Testing for Malignant Hyperthermia

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THE first descriptions of the syndrome that would eventually be named malignant hyperthermia (MH) were made in the early 1960s.1 By 1970, it became clear that alterations in skeletal muscle constituted the primary defect in MH. Through the collaboration of an outstanding pharmacologist, Werner Kalow, and a thoughtful clinical anesthesiologist, Beverly Britt, the basic elements of the common test for MH were described. Kalow and Britt subjected biopsied muscle to graded increments of caffeine, demonstrating a leftward shift of the dose–response curve in muscle from patients who had experienced an episode of MH.2 Ellis et al.3 described how muscle from these patients developed contractures to clinical concentrations of halothane in vitro. Between 1970 and 1985, testing was standardized (e.g., which muscle to biopsy, exposure regimens, diagnostic thresholds) to develop an accurate diagnostic test for MH. Two slightly different protocols emerged: a European version and a North American version, both using halothane alone and caffeine alone as the testing reagents.4,5 Meanwhile, in Japan, a slightly different approach was developed using muscle fibers that were chemically or mechanically "skinned." This review summarizes the clinical evaluation of the patient with presumed MH susceptibility (MHS) and describe tests that are used in this evaluation.

Appropriate Candidates for Biopsy

In general, suitable patients for biopsy include those with a family history of MH or who display unexplained hypercarbia, and those who exhibit perioperative rhabdomyolysis (table 1). By itself, massive rhabdomyolysis in association with hyperkalemia and cardiac arrest is usually not MH. Nonetheless, some experts advise muscle biopsy, as much for histology and analysis for dystrophin to detect muscular dystrophy as to exclude MH. Exercise-induced rhabdomyolysis has also been associated with MH.6 MH is a hypermetabolic disorder, and many other diseases and conditions can mimic it. Modern anesthetic techniques and drugs seem to retard the development of an MH episode, relative to the classic fulminant episode, e.g., marked hypermetabolism, hyperthermia, acidosis, rhabdomyolysis, and cardiac arrhythmias. This led to the development of a clinical grading scale to estimate the likelihood that an MH episode has occurred.7 The scale incorporates six clinical criteria: evidence of muscle rigidity, muscle breakdown, respiratory acidosis, temperature increase, cardiac involvement, and family history (table 2). The likelihood of MH increases if the patient has manifested multiple signs of MH. However, in the absence of certain laboratory values or incomplete vital signs, the grading scale loses power and might underestimate the probability of MH. The appropriate course is to have the patient and family evaluated for MHS at an MH biopsy center; this might include biopsy of both parents of a patient with possible MHS.

Anesthesia for the Biopsy

Regional anesthesia using nerve blocks or neuroaxial blocks is usually employed for muscle biopsy, supplemented with sedation as needed. Some clinicians supplement a nontrigger general anesthetic with a nerve block. Direct muscle infiltration with local anesthetic is contraindicated because it could adversely affect tissue viability. Most centers do not perform biopsies in patients aged less than 5 yr because of the amount of muscle required to be harvested. The procedure is usually performed on an outpatient basis. Proper surgical technique is crucial. The surgeon must not use electrocautery or stretch the muscle.

Tests for MHS

The Caffeine–Halothane Contracture Test

The caffeine–halothane contracture test (CHCT) requires approximately 2 g of muscle excised from the vastus lateralis or vastus medialis muscle. In the laboratory, the muscle is longitudinally dissected into strips. Small sutures are placed at both ends, and the strips are mounted into baths, with one end attached to a station-
ary hook and the other to a force transducer. Electrical stimulation resulting in muscle contraction (twitching) confirms tissue viability. Six muscle strips are mounted. In the North American protocol, halothane (3%) is added to the gas flow to three baths via an in-line vaporizer, while caffeine is added incrementally to the other three.

The diagnostic end point is the development of a contracture, which is an increase in baseline muscle tension. If a contracture of 0.7 g or greater develops in any halothane-exposed muscle strip or if a contracture of 0.3 g or greater develops in any strip exposed to caffeine at 0.5, 1, or 2 mM, then the test is considered to be...
positive, and the patient has MHS (figs. 1A and B). Contractures of 0.5–0.69 g to 3% halothane are sometimes classified as MH equivocal. The addition of ryanodine or 4-chloro-m-cresol to the baths can increase the accuracy of the CHCT.8

It is important that a small amount of muscle undergoes microscopic examination to detect structural abnormalities, which might include central cores, internal nuclei, and marked variation in fiber diameter. Histologic examination by itself cannot be used to diagnose MHS specifically—the defect is functional, not structural.

Occasionally, the biopsy results might be normal, but microscopic examination reveals muscular dystrophy, in which case, the patient must not be administered succinylcholine because this could lead to massive rhabdomyolysis and life-threatening hyperkalemia. Many recommend avoidance of volatile anesthetics.

**European, Japanese, and North American Testing Protocols**

The European MH CHCT protocol differs slightly from the North American CHCT protocol.9 The European protocol uses more increments in the caffeine and halothane concentrations, which lead to lower diagnostic thresholds. Several studies have evaluated the outcome of the testing procedures when applied to muscle from the same patient. In one study, 1 of 48 patients was positive to the North American protocol but negative to the European protocol.9 In a second study, 5 of 74 patients tested positive with the European protocol but negative with North American protocol.10 Conversely, 8 of 48 patients who tested negative with the North American protocol were MH equivocal with the European testing protocol. In the second study, 9 of 74 patients

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**Fig. 1.** (A) Abnormal (positive) response to 3% halothane. Each small box represents 0.1 g tension. The contracture response in this case is 1.6 g. A normal response to 3% halothane is a contracture up to 0.7 g. After exposure to halothane, 32 mM caffeine is added to the bath to determine maximal response. (B) Abnormal (positive) response to caffeine. Caffeine exposure is to 0.5, 1.0, 2.0, 4.0, 8.0, and 32 mM for 4 min. A contracture of greater than 0.3 g to 2 mM caffeine or less indicates susceptibility.
who tested negative with the North American protocol were MH equivocal with the European version.\textsuperscript{10} In a recent study in which muscle was harvested and tested in one European center and a portion was transported and tested in a nearby center, there was a 12\% discordance in diagnostic conclusions for MHS. That is, one center determined that the patient had MHS, and the other determined that the patient was MH negative. This finding indicates that refinements in testing protocols are still required.\textsuperscript{13} In Japan, a different method has been used. Skeletal muscle fibers are chemically or mechan-ically “skinned,” \textit{i.e.}, the sarcolemma is removed, leaving the sarcoplasmic reticulum, actin, and myosin and other proteins intact. Contractures to halothane and caffeine are assessed. Insufficient data exist comparing the Japa-nese protocol to the European or North American protocols.

**Sensitivity, Specificity, and Other Problems**

To define the sensitivity, specificity, and predictive value of a diagnostic test, known positive subjects and known controls for the disorder must be studied, and the probability that the individual being tested has MH should also be estimated. Inasmuch as the phenotypic and clinical presentation is variable, researchers have relied on using individuals who have had an unequivocal MH episode. The diagnostic thresholds are adjusted to maximize sensitivity, thereby minimizing false negatives. Currently, the North American and European tests are 97–99\% sensitive (\textit{i.e.}, 1–3\% false negatives). This sacrifices specificity, so that 10–15\% of normal patients will have false-positive tests.\textsuperscript{12} The specificity of the European protocol (\approx 90\%) is greater than that of the North American protocol (\approx 80–85\%). For unclear reasons, the lower specificity in the North American protocol is related to center-to-center variability and not within-center variability. This specificity and low prevalence of susceptibility for MH limits the test as a screening tool.\textsuperscript{15} The consensus among MH experts is that it is probably safer to label a healthy person MH susceptible than to label an MH patient nonsusceptible. This is based on the thought that it is easier to administer a nontriggering anesthetic than to treat a patient with full-blown MH. The disadvantage of this approach is that not only are some patients falsely labeled, but so too are members of their families. Because there is a significant false-positive response and a low prevalence of MH (perhaps 1 in 50,000), the positive predictive value of the contracture test is low.\textsuperscript{15} By selecting those patients with a high likelihood of MH based on the clinical grading scale,\textsuperscript{7} the positive predictive value may be improved. However, the negative predictive value is excellent because of the test’s high sensitivity and the relative lack of effect of pretest probability.\textsuperscript{15} Other pharmacologic agents (primarily ryanodine and chlorocresol) that alter intracellular calcium homeostasis have been integrated into the standard testing procedures in hopes of minimizing intermediate responses.

**Other Considerations**

The CHCT has been standardized using freshly har vested tissue from the vastus lateralis or medialis mus cles, so the patient must present to the biopsy center. The test must be completed within approximately 5 h after muscle harvest. After the biopsy, there is a period of relative disability of 2–7 days, depending on one’s occupation and activity level. The cost of the procedure, based on operating room time, surgical and pathology fees, and testing costs, can easily reach $6,000. There are 22 biopsy centers in Europe and 12 in North America. Each center performs 10–40 biopsies per year.

For one of the few (if not only) remaining physiologic \textit{in vitro} diagnostic tests in medicine, the CHCT has served well. However, refinements of the testing procedure have continued for several reasons:

1. Although most specimens from a biopsy respond in an identical manner, some (perhaps 30\%) do not.\textsuperscript{11}
2. There is not a distinct separation of MH nonresponders and MH-susceptible patients; intermediate values are found in 10–15\% of patients. Therefore, it is necessary to determine a threshold to separate the groups.\textsuperscript{15}
3. The test involves biologic material that degrades with time, and it is not possible to create a standard that can be shared among all laboratories.
4. Patients with myopathies whose basic defect entails increased intracellular calcium, such as muscular dys trophy, may display abnormal contractures to halothane and caffeine.
5. The lack of a common structural defect in MH muscle diminishes the usefulness of histologic examination as a diagnostic test.

**Molecular Genetic Testing**

Most recently, the demonstration that a mutation in the gene that encodes the calcium release channel (the ryanodine receptor, RYR-1) underlies porcine MH increased the expectation of a simple DNA-based test for MH in humans as well.\textsuperscript{14} That expectation has not yet been realized. There are many mutations in skeletal mus cle that may be causal for different forms of the syndrome. However, with time, an accurate DNA-based test that is applicable to most patients will become available. A significant international effort is underway to clarify the molecular genetic basis for MH. At least four gene loci have been associated with MHS. However, linkage analysis reveals that approximately 50\% of MH families...
are genetically linked to the RYR-1 gene located on chromosome 19q 12-13. To date, 23 missense mutations in the gene have been reported to segregate with MHS in European and North American families. Most are clustered in two regions. Brandt et al.\(^\text{15}\) have reported that 25% of patients from 105 families diagnosed as MH susceptible by the CHCT have RYR mutations. Sambuughin et al.\(^\text{16}\) have reported similar findings, but the distribution of mutations in these North American families differed from those reported by Brandt et al.\(^\text{15}\) from Germany. The largest genetic analysis from one family showed variable concordance between genetic mutation in the RYR-1 gene and the CHCT, depending on the thresholds used for the CHCT.\(^\text{17}\) Discordance of phenotype and genotype in this family was likely due to a second mutation causative for MHS.\(^\text{17}\) A recent article and its accompanying editorial highlighted the quandary of genetic testing of MHS. Urwyler et al.\(^\text{18}\) described the heterogeneity of genetic defects in European patients with MHS. They emphasized that genetic testing cannot currently stand alone in the absence of contracture testing. Likewise, Robinson and Hopkins\(^\text{19}\) stated that although genetic testing must continue, contracture testing must continue to be the basis of MH evaluation. Therefore, we believe that when possible and appropriate, molecular genetic analysis should be used in some families to supplement the CHCT.

### Other Proposed Tests for MH

Several other tests have been proposed or are being investigated as less-invasive diagnostic tests. Approaches with limited or no value (calcium uptake–adenosine triphosphatase, lymphocyte calcium test, platelet adenosine triphosphatase) have been abandoned. Other tests show some promise of at least providing a less-invasive test to determine susceptibility of relatives of known MH positive individuals.

**Creatine Kinase**

Creatine kinase concentrations are chronically increased in perhaps 50% of MH patients. However, creatine kinase is increased in many myopathic diseases and may be increased secondary to trauma. Therefore, its sensitivity and specificity are not satisfactory for it to be used for routine testing. If a first-degree relative of a patient with known MHS has increased creatine kinase, they are highly likely to have MHS as well.\(^\text{20}\)

**Nuclear Magnetic Resonance Spectroscopy**

Nuclear magnetic resonance spectroscopy measures the concentrations of adenosine triphosphate, phosphocreatine, and other phosphononoesters, along with pH, both in vivo and noninvasively, in muscle and other tissue. Several studies have shown delayed reconstitution of pH, adenosine triphosphate, and increased phosphocreatine in MH patients during and after graded exercise. A recent study reports 100% concordance between abnormalities in adenosine triphosphate and high-energy phosphates produced by a specific exercise protocol and the results of muscle biopsy.\(^\text{21}\) Most patients were analyzed post hoc with nuclear magnetic resonance spectroscopy techniques; only 10 were analyzed prospectively using both nuclear magnetic resonance spectroscopy and halothane–caffeine testing. One drawback of nuclear magnetic resonance spectroscopy is the requirement for expensive, technologically sophisticated equipment, along with personnel trained in the interpretation of the spectra. It is also known that changes similar to those found in MH patients can be found in a variety of myopathies.

**Lymphocyte Tests**

Recently, Sei et al.\(^\text{22}\) have shown RYR-1 receptors on B lymphocytes in humans. This implied that these cells might demonstrate changes in calcium flux similar to those demonstrated in muscle. They found that lymphocytes from MH patients, when incubated with 4-chloro-m-cresol, showed increased intracellular calcium concentrations. However, halothane did not affect intracellular calcium concentrations.

**Cultured Muscle Cells**

Censier et al.\(^\text{23}\) have also shown enhanced intracellular calcium release from muscle cultured from MH-susceptible patients. Further studies (including patients with other muscle diseases) are required to define the sensitivity and specificity of the technique.

### Conclusions

The CHCT, by diagnosing MHS in a meaningful way, provides clinical information for patients and anesthesia providers. Genetic testing has emerged as a viable supplement to the CHCT. Far too much emphasis has been placed on the limitations of the contracture test (e.g., the 10–15% false-positive rate and the need for fresh viable muscle) without recognition of the test’s value. The following considerations are useful when judging the importance of the CHCT in evaluation of MHS:

1. In the vast majority of circumstances, differentiation can be made between normal and abnormal responders.
2. Establishing an MH-positive or -negative diagnosis has clinical significance for the individual and his or her family in terms of subsequent anesthetic treatment.
3. There are many families in which relatives of a patient who died from MH or presumed MH have clearly abnormal responses on the CHCT.
4. The inheritance of MHS as determined by the CHCT
follows a specific autosomal dominant pattern, just as the clinical syndrome does.

5. Similar patterns of response to halothane, caffeine, ryanodine, and 4-chloro-m-cresol have been found in testing centers throughout the world.

6. The objective responses observed in muscle tissue form the basis for identifying genetic mutations causal for MH. Although there are discrepancies between presence of ryanodine mutations and contracture responses to test agents, there are families in which there is excellent agreement. Identification of the genetic defect or defects in MH and related disorders will ultimately lead to a better understanding of the biochemical changes that underpin an MH reaction. Progress in identifying the genetic basis for MH will be limited without the CHCT. Continued testing is essential for the development of the next generation of less-invasive, hopefully more accurate techniques, such as genetic or lymphocyte testing based on blood samples.

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References

13. Loke JC, MacLennan DH: Bayesian modeling of muscle biopsy contracture testing for malignant hyperthermia susceptibility. Anesthesiology 1998; 88:589–600

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