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False-negative Results with Muscle Caffeine Halothane Contracture Testing for Malignant Hyperthermia

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Background: During the period 1985–1991, 350 muscle contracture studies have been performed in the authors' laboratory, and during this period, they became aware of an occasional false-negative result. The findings pertaining to the four cases so classified are presented in detail.

Methods: In 1985 the protocol for the muscle strip caffeine halothane testing procedure adopted was that of the European Malignant Hyperthermia Society.

Results: Thirty-six percent of the cases tested susceptible for malignant hyperthermia, 15% tested equivocal in that they responded either to halothane or to caffeine singularly, and 49% gave a normal response. In this latter group, the authors believe they identified four false-negative results.

Conclusions: This study documents the rarity of false-negative results and substantiates the reliability of caffeine halothane testing as a biologic test in diagnosing the presence of a potentially serious problem. (Key words: Anesthetics, volatile; halothane. Hyperthermia: malignant. Muscle, skeletal; caffeine; contracture.

A large variety of tests have been proposed for the detection of malignant hyperthermia (MH) susceptibility that have, unfortunately, lacked reliability.¹ The cre-

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atine kinase test, although useful in family studies, is unsatisfactory in that 30% of carriers are not detected. The first reliable test became available with Kalow's introduction of muscle strip testing.² This biologic test observes that the degree of caffeine-induced contracture of muscle strips was more sensitive in MH-susceptible people. Ellis *et al.*³ subsequently demonstrated increased sensitivity of MH muscle strips to halothane and the combination of these tests became the caffeine halothane contracture test (CHCT). The technique adopted in our laboratory complies with the European protocol.⁴ Because false-negative results with muscle strip testing have not been documented, this improbable situation prompted us to report these four false-negative results obtained in our diagnostic laboratory in patients who are almost certainly MH-susceptible by clinical criteria alone.

Materials and Methods

The European protocol was used exclusively in this study as we found it to be highly reliable in accordance with our European colleagues.⁵

Over the period of this study, 49 control biopsies have been taken from normal subjects, who had no personal or family history of MH. In none of these did the exposure to 2 mM caffeine or 0.44 mM halothane, equivalent to a gas flow of 2% in the water bath, produce a significant contracture, *i.e.*, a contracture of 0.2 g, thus establishing the reliability of CHCT in our laboratory.

The muscle strips are preloaded by approximately 2 g to produce a minimum twitch tension of 1.5 g. Before halothane or caffeine are added to the perfusate, the muscle strip for testing is stimulated to contract for a period of 5 min and at the end of each study is shown to develop rigor in response to 16 mM caffeine. Other biopsies frequently are run at the same time as the test specimen and used as controls for that testing day. With respect to the four cases described in the paper, other

CHCTs run on the same day were positive in Case 1 and negative in the other three. Only the free base of caffeine is used, and the concentration of halothane in the water bath at the 2% delivery is calculated regularly and adjusted to be equivalent to 0.44 mM/L, as checked by gas chromatography in liquid medium.

A positive response to caffeine is recorded when a sustained increase in basal tension (contracture) of 0.2 g or more occurs with a caffeine concentration of 2 mM/L or 2% halothane in the gas phase. A minimum of three separate tests are performed on individual muscle strips for both the halothane and the caffeine tests and a positive response is obtained in at least one of the strips in each case. When the strips respond only to either caffeine or halothane, the result is tabulated as equivocal, *i.e.*, for halothane alone or for caffeine alone.

We restrict the biopsy procedure whenever possible to patients older than 3 yr. Biopsies on patients older than 13 yr are carried out under femoral nerve block. Younger patients are anesthetized by ketamine HCl administered intravenously or intramuscularly.

Muscle of suitable size located in the vastus lateralis in the region of the motor endpoint is sutured at both ends and removed. The biopsy adequately provides for a minimum of nine muscle strips. The muscle is in every Case examined histologically; fresh frozen sections are stained for hematoxylin and eosin, modified trichrome, and periodic acid-Schiff for glycogen. The histochemistry involves oxidative, phosphorylative, myoadenylate deaminase and ATPase stains at pH 4.3, 4.6, and 9.4. Specimens also are prepared for electron

microscopy, which is seldom needed; however, the blocks are stored and approximately 0.5 g of muscle tissue is stored at -70°C to be used as needed for research studies. After biopsy, all patients are issued with an information brochure addressed to us to inform us of the outcome of any future anesthetic and of the agents used.

Case Histories

Case 1

An 18-yr-old man was referred for investigation; his mother died 10 yr earlier when she became hyperthermic during anesthesia given for a bunionectomy. No further details were available. The rest of the family history was noncontributory. The results of the physical examination and routine investigations were normal, apart from the creatine kinase test of 311 IU/L (normal 0–195 IU/L).

Conducting CHCT in 1988 was standard for us (table 1), as was the muscle histology and histochemistry.

Approximately 18 months later, the patient underwent surgery to the right knee for the removal of a fragmented meniscus. Drugs administered during anesthesia were atropine, thiopental, succinylcholine, halothane, nitrous oxide, and oxygen. Approximately 30 min after commencing anesthesia, the patient developed severe tachycardia and hyperpnea, multiple extrasystoles were noted, and the temperature increased rapidly to 42.7°C . Generalized muscle rigidity and mottling of the skin developed, and cardiac arrest occurred. The anesthetic was stopped and cardiopul-

Table 1. Contracture to Varying Concentrations

Patient No.	Caffeine Concentration (mM)					Halothane Concentration (%)					Result
	0.5	1.0	2.0	4.0	8.0	0.5	1.0	2.0	3.0	4.0	
1*	0	0	0	0	0.5	0	0	0	0	0	MHN
1a*	0	0	0	0.3	2.1	0	0	0	0	0	MHN
2*	0	0	0	1.0	3.5	0	0	0	0	0	MHN
3*	0	0	0	0.2	1.3	0	0	0	0	0	MHN
4	0	0	0.2	0.4	0.7	0.2	0.6	1.3	1.5	1.8	MHS
5	0	0.5	0.9	1.6	4.2	0	0.6	1.5	2.0	2.5	MHS
6	0	0	0	0	2.5	0	0	0	0	0	MHN
7	0	0.7	2.8	8.0	0	0.9	1.8	5.5	0	0	MHS
8	0	0.2	0.5	0.8	2.5	0.1	0.3	0.8	1.6	2.1	MHS
9*	0	0	0	0	0.6	0	0	0	0	0	MHN
10	0	0	0	0	0.6	0	0	0	0	0	MHN

Values are expressed in grams.

MHN = normal, all six muscle strips have a normal response; MHS = malignant hyperthermia-susceptible, at least one strip gave an abnormal contracture result.

* False-negative case.

FALSE-NEGATIVE RESULTS WITH CHCT FOR MH

monary resuscitation commenced while dantrolene was infused in repeated doses of 2 mg/kg over 30 min to a total dose of 14 mg/kg. In addition, intravenous bicarbonate and external cooling was administered. The resuscitated patient was monitored in the intensive care unit over the next 5 days. The arterial blood results taken approximately 5 min after cardiac arrest were as follows: pH 6.9, base deficit -22 mEq/L, arterial carbon dioxide tension 68 mmHg, arterial oxygen tension 84 mmHg, serum sodium 134 mEq/L, potassium 6.0 mEq/L, chloride 95 mEq/L, and calcium 3.9 mEq/L. The creatine kinase test estimated 24 h later was 21,560 IU/L. Muscle pain and weakness persisted for approximately 8 weeks.

A repeat biopsy was performed in 1990 on the opposite vastus lateralis muscle approximately 5 months after the MH reaction and again tested normal (table 1). The muscle histology and histochemistry results were normal; occasional regenerating fibers were noted.

The patient and attending doctors were informed of the negative result but were advised to regard the patient as MH-susceptible. Despite this warning, the patient was given succinylcholine and halothane for another minor surgical procedure and suffered and survived another MH crisis. It was established that the MH crisis was associated with tachypnea, tachycardia, pyrexia of 41.5° C, and early generalized rigidity. Intravenous dantrolene and sodium bicarbonate were administered and the patient externally cooled. No further details were available; however, 6 months passed before the patient's strength returned to normal.

Case 2

A healthy 4-yr-old boy was referred in 1991 for investigation for an MH reaction to anesthesia administered during minor but extensive dental surgery. There was a history of delayed recovery after a previous anesthetic comprising succinylcholine and halothane a year earlier. On that occasion, his temperature increased to 39.1° C and gradually subsided to normal over the next 5 h. There was no record of rigidity.

For the second anesthetic, he received 5 mg promethazine HCl intramuscularly; anesthesia was induced and maintained with 2% halothane, 40% O₂, and 60%

N₂O. Succinylcholine was not used. The operative procedure was discontinued at 50 min when the patient developed a tachycardia of 230/min and was tachypneic. Temperature increased to 40.5° C, and skin mottling, cyanosis, and generalized muscle rigidity occurred and were noted to commence in the arms. Blood gases at this stage indicated a base deficit of -15 mEq/L, arterial carbon dioxide tension 55 mmHg, arterial oxygen tension 87 mmHg, pH 7.1, and serum potassium 5.5 mEq/L. The patient responded to cold Darrow's solution[‡], given intravenously and with dantrolene added in an initial dose of 2 mg/kg, and was actively, externally cooled. After three more doses of dantrolene, the patient's condition stabilized. He was discharged from the hospital 5 days later. The creatine kinase test result was 16,750 IU/L, and the patient complained of aching muscles and weakness.

Three months after the hyperpyrexial crisis, the patient was subjected to a muscle biopsy to substantiate his MH status. Muscle was taken from the left vastus lateralis while under ketamine HCl anesthesia. CHCT was normal (table 1). Apart from an increase in the number of type 1 fibers (78%), the muscle was normal as ascertained by histology and histochemistry.

Case 3

A 30-yr-old woman gave a history of pyrexia that occurred toward the end of the 90-min anesthetic administered for hysterectomy. The anesthetic comprised succinylcholine, halothane, nitrous oxide, and oxygen. She developed a tachycardia of 140/min and became hyperpneic, and her temperature gradually increased to 41° C.

The patient was covered with wet blankets and received cooled intravenous solutions by way of normal saline and 5% dextrose in water. The temperature gradually decreased to normal over the next 6 h. No further investigations were carried out at that time. The patient suffered severe muscle ache, and on the second postoperative day, the creatine kinase test measured 14,130 IU/L. Three months later, the patient underwent muscle biopsy for CHCT with negative results. The muscle histology and histochemistry results were normal (1989).

The patient is a member of a family with an established history of MH: her 69-yr-old father, while undergoing appendectomy, had experienced a presumed MH crisis characterized by trismus, pyrexia during and after surgery, and severe postoperative myalgia. The

[‡] Darrow's solution: 2.7 g potassium chloride, 5.8 g sodium lactate, and 4 g/L sodium chloride; one volume of solution diluted in two volumes isotonic glucose.

anesthetic agents comprised succinylcholine, halothane, nitrous oxide, and oxygen. Malignant hyperthermia was confirmed subsequently by CHCT. The patient's 4-yr-old daughter underwent muscle biopsy that tested MH-susceptible. The tests on both these subjects were performed in our laboratory in 1990 and 1989, respectively (table 1).

To eliminate the remote possibility of the positive response in the patient's daughter as emanating from the husband, he subsequently underwent a muscle biopsy in the same year for CHCT and was diagnosed as normal (table 1), and paternity was established. It was unnecessary to establish maternity as the patient's daughter was delivered at home.

Case 4

A 35-yr-old man is a member of a large family carrying the gene for MH in whom expression of the disorder has been documented over four generations. The investigation of this family was prompted initially by the death under anesthesia of the patient's 4-yr-old fraternal niece. The anesthetic was administered in the dentist's office and consisted of succinylcholine, halothane, nitrous oxide, and oxygen. Death was preceded by severe tachycardia, tachypnea, hyperpyrexia (41.9°C), and generalized muscle rigidity culminating in cardiac arrest. No dantrolene or intravenous fluids were administered, and attempts at cardiopulmonary resuscitation by way of external cardiac massage and ventilation with 100% O_2 failed. Blood analysis was not performed.

When the patient's 5-yr-old son required surgery, he underwent skeletal muscle biopsy for CHCT and was found to be MH-susceptible, as was the patient's brother (1987; table 1).

Later in the same year, a biopsy of the patient's vastus lateralis was performed under femoral block, and CHCT result was negative; muscle histology and histochemistry results were normal. To exclude the remote possibility of the MH trait having been introduced to the children by the patient's wife, she underwent skeletal muscle biopsy a month later and tested negative. Tests for paternity confirmed the father-son relationship.

Results

Thirty-six percent (126) of the patients tested positive, 49% (171) tested normal, and 15% (53) tested

equivocal; of the latter, 7% (25) were equivocal in that they responded only to halothane and 8% (28) were equivocal in that they responded only to caffeine.

Replies received from 112 patients who tested MH-negative indicated that they had received triggering anesthetics consisting of halothane and succinylcholine, and of these, 21 patients had been subjected to more than one exposure. There were no adverse effects recorded apart from Case 1. The MH-susceptible patients established by CHCT in accordance with our results who carried evidence by way of a letter and Medic Alert medal were never subjected to a provocative anesthetic, so that we have no evidence of a false-positive result. Four of the 171 MH-negative patients have been identified on clinical evidence as false-negative CHCT results.

Discussion

As vast experience is now at hand with the muscle strip testing techniques, it is improbable that false-negatives have not occurred; the absence of such documentation occasioned the presentation of these cases. We believe that all clinical biologic tests will have false-negative results; however, in the case of CHCT, the numbers are extremely low and so accurately document the reliability of this useful procedure in the identification of patients who carry a potentially fatal disease.

Using the European protocol, several cases were classified as MH-equivocal; these cases are regarded as clinically susceptible.

The North American Malignant Hyperthermia Group standardized CHCT by defining a halothane contracture as being greater than 0.7 g after nonincremental exposure to 3% halothane bubbled through the tissue bath for 10 min. The caffeine test is incremental with doses increasing from 0.5 to 32 mM, the concentration being changed every 4 min if no contracture develops. Allen *et al.*,⁶ while assessing the accuracy of the 3% halothane test in control swine, found one false-negative result, which decreased the test sensitivity to 90%.

The specificity to a 0.2 g contracture with 2 mM caffeine was 100% and the sensitivity 100%, indicating that the concentrations were ideal for their studies on normal and susceptible swine.⁶ Results in swine, however, are not entirely applicable to humans. Larach *et al.*⁷ prospectively studied 824 muscle fascicles from 109 subjects at low risk and 24 subjects at high risk

for MH susceptibility. The information was collected from nine MH diagnostic centers. The high- and low-risk subjects were classified by the ordinal MH clinical grading scale, developed by an international MH expert panel and proposed and discussed at the VIth International Meeting on Malignant Hyperthermia held in Hershey, Pennsylvania, in September 1992. Larach *et al.* concluded that a highly sensitive and adequately specific MH diagnostic test can be achieved if current contracture cutoff points of the North American CHCT protocol were modified. The cutoff point for 3% halothane would decrease from >0.7 g to ≥ 0.5 g, and the 2 mm caffeine cutoff point would increase from ≥ 0.2 g to ≥ 0.3 g. However, the authors state that further study is needed to determine whether these modified points would continue to be highly sensitive and acceptably specific when study subjects are matched for age and sex.

Serendipitously, a recent report[§] commented on six negative results that occurred when the European protocol criteria were applied to a series of 139 patients who tested positive to the North American protocol. We find this difference difficult to explain.

We contend that there can be no doubt about the validity of our reported false-negative results established by the clinical criteria. The negative response to muscle testing in Case 1 on two separate occasions, though inexplicable, must carry a message of clinical importance. The lesson, we believe, is that a patient in whom the clinical and biochemical findings occur in reaction to anesthetic challenge warrants a diagnosis of MH and negative results to testing must be disregarded.

The possibility of laboratory procedural error is highly improbable as technical expertise has not

changed and apparatus and chemicals are checked continually. Furthermore, on the same day as the test pertaining to Case 1 proved negative for the second time, another patient's biopsy study proved positive with the same testing apparatus.

We further established that the four patients tested and classified as false-negative were not receiving any form of medication that may affect the result, *e.g.*, calcium channel blockers.

CHCT is at present the best test for MH and is of inestimable value. It will be some time before DNA studies attain the same degree of accuracy and availability. We wish to stress that the results obtained with the European protocol are specific to our laboratory and, furthermore, cannot be extrapolated into laboratories using the North American protocol.

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