EDITORIAL VIEWS

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Should We Use Muscle Biopsy to Diagnose Malignant Hyperthermia Susceptibility?

In this issue of ANESTHESIOLOGY, Isaacs and Badenhorst[1] report, for the first time, on caffeine halothane muscle contracture test false-negative results. Although the caffeine halothane contracture test (CHCT) is the "gold standard" for diagnosing malignant hyperthermia (MH) susceptibility, the authors convincingly present four patients with negative CHCT results despite clear-cut clinical evidence of MH susceptibility. Case 1 is particularly compelling because this patient survived two fulminant MH episodes (including a cardiac arrest), had a positive family history for MH (maternal death from presumed MH), and yet tested negatively on two separate caffeine halothane contracture assays. Isaacs and Badenhorst conclude their paper with the assertion that CHCT is still ". . . the best test for MH and is of inestimable value."[1] How can the evidence presented possibly support such a conclusion? To answer this question, we need to review the way we evaluate the performance of any clinical diagnostic test.

First, we must define the disease or the condition under evaluation before evaluating the performance of a diagnostic test. For example, clinicians need to reach agreement on the clinical definition of eosinophilia-myalgia syndrome before they can determine the sensitivity and specificity of an elevated sedimentation rate for this syndrome's diagnosis.[2] Fortunately, international MH experts recently agreed on an MH clinical case definition that will facilitate the evaluation of MH diagnostic tests.[3]

Second, we must obtain test specimens from those individuals who clearly demonstrate the disease condition (positive controls) and from those who clearly are free of the disease condition (negative controls). Ideally, control specimens should be exchanged among different diagnostic laboratories and used multiple times to standardize test outcomes. This cannot be done with CHCT because large specimens of fresh muscle are required within 5 h of muscle excision.[4,5] Negative controls rarely are tested at the same time as diagnostic MH specimens. Since it is impossible to routinely and repeatedly excise large specimens of muscle from known MH-susceptible individuals, MH diagnostic laboratories rely on few human and many porcine positive control specimens to validate their diagnostic methods. Epidemiologic studies by international MH registries may be used to compare the performance of one diagnostic center with another[6] because exchange of control specimens among laboratories is difficult.

Third, we must use positive and negative control specimens to calibrate a diagnostic test. A positive diagnostic test result (true-positive) should be obtained when positive control subjects (diseased individuals) are tested. A negative diagnostic test result (true-negative) should be obtained when negative control subjects (healthy individuals) are tested. A false-negative result occurs when a diseased individual has a negative test result. A false-positive result occurs when a healthy individual has a positive test result. Sensitivity of a test is defined as the percentage of positive test results in a diseased population and is calculated from the formula: 100 × [true-positives/(true-positives + false-negatives)]. Specificity of a test is defined as the percentage of negative test results in the absence of disease and is calculated from the formula: 100 × [true-negatives/(true-negatives + false-positives)]. Sensitivity and specificity are stable properties of a test that are uninfluenced by disease prevalence, *e.g.*, the number of subjects with the disease per 100,000 population.

Fourth, when we select a particular diagnostic test or test cutoff points to delineate positive from negative results, we must make a tradeoff between test sensitivity and specificity. A test rarely achieves both 100% sensitivity and 100% specificity. Test sensitivity should be high (preferably 100%, eliminating false-negatives) at the expense of reduced test specificity if the following conditions apply: (1) failure to detect the disease causes potentially severe or fatal outcomes, (2) ability to detect the disease leads to effective prevention or

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treatment, and (3) false-positive results do not produce significant emotional or economic harm to the patient. Thus, we wish to select a test with nearly 100% sensitivity for diseases such as pheochromocytoma and MH. On the other hand, test specificity should be high (preferably 100%, eliminating false-positives) at the expense of reduced test sensitivity if the following conditions apply: (1) a disease is serious but without effective treatment or cure, (2) knowledge that the disease is absent improves emotional or public health, and (3) false-positive results can produce serious emotional or economic harm to the patient.[7] An example of such a disease might be Huntington's chorea.

The North American Malignant Hyperthermia Registry has published, in a preliminary form, data demonstrating CHCT sensitivity of 100% (one-sided 95% confidence interval 88.3%) and specificity of 78% (one-sided 95% confidence interval 70.9%) when North American contracture cutoff points are modified.[3] This sensitivity and specificity compares favorably with elevated creatine phosphokinase-MB enzyme values for sensitivity (100%) and specificity (85%) for the diagnosis of acute myocardial infarction.[8]

Fifth, we should determine the predictive value of positive and negative test results, which also requires study of disease prevalence. With the exception of Denmark[9] and Austria,[10] the prevalence of MH in most of the world has not been scientifically reported but is thought to be very low. The predictive value of a positive test result is defined as the percentage of positive results that are true-positives as measured by a "gold standard" and is calculated from the formula: 100 × [true-positives/(true-positives + false-positives)]. The predictive value of a negative test result is defined as the percentage of negative results that are true-negatives as measured by a "gold standard" and is calculated from the formula: 100 × [true-negatives/(false-negatives + true-negatives)].

As prevalence of a disease decreases, the predictive value of a positive test result will decrease even for highly sensitive and adequately specific diagnostic tests. If the prevalence of a disease within the test population is increased through careful selection without improving test sensitivity or specificity, then the predictive value of a positive test result will increase. The predictive value of a positive CHCT result can be improved by selecting for biopsy only those individuals who have significant clinical risk factors for MH susceptibility. However, as prevalence of a disease increases within a test population, the predictive value of a negative test result will decrease even if test sensitivity and specificity are unchanged. Given the low prevalence of MH within the general population, the false-negative test results reported by Isaacs and Badenhorst would be remarkable unless they had appropriately increased MH prevalence within their test population through prescreening subjects for high risk of MH susceptibility. Since prevalence of MH is unknown, the predictive value of CHCT cannot be calculated.

The European and North American Malignant Hyperthermia Groups have developed somewhat different protocols for the caffeine halothane muscle contracture test.[4,5] Isaacs and Badenhorst use the European protocol, which differs from the North American in several ways. European group members expose muscle to incremental doses of halothane rather than a single dose of 3% halothane. All diagnostic centers using the European protocol agree that a muscle contracture response of ≥ 0.2 g is abnormal. In contrast, North American diagnostic group members have proposed a range of values for abnormal threshold responses with the individual diagnostic laboratory director responsible for choosing the specific cutoff point for his or her laboratory. With the European protocol, muscle must contract abnormally to the separate administration of both halothane and caffeine test agents for the test to be interpreted as positive and the patient designated MH-susceptible. If muscle contracts abnormally to only one test agent, then a European laboratory will designate the test result as equivocal even though the patient with this test result will be managed clinically as MH-susceptible. North American protocol designates even one abnormal contracture response to a single test agent as a positive result. In contrast to the North Americans, the Europeans do not have a central registry to collect and analyze individual diagnostic laboratory CHCT responses.

Will other MH diagnostic tests, including molecular genetic techniques, be developed in the next decade to replace the CHCT? Gillard *et al.* have found that a substitution of cysteine for arginine 614 in the ryanodine receptor (calcium release channel of skeletal muscle sarcoplasmic reticulum) on chromosome 19q13.1 cosegregates with MH susceptibility in 1 of 35 MH-susceptible families.[11] Levitt *et al.* have demonstrated that MH susceptibility in 5 of 16 families appears to be linked to a different chromosome, 17q11.2-q24.[12] Further work by Olckers *et al.* suggests that a gene, localized to chromosome 17, that encodes the adult muscle sodium channel α-subunit may harbor

a primary mutation in certain forms of MH susceptibility.[13] It is probable that additional mutations causative for MH will be identified. If MH genetic heterogeneity is extensive, then molecular genetics may never provide anesthesiologists with a preoperative screening test for MH susceptibility, and genetics testing may be restricted to selected, well characterized families.

Multiple researchers have investigated other noninvasive techniques that fail to discriminate between MH-susceptible and -nonsusceptible individuals, including measurement of cytosolic-free calcium concentrations in lymphocytes,[14] spin resonance spectroscopy of erythrocyte membranes,[15] and phosphorus magnetic resonance spectroscopy of muscle.[16] CHCT likely will remain the sole clinical MH diagnostic test for discriminating between MH-susceptible and -nonsusceptible individuals for the next decade.

What are the implications and limitations of the Isaacs and Badenhorst report of false-negative CHCT results? I agree with the authors that all clinical biologic tests will have false-negative results. No single clinical test for any disease will diagnose successfully all human subjects, because the subjects have a mixed genetic structure and are exposed to many environmental influences. The Isaacs and Badenhorst report emanates from a single diagnostic laboratory that uses the European protocol. This report of a false-negative rate of at least 2.3% (4 of 171 patients) cannot be extrapolated to other MH diagnostic laboratories, especially to those using the North American protocol, because the European and North American protocols differ in several ways. The false-negative rate for other individual European and North American diagnostic centers is thought to be low,[17,18] but has not been reported extensively.

Should we continue to subject patients with a clear-cut history of a fulminant MH episode to caffeine halothane contracture testing? Patients who have experienced severe MH reactions similar to Case 1 are rare and have represented less than 1% of all patients undergoing diagnostic testing in North America. I believe that this rare subgroup of patients may, with informed consent, be subjected to caffeine halothane contracture testing so that they may serve as positive controls for MH diagnostic testing. I agree with Isaacs and Badenhorst that, for patients who experience fulminant MH episodes similar to that of Case 1, a negative CHCT result should not lead to rechallenge with MH triggering anesthetic agents, because another life-threatening event may occur. We urge continued reporting of these patients to The North American Malignant Hyperthermia Registry* so that we can study further risk factors associated with their presentation and devise better treatment modalities to decrease MH morbidity and mortality.

If CHCT can yield false-negative results, why bother testing any patients for MH susceptibility? Wouldn't it be safer to label as susceptible all patients at possible risk since one can anesthetize patients with nontriggering agents? I believe that this is an incorrect course because whenever one labels an individual susceptible to an inherited disease, the family is labeled susceptible as well. Indiscriminate labeling soon leads to significant numbers of patients who will have to be managed as MH-susceptible requiring deviation from current practice of frequent potent inhalational anesthetic administration. The risks of anesthesia for patients labeled as MH-susceptible increase further when they have coexisting difficult airways (including epiglottitis), full stomachs, asthma, or tetralogy of Fallot. Individuals labeled as MH-susceptible are not eligible for military service and may have difficulty obtaining medical care, dental services, and insurance coverage.

I would assert that, as with other medical conditions, diagnostic testing should continue to be performed on those judged to be at significant risk for MH susceptibility. The Malignant Hyperthermia Association of the United States MH experts† may be consulted to help evaluate which patients are at significant risk and deserve further diagnostic evaluation. Although the predictive value of a negative CHCT result is likely to be very high, patients receiving triggering anesthetic agents after negative tests should have appropriate monitoring (including continuous core temperature and expired carbon dioxide monitoring), and their anesthesiologists should have immediate access to adequate supplies of dantrolene. We urge anesthesiologists to continue to report anesthetic outcomes for contracture-negative patients so that we can study test sensitivity further and evaluate the wisdom of these recommendations.*

* Confidential reporting forms can be obtained from The North American Malignant Hyperthermia Registry by writing the author or calling 717-531-6936.

† Malignant hyperthermia experts may be reached for nonemergent questions by phoning the Malignant Hyperthermia Association of the United States at 203-847-0407. In emergencies, malignant hyperthermia experts may be reaching by calling Medic Alert, Index Zero at 209-634-4917.

Marilyn Green Larach, M.D., F.A.A.P.
Director
The North American Malignant Hyperthermia Registry
Associate Professor of Anesthesia and Pediatrics
Pennsylvania State University College of Medicine
P.O. Box 850
Hershey, Pennsylvania 17033

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