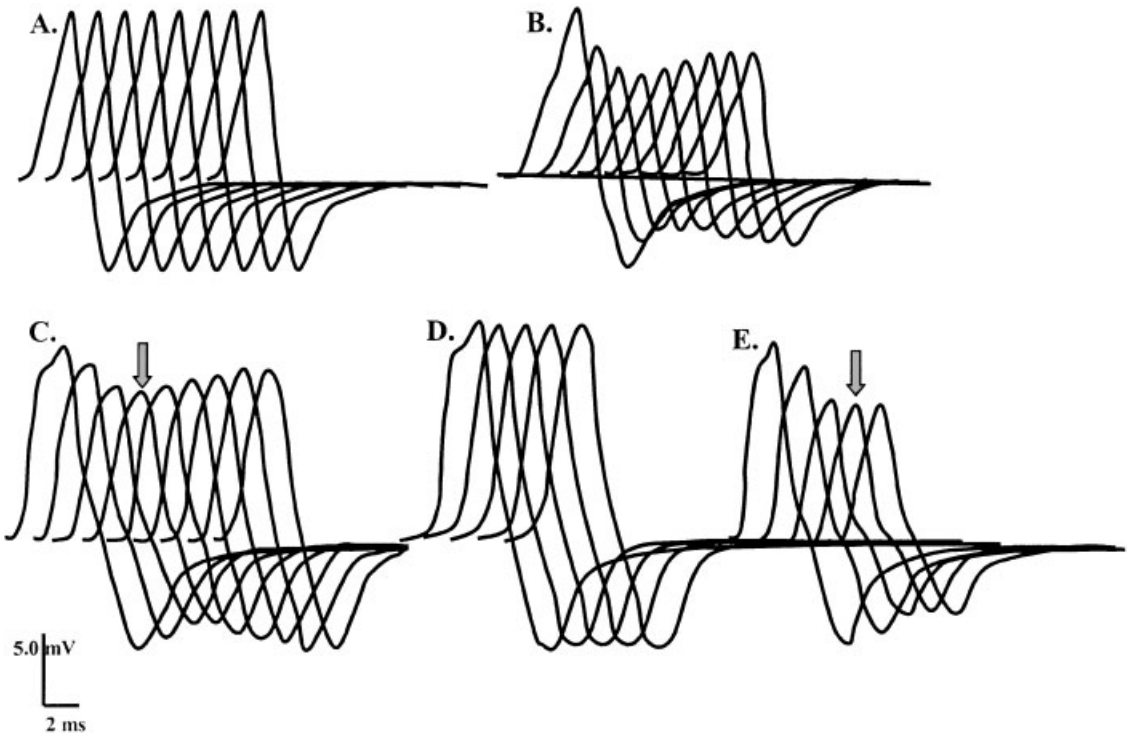

Figure 1 Repetitive nerve stimulation tracings from a normal control subject (A) and a patient with myasthenia gravis (B) illustrating a classic decremental response. Responses were obtained with repetitive stimulation of the ulnar nerve at 3 Hz, recording from the abductor digiti minimi muscle. (C) A prominent decrement is seen in another patient with MG. Comparing the amplitude of the first potential with the fourth potential (arrow), there is a 24% decrement. (D) Immediately after 30 seconds of exercise, the decrement is now much less (''repair of the decrement''). (E) Four minutes after exercise the decrement is now worsened (32%) compared with the resting baseline (postactivation exhaustion).

between trials. The average decrement after three trials may be reported and is often more accurate than the measurement after a single trial. Widely varying measurements from one trial to another are an indication of poor technique or may be caused by incomplete muscle relaxation.

2. *Response (envelope) pattern.* Movement of the stimulating or recording electrodes, or the muscle itself, may produce irregular patterns of change in CMAP size and configuration during repetitive stimulation. It is important to recognize these patterns as not conforming to those expected in disease of the neuromuscular junction. Stimulating and recording electrodes and the appropriate joints should be stabilized to minimize these artifactual changes.
3. *Muscle temperature.* The decremental response in endplate diseases is less when the muscle is cool.[11] Hand or foot muscles should be warmed to at least 36°C to ensure the maximum diagnostic sensitivity.
4. *AChase medications* may mask a decrement and should be stopped at least 12 hours prior to the study. Caution is required in certain patients who are dependent on these medications, as this may produce a disease exacerbation.

The typical pattern seen with repetitive nerve stimulation at 2 to 3 Hz in a patient with MG is a progressive decrement of the second through the fourth or fifth response with some return toward the initial CMAP size during the subsequent responses to a train of stimuli, the so-called "U-shaped pattern" (Fig. 1). Voluntary activation of the tested muscle by sustained contraction for 30 to 60 seconds or longer often produces characteristic repetitive nerve stimulation findings in patients with postsynaptic disorders such as MG. Immediately after activation of a muscle in a patient with MG, there may be a modest increase in the size of the baseline CMAP and a "repair of the decrement" (Fig. 1D). After 2 to 5 minutes, a worsening of the decremental response compared with preexercise values or postactivation exhaustion (PAE) is seen (Fig. 1E). In some muscles, particularly in patients with mild disease, an abnormal decrement may be seen only during the PAE stage.

Repetitive nerve stimulation studies demonstrate an abnormal decrement in an extremity muscle (hand or shoulder) in only ~60% of patients with MG, being more sensitive in generalized disease. Repetitive nerve stimulation is more likely to be abnormal in a proximal or facial muscle in patients with MG,[9] and multiple muscles should be tested to obtain the maximal diagnostic yield. A list of commonly tested nerves and the advantages and disadvantages of each is given in Table 1.

The ulnar, median, and musculocutaneous nerves are useful if the patient's symptoms are most prominent in the extremities. When signs and symptoms are limited to ocular or bulbar muscles, it is not unusual for RNS studies to be completely normal if only extremity muscles are tested. The yield may be improved by testing facial muscles or the masseter, particularly in patients with purely ocular or oculobulbar weakness.[12] Most laboratories consider that a complete evaluation for neuromuscular junction disease includes repetitive nerve stimulation testing of at least three muscles. A reproducible decrement of greater than 10% in two muscles constitutes a definite electrodiagnosis of a primary defect in neuromuscular transmission, as long as primary disorders of nerve and muscle are excluded. Patients with neuropathic disease, particularly when associated with active denervation/reinnervation (e.g., amyotrophic lateral sclerosis) may have an abnormal decrement on RNS testing.[13] Once a proximal or facial muscle is tested and is negative in a patient with predominantly oculobulbar symptoms, it is reasonable to proceed directly to SFEMG.

SFEMG

SFEMG is a selective recording technique in which a specially constructed concentric needle is used to identify and record action potentials from individual muscle fibers. SFEMG is the most sensitive clinical test for detection of a defect in neuromuscular transmission. Its sensitivity allows for demonstration of abnormalities in

Table 1 Nerves and Muscles Tested with RNS

Nerve	Muscle	Advantages	Disadvantages
Ulnar	ADM	Well tolerated, easy, reliable recordings	Insensitive
Median	APB	Well tolerated, easy, reliable recordings	Insensitive
Spinal accessory	Trapezius	Sensitive, well tolerated	Difficult to exercise
Musculocutaneous	Biceps	Sensitive	Difficult to immobilize
Axillary	Deltoid	Sensitive	Immobilization and stimulation difficult
Peroneal	EDB	Easy to stimulate and exercise	Insensitive
Facial	Nasalis or orbicularis oculi	Sensitive	Poorly tolerated, immobilization and stimulation difficult
Phrenic	Diaphragm	Probably sensitive	Poorly tolerated, technically difficult

RNS, repetitive nerve stimulation; ADM, adductor digit minimi; APB, abductor pollicis brevis; EDB, extensor digitorum brevis.

clinically unaffected muscles.[14] When the motor nerve is stimulated or activated voluntarily, the latency from nerve activation to muscle action potential varies from discharge to discharge. This variation is the neuromuscular jitter and is produced by fluctuations in the time it takes for the EPP at the neuromuscular junction to reach the threshold for action potential generation. These fluctuations are in turn due to the normally varying amount of ACh released from the nerve terminal after a nerve impulse. A small amount of jitter is seen in normal muscles due to this phenomenon. An increase in the magnitude of this jitter is the most sensitive electrophysiological sign of a defect in neuromuscular transmission. When the defect in neuromuscular transmission is more severe, some nerve impulses fail to elicit action potentials and SFEMG recordings demonstrate intermittent impulse blocking. SFEMG recordings demonstrate this as an intermittent absence of one or more single muscle fiber action potentials on consecutive firings (Fig. 2).

SFEMG studies can be performed during either mild voluntary activation of the muscle under study or with axonal microstimulation. Jitter measurements performed during voluntary activation of the muscle are less subject to technical problems but are more dependent on patient cooperation. As the patient minimally contracts the muscle under study, the examiner positions the recording electrode to record two or more time-locked action potentials. A constant recording position is maintained until at least 50 discharges are recorded (Fig. 3). At least 20 potential pairs from different areas in the muscle should be sampled, taking care not to measure the same pair of potentials more than once. Jitter is measured as the variation in the time interval between the two action potentials in the pair (interpotential interval) and represents the combined jitter in two end plates.

In jitter studies performed with axonal microstimulation, action potentials from single muscle fibers are recorded during stimulation of motor nerve fibers with a monopolar needle inserted near the nerve.[15] The stimulus to response interval is determined for a series of 50 to 100 stimulations, and the variability of these intervals is a measure of the jitter in a single end plate. This technique is prone to several pitfalls and misinterpretations but is useful when patients cannot sustain muscle contraction.[16] Jitter is calculated as the mean difference between consecutive interpotential intervals (or stimulus response intervals).The mean jitter of all fiber pairs or end plates, the percentage with normal jitter, abnormal jitter, and

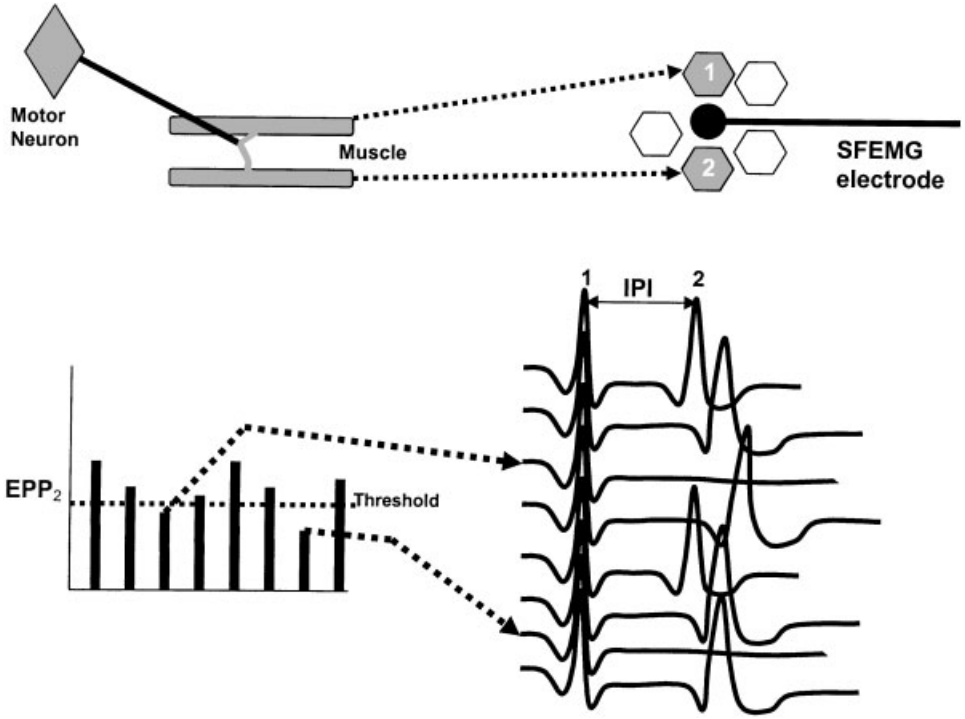

Figure 2 Method of single-fiber electromyography with voluntary activation. The single-fiber needle is inserted into voluntarily activated muscle and is positioned so that recordings are obtained from two or more single muscle fibers belonging to the same motor unit. One single muscle fiber action potential serves as a time reference and the interpotential interval (IPI) is measured after consecutive discharges between the reference potential and subsequent time-locked potentials. In disorders of the neuromuscular junction, there may be marked variability of the IPI (abnormal jitter). If severe, neuromuscular transmission failure may occur in which the EPP amplitude fails to reach the threshold for action potential generation. This is demonstrated here by the absence of the second recorded fiber pair when the EPP in the second muscle fiber (EPP_2) is subthreshold (dotted lines and arrows).

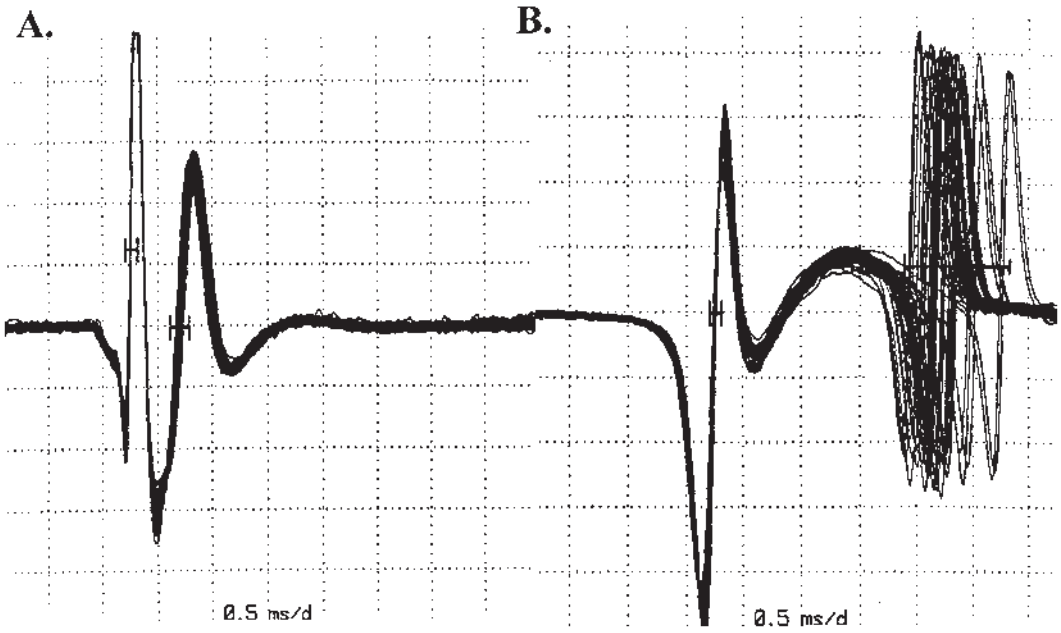

Figure 3 Jitter recordings obtained during voluntary activation from the extensor digitorum communis muscle in (A) a normal control subject (100 consecutive discharges are superimposed) and (B) a patient with myasthenia gravis (52 consecutive discharges are superimposed). Horizontal lines with brackets indicate the trigger windows within which the interpotential intervals are measured.

impulse blocking are calculated and reported for each muscle tested. A study is abnormal if the mean jitter of all fiber pairs (or end plates) exceeds the upper limit of normal for that muscle, or if more than 10% of pairs or end plates have jitter that exceeds the upper limit of normal for jitter in that muscle. Reference values for jitter during voluntary activation have been determined for several muscles in a multicenter collaborative study.[17] Normal jitter values for axonal stimulation studies have been determined for some muscles.[18] For other muscles, the normal values for stimulated jitter can be calculated by dividing the values for voluntarily activated jitter by 1.4.

SFEMG demonstrates increased jitter in virtually all patients with MG if appropriate muscles are tested. Jitter is greatest in weak muscles but is usually increased even in muscles with normal strength. A list of frequently tested muscles is given in Table 2. Facial muscles are often more abnormal than limb muscles in most MG patients, and it may be necessary to test several muscles

Table 2 Muscles Tested with SFEMG

Muscle	Advantages	Disadvantages
EDC	Easy to activate and test, relatively free of age-related changes	Not as sensitive in ocular or oculobulbar disease
Orbicularis oculi	Sensitive in generalized or purely ocular disease	Thin muscle: five to six separate insertions necessary to prevent duplicate testing of fiber pairs; difficult to maintain steady activation
Frontalis	Sensitive in generalized or purely ocular disease, easily activated	Thin muscle: five to six separate insertions necessary to prevent duplicate testing of fiber pairs
Masseter	Potentially useful in predominantly bulbar weakness; easily activated	Normal values not determined, sensitivity not established
Neck extensors	May be most abnormal muscle in some patients with MuSK-positive MG; easily activated	Normal values not determined, sensitivity not established
Deltoid	Alternative to facial muscle in generalized disease	Sensitivity not established, difficult to activate selectively
Biceps	Alternative to facial muscle in generalized disease	Sensitivity not established, difficult to activate selectively
Quadriceps	Potentially useful with predominant weakness in lower extremities	Sensitivity not established, difficult to activate selectively
TA	Potentially useful with predominant weakness in lower extremities	Sensitivity not established, false-positive result a concern as often involved in root/peripheral nerve disease

SFEMG, single-fiber electromyography; EDC, extensor digitorum communis; TA, tibialis anterior.

to demonstrate abnormal jitter in patients with mild or purely ocular weakness. The finding of normal jitter in a clinically weak muscle essentially rules out a defect in neuromuscular transmission as a cause for the weakness. It is important to understand that the enhanced sensitivity of SFEMG comes at the price of reduced specificity. Jitter may be increased in primary nerve or even muscle disease, and these disorders must be excluded by the appropriate electrophysiological and clinical examinations before concluding that the patient has MG.[19,20]

SEROLOGICAL (IMMUNOLOGICAL) TESTING

Anti-AChR Antibodies

Antibodies to the AChR and their effects on AChR number and function are generally regarded as the most specific diagnostic markers for MG. Antibodies binding to the AChR are present in ~85% of patients with MG as measured by the conventional radioimmunoprecipitation assay.[21,22] The serum level of AChR binding antibodies varies widely among patients with similar degrees of weakness. The concentration of antibodies may be low at symptom onset and become elevated later; thus, repeat testing may be appropriate when initial values are normal. Normal AChR binding antibody concentrations do not exclude the diagnosis, even in generalized MG. Virtually all MG patients with thymoma will have elevated AChR binding antibodies. The diagnostic specificity for MG is quite good, although increased titers may rarely be found in autoimmune liver disease, systemic lupus, inflammatory neuropathies, amyotrophic lateral sclerosis, as well as in patients with thymoma without MG, in first-degree relatives of patients with acquired autoimmune MG, and in patients with Lambert-Eaton myasthenic syndrome.[23]

AChR modulating antibodies bind to exposed segments of the AChR on skeletal muscle membranes.[24] Elevated levels of these antibodies are typically found in generalized MG and MG associated with thymoma, but are most useful when the AChR binding assay is negative, which occurs in ~3 to 4% of patients.[24] AChR blocking antibodies bind at or near the neurotransmitter binding site on the skeletal muscle AChR and are detectable by a modified immunoprecipitation assay.[24] They are found in only 1% of MG patients without AChR binding antibodies, making them of limited diagnostic utility.

Antibodies to Striated Muscle or Striational Autoantibodies

Antibodies to striated muscle (StrAb) were the first autoantibody discovered in MG.[23] They are reactive with contractile elements of skeletal muscle. They are elevated in 30% of all adult-onset MG and are highly associated with thymoma, being positive in 80% of MG patients with thymoma and 24% of patients with thymoma without MG.[23,25] The absence of StrAbs does not exclude thymoma, and their presence is not absolutely indicative of thymoma, particularly in elderly patients.[23] Antibodies to striated muscle are most useful as a marker of thymoma in patients with MG onset before age 40. Antibodies to striated muscle may also be a valuable marker in middle-aged or elderly patients with mild MG, where they can be the only serological abnormality. False-positives rarely occur in patients with rheumatoid arthritis who are treated with penicillamine, in 3 to 5% of patients with Lambert-Eaton myasthenic syndrome, and in recipients of bone marrow allografts with graft-versus-host disease.[23]

Antibodies to Other Skeletal Muscle Proteins

Some patients with MG have antibodies directed against skeletal muscle antigens in addition to the AChR. Antibodies to the intracellular striated muscle protein titin and antibodies to the ryanodine receptor may be found in MG patients with thymoma and in a proportion of patients with late-onset MG. Approximately 95% of MG patients with thymoma have titin antibodies, but so do 50% of patients with late-onset nonthymomatous MG.[26] Ryanodine antibodies are found in 75% of MG patients with thymoma but are more specific and are often associated with the presence of a malignant thymoma.[26] The role of these antibodies in disease pathogenesis has not been determined, although patients with titin and/or ryanodine antibodies tend to have more severe disease and may be less responsive to treatment. Therefore, the presence of these antibodies may potentially be useful in assessing disease prognosis and treatment.

"Seronegative MG"

It is becoming more apparent that patients with "seronegative" generalized MG (SNMG) have distinctive forms of disease compared with AChR antibody-positive MG. A proportion of patients with generalized SNMG have been found to have immunoglobulin G antibodies to the MuSK, a neuromuscular junction protein that plays an important role in the clustering of AChRs. MuSK antibodies have been reported in 38 to 71% of patients with generalized SNMG[27–29] but have not been found in AChR antibody-positive or ocular MG. More recent studies indicate that the true incidence is probably on the order of 40 to 50% of AChR antibody-negative, generalized MG patients. MuSK-positive patients may have atypical presentations characterized by prominent facial, bulbar, neck, shoulder, and respiratory muscle involvement with relative sparing of

ocular muscles. In many of these patients, abnormal neuromuscular transmission was preferentially demonstrated by RNS or SFEMG testing of weak muscles, particularly those with weakness restricted to facial or shoulder muscles. They frequently do not respond to cholinesterase inhibitors but improve with plasma exchange and selected immunotherapy.[29] MuSK antibodies may alter the normal maintenance of a high density of AChRs at the neuromuscular junction, but the confirmation and mechanism of their pathogenic effect has not been demonstrated. Studies in SNMG patients without MuSK antibodies indicate that their plasma reduces AChR function in cell culture assays.[21] The mechanism of this action and the circulating factor(s) responsible for this effect remain to be defined.

COMPARISON OF DIAGNOSTIC TESTS

The edrophonium (Tensilon) test is readily accessible, easy to perform, and abnormal in most patients with MG who have ptosis or ophthalmoparesis. Unfortunately, the results are not entirely objective and a positive test should be confirmed by more objective means (electrophysiological or serological testing). RNS is the least sensitive of the diagnostic techniques, but has the advantage of being widely available and relatively simple to perform. Although SFEMG is clearly the most sensitive technique, it requires special equipment and training and is time- and labor-intensive. It also detects neuromuscular transmission abnormalities in primary disorders of nerve and muscle, and these must be excluded before a diagnosis of MG is made. SFEMG is most valuable in patients with mild or purely ocular or oculobulbar disease, particularly when immunological tests are negative. It is also valuable in excluding a disorder of neuromuscular transmission, as the finding of normal jitter in a weak muscle indicates the weakness is not due to a defect in neuromuscular transmission. Currently, detection of elevated levels of AChR binding antibodies is the most specific test for MG, although levels are normal in up to 15% of patients. A proportion of MG patients with AChR antibody-negative, generalized disease may have detectable MuSK antibodies. The sensitivity of the various diagnostic tests in MG[30] is shown in Table 3.

Table 3 Comparison of Diagnostic Tests in 550 Patients with MG[31]

	Edrophonium	RNS	AChR-ab	SFEMG
Generalized (%)	91	76	80–85	99
Ocular (%)	84	48	55	97

MG, myasthenia gravis; RNS, repetitive stimulation in distal hand and shoulder muscle; AChR-ab, acetylcholine receptor binding antibodies; SFEMG, single-fiber electromyography of at least one muscle.

DIAGNOSTIC APPROACH

Once the clinical diagnosis of MG is made, blood samples for AChR binding antibodies and striational antibodies should be obtained. The latter is obtained mainly to increase diagnostic yield in late-onset MG and to assess for thymoma in younger patients. In most cases, these tests will require up to 10 days for results to become available. A provisional diagnosis may be made using the edrophonium test if the patient has clear eyelid ptosis or ophthalmoparesis that can be serially followed. Electrophysiological testing offers the opportunity to make a more definitive diagnosis. Patients with generalized MG, particularly with limb weakness, may be evaluated using RNS studies, which should optimally be performed in a weak muscle. Once a proximal or facial muscle is negative, it is reasonable to proceed to SFEMG. In patients without clinical limb weakness, initial evaluation with SFEMG is appropriate. Once the diagnosis is confirmed by either electrophysiological or serological testing, computed tomography (CT) of the chest should be obtained to rule out the presence of an underlying thymoma. Titin and/or ryanodine antibodies may be useful in predicting the presence of thymoma in certain cases, but the added yield compared with a chest CT is questionable. MG patients with generalized disease who do not have AChR antibodies should be tested for MuSK antibodies, particularly if their clinical presentation is atypical.

REFERENCES

1. Osserman KE, Kaplan LI. Rapid diagnostic test for myasthenia gravis: increased muscle strength, without fasciculations after intravenous administration of edrophonium (Tensilon) chloride. J Am Med Assoc 1952;150:265–268
2. Katz B, Miledi R. The binding of acetylcholine to receptors and its removal from the synaptic cleft. J Physiol 1973;231: 549–574
3. Daroff RB. The office Tensilon test for ocular myasthenia gravis. Arch Neurol 1986;43:843–844
4. Dirr LY, Donofrio PD, Patton JF, Troost BT. A false-positive edrophonium test in a patient with a brainstem glioma. Neurology 1989;39:865–867
5. Fierro B, Croce G, Filosto L, Carbone N, Lupo I. Ocular pseudomyasthenia: report of a case with a pineal region tumor. Ital J Neurol Sci 1991;12:593–596
6. Branley MG, Wright KW, Borchert MS. Third nerve palsy due to cerebral artery aneurysm in a child. Aust N Z J Ophthalmol 1992;20:137–140
7. Rowland LP. Prostigmine-responsiveness and the diagnosis of myasthenia gravis. Neurology 1955;5:612–624
8. Viets HR, Schwab RS. Prostigmine in the diagnosis of myasthenia gravis. N Engl J Med 1935;213:1280–1283
9. Howard JF, Sanders DB, Massey JM. The electrodiagnosis of myasthenia gravis and the Lambert-Eaton myasthenic syndrome. Neurol Clin 1994;12:305–329
10. McComas AJ, Galea V, Einhorn RW. Pseudofacilitation: a misleading term. Muscle Nerve 1994;17:599–607

11. Borenstein S, Desmedt JE. Local cooling in myasthenia. Improvement in neuro- muscular failure. Arch Neurol 1975; 32:152–157
12. Niks EH, Badrising UA, Verschuuren JJ, van Dijk JG. Decremental response of the nasalis and hypothenar muscles in myasthenia gravis. Muscle Nerve 2003;28:236–238
13. Killian JM, Wilfong AA, Burnett L, Appel SH, Boland D. Decremental motor responses to repetitive nerve stimulation in ALS. Muscle Nerve 1994;17:747–754
14. Sanders DB, Stlberg E. AAEM minimonograph # 25: single-fiber electromyography. Muscle Nerve 1996;19:1069–1083
15. Trontelj JV, Stlberg EV. Jitter measurement by axonal microstimulation. Guidelines and technical notes. Electroencephalogr Clin Neurophysiol 1992;85:30–37
16. Stlberg E, Trontelj JV, Mihelin M. Electrical microstimulation with single-fiber electromyography: a useful method to study the physiology of the motor unit. J Clin Neurophysiol 1992;9:105–119
17. Gilchrist JM. ad hoc Committee. Single fiber EMG reference values: a collaborative effort. Muscle Nerve 1992;15:151–161
18. Trontelj JV, Khuraibet A, Milhelin M. The jitter in stimulated orbicularis oculi muscle: technique and normal values. J Neurol Neurosurg Psychiatry 1988;51:814–819
19. Mercelis R. Abnormal single fiber electromyography in patients not having myasthenia. Ann N Y Acad Sci 2003; 998:509–511
20. Sanders DB. Clinical impact of single-fiber electromyography. Muscle Nerve 2002;11(suppl):S15–S20
21. Vincent A, McConville J, Farrugia E, et al. Antibodies in myasthenia gravis and related disorders. Ann N Y Acad Sci 2003;998:324–335
22. Vincent A, Newsom-Davis J. Acetylcholine receptor antibody as a diagnostic test for myasthenia gravis: results in 153 validated cases and 2967 diagnostic assays. J Neurol Neurosurg Psychiatry 1985;48:1246–1252
23. Lennon VA. Serological diagnosis of myasthenia gravis and Lambert-Eaton myasthenic syndrome. In: Lisak RP, ed. Handbook of Myasthenia Gravis and Myasthenic Syndromes. New York: Marcel Dekker; 1994:149–164
24. Howard FM, Lennon V, Finley J, Matsumoto J, Elveback LR. Clinical correlations of antibodies that bind, block or modulate human acetylcholine receptors in myasthenia gravis. Myasthenia gravis: biology and treatment. Ann N Y Acad Sci 1987;505:526–538
25. Cikes N, Momoi MY, Williams CL, et al. Striational autoantibodies: quantitative detection by enzyme immunoassay in myasthenia gravis, thymoma, and recipients of D-penicillamine or allogeneic bone marrow. Mayo Clin Proc 1988;63:474–481
26. Skeie GO, Romi F, Aarli JA, Bentsen PT, Gilhus NE. Pathogenesis of myositis and myasthenia associated with titin and ryanodine receptor antibodies. Ann N Y Acad Sci 2003; 998:343–350
27. Hoch W, McConville J, Helms S, Newsom-Davis J, Melms A, Vincent A. Auto-antibodies to the receptor tyrosine kinase MuSK in patients with myasthenia gravis without acetylcholine receptor antibodies. Nat Med 2001;7: 365–368
28. Scuderi F, Marino M, Colonna L, et al. Anti-P110 autoantibodies identify a subtype of "seronegative" myasthenia gravis with prominent oculobulbar involvement. Lab Invest 2002;82:1139–1146
29. Sanders DB, El-Salem K, Massey JM, McConville J, Vincent A. Clinical aspects of MuSK antibody positive seronegative MG. Neurology 2003;60:1978–1980
30. Sanders DB. Diseases associated with disorders of neuromuscular transmission. In: Brown WF, Bolton CF, Aminoff MJ, eds. Neuromuscular Function and Disease. Basic, Clinical, and Electrodiagnostic Aspects. Philadelphia: W.B. Saunders; 2001:1346–1347