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In vitro contracture test for diagnosis of malignant hyperthermia following the protocol of the European MH Group: Results of testing patients surviving fulminant MH and unrelated low-risk subjects

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Background: Determination of sensitivity and specificity of the *in vitro* contracture test (IVCT) for malignant hyperthermia (MH) susceptibility using the European MH Group (EMHG) protocol has been performed in some laboratories but only on a small sample from the combined EMHG. Thus, the purpose of the present study was to determine combined EMHG sensitivity and specificity of the test.

Methods: Results of IVCT of patients with previous fulminant MH and normal, low-risk subjects (controls) were collected from 22 centres of the EMHG. IVCT was performed according to the EMHG protocol. Patients were included in the study if the clinical crisis had a score of at least 50 points with the Clinical Grading Scale. Low-risk subjects were included provided they did not belong to a family with known MH susceptibility, they had not developed any signs of MH at previous anaesthetics, and they did not suffer from any neuromuscular disease. For inclusion of both MH patients and low-risk subjects, at least 1 muscle bundle in the IVCT should have twitches of 10 mN (1 g) or more. For evaluation of individual tests, only muscle bundles with twitch heights of 10 mN (1 g) or more were used.

Results: A total of 1502 probands had undergone IVCT because of a previous anaesthesia with symptoms and signs suggestive of MH. Of these, 119 had clinical scores of 50 and above. From these 119 MH-suspected patients and from 202 low-risk subjects, IVCT data were collected. Subsequently, 14 MH-suspected patients were excluded from further analysis for the following reasons: In 3 patients, the suspected MH episode could be fully explained by diseases other than MH; in 11 MHS patients, IVCT was incomplete (n=1), data were lost (n=3), or none of the muscle bundles fulfilled twitch criteria (n=7). Of the remaining 105 MH-suspected patients, 89 were MHS, 10 MHEh, 5 MHEc, and one MHN. Thus, we observed a diagnostic sensitivity of the IVCT of 99.0% if the MHE group is considered susceptible (95% confidence interval 94.8–100.0%). Of the 202 low-risk subjects, 3 were MHS, 5 MHEh, 5 MHEc, and 189 MHN. This gives a specificity of the IVCT of 93.6% (95% confidence interval 89.2–96.5%).

Conclusion: The IVCT for diagnosis of MH susceptibility in Europe has a high sensitivity and a satisfactory specificity.

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Key words: Anesthesia, complication, inhalational, halothane; caffeine; malignant hyperthermia, diagnosis; ryanodine receptor.

MALIGNANT hyperthermia (MH) is a pharmacogenetic disease of skeletal muscle which is mainly of concern during and following anaesthesia. Diagnosis of susceptibility to MH may be established using an *in vitro* contracture test (IVCT) with halothane and caffeine. Two international protocols for the performance of such tests have been published, one by the European MH Group (EMHG) (1, 2) and the other by the North American MH Group (NAMHG) (3). The two protocols are similar in many ways, both including a halothane and a caffeine test. They differ in some details, and apparently these minor differences are enough to account for some variations in the results of the tests (4, 5).

The ability of a test to discriminate between those who have a disease and those who are disease-free is measured by the sensitivity and specificity of the test (6). Sensitivity and specificity are inherent characteristics of the test and in principle independent of the prevalence of the disease tested for. The sensitivity measures the proportion of those with the disease who are correctly identified by the test and the specificity measures the proportion of those without the disease who are correctly called disease-free by the test (6). In both Europe and North America the threshold between a normal and an abnormal test result, for reasons of safety, has been deliberately chosen so as to secure a high sensitivity of the test, well knowing that such a step will sacrifice specificity.

In a preliminary evaluation of the test results from a few centres in Europe, the sensitivity and specificity of the test was found to be 100% and 93%, respectively (7). Similar figures have been observed in some individual European centres (4, 8). Apparent false negative results of the IVCT have been reported (9, 10) although it must be questioned if the cases described are truly false negatives. Other formal studies have not found evidence of false negative test results (11–13). One of the problems encountered when determining sensitivity of the IVCT is that the symptoms and signs of MH are non-specific. Thus, other diseases and conditions may mimic the clinical presentation of MH (14). Another problem is that individuals with the MH phenotype may be anaesthetised with triggering agents without developing clinical MH (15, 16). Recently, a Clinical Grading Scale was developed by an international panel of MH experts for assessment of the likelihood that any adverse clinical event could be considered to be MH (17). This grading scale cannot be specific for MH but allows a comparison of the severity of the observed clinical episode, and therefore could help to categorise patients when it is impractical to describe in detail the individual case histories.

The European protocol for the IVCT has been published (1, 2), is used widely and has been upgraded regularly (see Appendix). Thus, viability criteria and drug concentrations have been specified. Also, a common database format for recording results has been agreed upon. In the protocol four tests are required: two halothane and two caffeine tests (see Appendix). The caffeine tests are static tests performed at optimal length of the fibre bundle. Concerning the halothane tests, at least one is a static test, whereas the second may be either a static or a dynamic test in which the length of the fibre bundle is cyclically changed (18). Few European centres use the dynamic test at present, and it is not settled if this test has any advantages compared to the static test (19, 20). However, more patients in Europe are tested with the dynamic halothane test than without it because it is used by the Leeds MH centre which is by far the largest MH centre in Europe.

A patient is considered susceptible to MH (MHS) if at least one halothane test result and one caffeine test result are abnormal (2). If the results of all 4 tests are normal, the patient is considered non-susceptible (MHN). In the case of abnormal test results to either caffeine or halothane but not to both agents, the result is categorised as MHE (equivocal) and for reasons of safety most of these patients are clinically treated as MH susceptible, whereas in genetic investigations they are assigned unknown disease status.

The present study was initiated to establish combined sensitivity and specificity of the test on data from many different European centres applying the viability criteria which are now part of the testing protocol on individual muscle bundles. For the clinical safety of patients it is essential to secure a high sensitivity of the IVCT but for research purposes it is important to estimate the specificity. Such data have hitherto not been available on a large scale for data obtained with the protocol of the European MH Group.

Material and methods

All centres performing IVCT in Europe were asked to forward results of IVCT in patients considered to have survived fulminant MH, and in normal, low-risk subjects (controls), using the database format of the EMHG for reporting results. Patients were assigned a diagnosis according to the result of the IVCT: MHS (MH susceptible) if the result of at least one halothane test and one caffeine test were abnormal; MHE (MH equivocal) if either the halothane or the caffeine test result was abnormal; and MHN (MH negative) if both

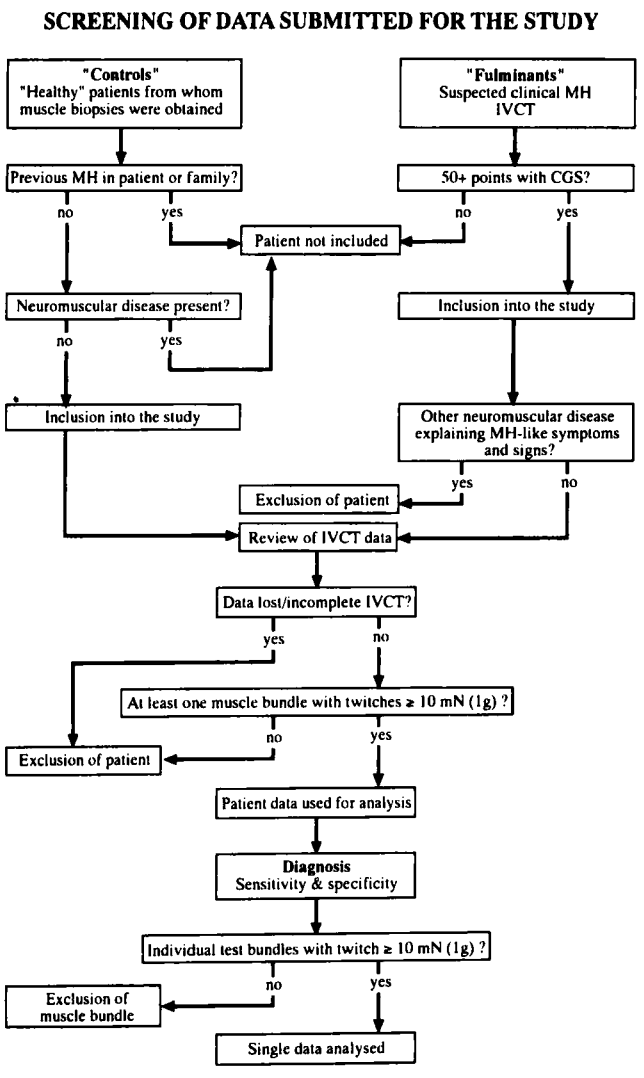

Fig. 1. Flowsheet showing how data were screened for inclusion into the study and at which levels patients or muscle bundles were excluded. Scoring with the Clinical Grading Scale (CGS) was performed in individual centres and checked for the patients with 50 points or more by the first author. See text for further explanation.

halothane and caffeine test results were normal. Abnormal results of IVCT were those with increases in muscle bundle force of at least 2 mN (0.2 g) at halothane 0.44 mmol l^{-1} or less and at caffeine 2 mmol l^{-1} or less. Contracture thresholds at higher concentrations than these were considered normal.

In order to avoid any bias in the selection of patients, we applied the Clinical Grading Scale (17) to the clinical episodes of the patients investigated with IVCT because of previous symptoms and signs of MH during or following anaesthesia. Only those cases with a score of 50 or more (i.e. the patients belonging to group 6 with the most severe and well-documented reactions) were accepted for inclusion into this study as fulminant MH cases. The flowsheet for evaluation of patient data is shown in Fig. 1.

Normal low-risk subjects (controls) were defined as patients undergoing surgery who had no symptoms or signs of neuromuscular disease themselves, and who were not genetically related to any such patient. If these low-risk subjects had previously undergone anaesthesia, it was a condition that no side effects which could resemble MH had been observed.

An additional criterion should be met by both MH patients and normal low-risk subjects in order for them to be included into the study: at least one muscle bundle should have a twitch height of 10 mN (1 g) or more (Fig. 1). For analysis of individual tests, only muscle bundles with twitch heights of at least 10 mN (1 g) were included because this viability criterion is the one applied in the protocol for the IVCT. Thus, inclusion into the study was determined on 2 levels: a subject level and a muscle bundle level (Fig. 1).

Patients were excluded if they were found to have another neuromuscular disease which could fully explain the MH-like symptoms and signs (Fig. 1). For both MH patients and low-risk subjects, patients were excluded if data were lost or the IVCT was not complete. In addition, results from individual muscle bundles were excluded if the bundle did not fulfil viability criteria (i.e. a twitch height of 10 mN (1 g)) (Fig. 1).

For statistical evaluation, the Mann-Whitney Rank Sum Test and the Chi-square test were used. Confidence intervals were calculated for binomially distributed data. $P<0.05$ was considered significant.

Results

A total of 22 MH centres from 13 countries in Europe participated in the project. Altogether, 1502 probands had been investigated with IVCT in these centres. The 1502 suspected MH reactions were scored according to the Clinical Grading Scale (17). The result of this scoring and the outcome of the IVCT performed in these 1502 patients are shown in Table 1. A significant correlation was found between the category of the clinical reaction and diagnosis ($P<0.001$).

IVCT data concerning the 119 patients scoring 50 points or more in the Clinical Grading Scale (i.e. group 6 patients with a likelihood of MH described as "almost certain") were collected. In addition, data were collected from 202 normal low-risk subjects. Results from 14 of the group 6 patients were subsequently excluded for the following reasons (see also Fig. 1): 3 patients were found to suffer from other neuromuscular diseases; these patients were MHN and

Table 1

Results of scoring the adverse anaesthetic reaction of all probands investigated for MH using the Clinical Grading Scale [17], and results of IVCT.

Category/diagnosis	MHS	MHE	MHE	MHN	Total
1 (0)	4	2	2	21	29
2 (3–9)	4	3	3	24	34
3 (10–19)	161	81	22	474	738
4 (20–34)	118	56	23	214	411
5 (35–49)	96	27	8	40	171
6 (50+)	99	11	5	4[a]	119
Total	482	180	63	777	1502

The proportion of individuals in each diagnostic category varies with scoring. Diagnostic category and scoring group are significantly related ($P<0.001$, Chi-square analysis).

[a]Three of these 4 MHN individuals were excluded from the study because other diseases fully explained the clinical episode (neuroleptic malignant syndrome in one patient, limb girdle muscle dystrophy in one, and myotonia fluctuans in one patient) – see text for further details.

their clinical episodes are reviewed below. In 4 patients, the IVCT was incomplete (n=1) or data were lost (n=3), and in 7 patients none of the muscle bundles for IVCT had twitch heights of at least 10 mN. These 11 patients were all MHS. Thus, test results from 105 patients with previously suspected fulminant MH and 202 low-risk subjects were evaluated in the study. The data finally included in the study originated from 20 centres for MH patients and 14 centres for low-risk subjects. Thirteen centres contributed data on both MH patients and low-risk subjects.

The case histories of the 3 patients who had scores of 50 or more in the Clinical Grading Scale, and who were excluded because of other diseases are summarised below to illustrate the appropriateness of the exclusion.

Patient 1: A 60-yr-old male was anaesthetised for a laparotomy because of a perforated peptic ulcer. Drugs given during anaesthesia included atropine, diazepam, thiopentone, droperidol, suxamethonium, fentanyl, and isoflurane in O_2/N_2O. The anaesthesia was uneventful. The following day the patient developed delirium tremens and was treated with haloperidol. Following this drug, the temperature started to increase. Within 2 days temperature reached 42°C, the patient became rigid and myoglobinuria was observed. CK was 20900 U/l. The patient was treated with dantrolene without any effect. Following symptomatic treatment in the ICU, the condition of the patient slowly improved during the next month. IVCT (result MHN) was made for a differential diagnosis of MH and malignant neuroleptic syndrome (NMS) and the patient is considered to have suffered from NMS.

Patient 2: A 13-yr-old boy was anaesthetised for dental surgery. Following suxamethonium, he developed masseter muscle rigidity, which did not impede intubation. Anaesthesia was maintained with halothane. He became acidotic with an arterial pH of 7.24 and a base excess of −9. Temperature increased to 38°C. Postoperatively, he had marked myoglobinuria but no renal failure. CK was 111740 U/l. Subsequently, resting CK was found to be increased to 1163 U/l. The patient had no clinical signs of a neuromuscular disease at the time. IVCT was normal (MHN), but muscle histology was abnormal, indicating limb girdle muscle dystrophy. This latent neuromuscular disease is considered to have caused the anaesthetic problems in this patient.

Patient 3: A 16-yr-old male was anaesthetised for torsion of the testis. Rapid sequence induction was performed. Following suxamethonium, masseter and generalised muscle rigidity was observed. A few minutes later oxygen saturation decreased to 26% and end- tidal pCO_2 increased to 12.7 kPa in spite of ventilation with 100% oxygen and a high flow. Heart rate increased to 120 bpm and temperature to 39.6°C. Arterial blood gases 30 min after induction showed acidosis with pH 7.09, pCO_2 8.9 kPa, and base excess −11.7. The patient was treated with dantrolene and sodium bicarbonate, and recovery was rapid. Serum potassium remained normal, CK increased to a maximum of 743 U/l. IVCT was normal. However, the pa-

Table 2

Type of anaesthetic agent triggering MH in 105 patients suspected of fulminant MH.

Anaesthetic	No. of patients
Halothane	44
Enflurane	12
Isoflurane	45
Other agent	4
Total	105

Table 3

The use of suxamethonium, clinical signs, and treatment of MH with Dantrolene in 105 patients suspected of fulminant MH.

Sign or treatment	Yes	No	Unknown
Suxamethonium	80	23	2
Masseter muscle rigidity	55	38	12
Generalised rigidity	64	24	17
Myoglobinuria	68	11	26
Ventricular extrasystoles	37	32	36
Dantrolene	77	26	2

Table 4

Clinical details of 105 patients suspected of fulminant MH.

	Mean (SD)	Maximum	Minimum	N
Age	21.3 (11.7)	65	1	105
Duration (min)	56.6 (60.9)	360	5	85
Temperature (°C)	39.9 (1.4)	44.0	37.1	100
CO_{2ET} (kPa)	11.0 (3.6)	28.0	6.3	42
CO_{2art} (kPa)	10.7 (4.8)	32.3	5.3	70
pH_{art}	7.09 (0.15)	7.40	6.60	83
CK (U/l)	29,840 (35,503)	225,000	300	94
K^+ (mmol l^{-1})	5.6 (1.2)	8.0	3.1	71
Score	63.5 (9.4)	88	50	105

Age: at the time of the MH crisis; duration: of anaesthesia until MH was diagnosed; temperature: the maximum temperature measured during the crisis; CO_{2ET}: maximum end-tidal tension of CO_2 during the crisis; CO_{2art}: maximum tension of CO_2 measured during the crisis in arterial blood; CK: maximum concentration of the enzyme creatine kinase measured in blood following the crisis; K^+: maximum concentration of potassium measured in blood during the crisis; score: result of application of the Clinical Grading Scale [17]; N: the number of patients in whom a given variable was measured.

Table 5

Clinical details relating to the muscle biopsy in 105 patients suspected of MH and 202 low-risk subjects.

	Fulminant	Control	*P* value
Sex, m/f/u	67/38/0	106/94/2	0.091
Age, yrs	23.7 (11.6) (4–66)	51.3 (17.9) (7–90)	<0.001
Anaesthesia, r/g/u	62/27/16	127/58/17	0.185
Neurology, p/a/u	11/79/15	0/143/59	<0.001
Histopathology, n/a/np	62/18/25	43/0/159	<0.001

Sex: m=male, f=female; anaesthesia for the biopsy: r=regional, g=general, u=unknown; abnormal neurological signs: p=present, a=absent, u=unknown; histopathology: n=normal, a=abnormal, np=not performed.

tient was found to suffer from myotonia fluctuans and a mutation in the sodium channel gene on chromosome 17 was identified (21).

Details of the clinical episodes of MH in the remaining 105 patients suspected of fulminant MH are summarised in Tables 2–4.

It is apparent that several variables were not measured or reported for all patients. Some measurements of end-tidal or arterial CO_2 tensions (Table 4) were done during hyperventilation or following other treatments. The reporting system did not allow for details concerning this problem.

Clinical details of the MH patients and low-risk subjects relating to the muscle biopsy are shown in Table 5. The low-risk subjects were significantly older than the patients surviving MH (Mann-Whitney test, $P<0.001$), whereas the sex distribution was similar in the two groups of patients ($P=0.091$). A significantly larger proportion of MH patients than controls had abnormal neurological signs and abnormal histopathology, although histopathology was not performed in the majority of control patients.

Details relating to the IVCT are shown in Table 6 (halothane test) and Table 7 (caffeine test). No significant differences were found between the two groups for any variables of the IVCT with the exception of the number and sizes of contractures, which were significantly more frequent and of larger size in the MH group ($P<0.001$).

Whereas 168 of 181 viable specimens from MH patients developed a contracture of ≥2 mN (0.2 g) in the halothane test, only 12 of 272 specimens from low-risk subjects did so ($P<0.001$) (Table 6). In the caffeine test, 128 of 143 viable specimens from MH patients developed contractures whereas only 8 of 258 viable

Table 6

Muscle bundle characteristics in the halothane test for those bundles included in the study (criterion: twitch heights of at least 10 mN).

		Halothane test				
		Median	Mean (SD)	Range	$N_{samples}$	$N_{individuals}$
Twitch (mN)	Fulm	35	43 (28)	10–132	181	103
	Contr	20	24 (15)	10–98	272	181
Length (mm)	Fulm	18.0	18.9 (5.5)	6–33	173	64
	Contr	17.0	19.9 (5.1)	6–33	231	107
Weight (mg)	Fulm	150.0	165.2 (81.8)	49–684	127	65
	Contr	153.5	156.7 (72.5)	22–340	168	98
Contracture (mN)	Fulm	17	20 (16)[a]	2–89[a]	168[a]	99[a]
	Cont	4	4 (3)[a]	2–12*	12[a]	8[a]

Fulm=bundles from patients with a score in the Clinical Grading Scale of 50 and above (n=105 patients). Contr=bundles from low-risk subjects (n=202 subjects). $N_{samples}$ for twitch are the number of specimens fulfilling viability criteria (twitch height of 10 mN or above). For length and weight, $N_{samples}$ signify the number of specimens fulfilling viability criteria and for which length and weight were reported. For contracture, $N_{samples}$ signify the number of viable specimens with a contracture of at least 2 mN at halothane 0.44 mmol l^{-1}. $N_{individuals}$ signify the number of individuals from whom the $N_{samples}$ come. All patients had at least one halothane test and one caffeine test performed. [a] $P<0.001$.

Table 7

Muscle bundle characteristics in the caffeine test for those bundles included in the study (criterion: twitch heights of at least 10 mN).

		Caffeine test				
		Median	Mean (SD)	Range	$N_{samples}$	$N_{individuals}$
Twitch (mN)	Fulm	27	38 (27)	10–146	143	92
	Contr	20	25 (17)	10–99	258	179
Length (mm)	Fulm	18.0	18.2 (5.2)	10–32	113	65
	Contr	18.0	19.5 (5.9)	5–35	172	141
Weight (mg)	Fulm	167.5	169.5 (74.8)	47–550	112	65
	Contr	160.0	155.1 (74.0)	27–350	166	130
Contracture (mN)	Fulm	11	14 (13)[a]	2–62	128[a]	94[a]
	Contr	2.8	3 (1)[a]	2–5	8[a]	8[a]

Fulm=bundles from patients with a score in the Clinical Grading Scale of 50 and above (n=105 patients). Contr=bundles from low-risk subjects (n=202 subjects). $N_{samples}$ for twitch are the number of specimens fulfilling viability criteria (twitch height of 10 mN or above). For length and weight, $N_{samples}$ signify the number of specimens fulfilling viability criteria and for which length and weight were reported. For contracture, $N_{samples}$ signify the number of viable specimens with a contracture of at least 2 mN at caffeine 2 mmol l^{-1}. $N_{individuals}$ signify the number of individuals from whom the $N_{samples}$ come. All patients had at least one halothane test and one caffeine test performed. [a] $P<0.001$.

Table 8

Diagnostic outcome of the IVCT in 105 patients suspected of fulminant MH and 202 normal low-risk subjects.

	MHS	MHEh	MHEc	MHN	N	Sensitivity, %	Specificity, %
Fulm	89	10	5	1	105	99.0 (94.8–100.0)	–
Contr	3	5	5	189	202	–	93.6 (89.2–96.5)

For calculation of the diagnostic sensitivity and specificity of the IVCT, patients with an MHE diagnosis are considered susceptible to MH, as most would be clinically for reasons of safety. For sensitivity and specificity, 95% confidence limits are given in brackets. If, for calculation of specificity, the MHS diagnosis is exclusively used, specificity would increase to 98.4%.

specimens from the low-risk subjects did so ($P<0.001$).

From the number of muscle bundles with contractures exceeding the threshold of 2 mN (0.2 g) at halothane 0.44 mmol l^{-1} or caffeine 2 mmol l^{-1}, the false positive or false negative rate of results may be calculated for the two tests. Altogether, 13 of 181 muscle bundles from MH patients did not develop contractures in the halothane test (Table 6), giving a false negative rate of 7.2% for individual muscle bundles. The false negative rate for muscle bundles in the caffeine test was 15/143 (Table 7), i.e. 10.5%. The false positive rate for the halothane test was 12/272 muscle bundles (table 6), i.e. 4.4%, whereas the false positive rate for the caffeine test was 8/258 (Table 7), i.e. 3.1%. It must be noted that these rates are not for patients, only for specimens tested.

Diagnoses resulting from the IVCT are shown in Table 8. For reasons of safety, the MHE group is considered to be at risk of MH. The sensitivity and specificity of the IVCT are 99.0% (104/105) (95% confidence interval 94.8–100.0%) and 93.6% (189/202) (95% confidence interval 89.2–96.5%), respectively. If the MHE group is omitted from the MH group, the specificity increases to 98.4% (189/192). However, we have agreed the MHE groups are under constant review.

One patient among those categorised by the Clinical Grading Scale as fulminant tested MHN in the IVCT. The MH-suspected episode occurred in 1978 and is reported below.

Patient 4: This patient was a 10-month-old boy requiring plastic surgery for cleft palate. The anaesthetic itself was uncomplicated and included halothane and atropine, but at recovery generalised rigidity and possibly convulsions were observed. The body temperature was 40.6°C and an arterial blood sample showed a combined metabolic and respiratory acidosis with pH 7.10 and $PaCO_2$ 8.7 kPa. No information about possible myoglobinuria, plasma increases in CK or K^+ were available. Treatment consisted of oxygen and cooling (the patient had been on a warming blanket throughout a 2-hour period in addition to being wrapped in blankets and completely covered by surgical drapes). Further recovery was uneventful. The patient had an IVCT performed in 1989 at the age of 12 years. He looked "peculiar" and was

Table 9

Effect on sensitivity and specificity of different contracture thresholds applied to the IVCT data presented in this study.

	Threshold, mN	MHS	MHEh	MHEc	MHN	N	Sensitivity, %	Specificity, %
Fulminant	2	89	10	5	1	105	99.0	–
	3	81	16	5	3	105	99.0	–
	4	77	16	6	6	105	94.2	–
	5	66	24	6	9	105	91.4[a]	–
Control	2	3	5	5	189	202	–	93.6
	3	2	3	2	195	202	–	96.5
	4	1	4	1	196	202	–	97.0
	5	0	3	0	199	202	–	98.5[a]
Fulminant	H 5, C 3	77	15	9	4	105	96.2	–
Control	H 5, C 3	2	1	2	197	202	–	97.5

Values are number of patients within a diagnostic category, given a particular threshold. If nothing is mentioned the threshold applies to both halothane and caffeine data. The currently used threshold for both halothane and caffeine tests is a 2 mN contracture with 0.44 mmol l^{-1} halothane or less and 2 mmol l^{-1} caffeine or less. [a]denotes a significant decrease in sensitivity or increase in specificity compared to that obtained with 2 mN.

intellectually somewhat retarded. There were no clinical signs of neuromuscular disease except for the treated cleft palate. IVCT was normal. Histology showed fibre type 1 predominance and centrally located nuclei but no cores, indicating the presence of a nonspecific myopathy. Following this result, the mother was investigated to rule out a false negative result of the IVCT. She was also MHN. The father has refused to undergo IVCT. Thus, it is impossible to determine if this is a false negative result of the IVCT or the episode was due to overheating with ensuing convulsions, hypoxia, hypercarbia, and acidosis in a patient with a latent myopathy.

The number of data reported from individual centres was too small to calculate centre-specific variability and the number of data was not evenly distributed from the 22 participating centres. Concerning the MH patients, the 16 non-MHS responses originated from 9 different centres, which does not hint at skewness in distribution. Likewise, the 13 non-MHN responses in the control group originated from 6 different centres out of the 14 centres contributing control data.

With the present large sample of data, it is possible to investigate the effect on sensitivity and specificity of other thresholds than the current of 2 mN (0.2 g). In Table 9 calculated sensitivity and specificity are shown for thresholds of 2–5 mN. It is apparent that increases in the threshold value above 3 mN reduces sensitivity. This is significant for the 5 mN threshold (P=0.023, Chi-square test with Yates correction). A threshold of 3 mN maintains sensitivity and increases specificity. However, this increase in specificity is not statistically significant (P=0.25, Chi-square test with Yates correction). In addition, data are given for different halothane and caffeine thresholds: 0.3 g for caffeine and 0.5 g for halothane, but this does not improve sensitivity and specificity.

Discussion

Safety for all patients undergoing anaesthesia is a main goal of anaesthetists. MH has been one of the severe complications of anaesthesia with an early mortality rate of 70% (22). In recent years, the number of deaths from MH has significantly decreased, and in some countries is now zero (4). Performing IVCT has several purposes: The main one is to eliminate the threat of MH from all those individuals who do not have the MH phenotype so that they can be anaesthetised without any specific precautions and be given volatile anaesthetics if the anaesthetist wishes to use these agents. Because MH is an inherited disease, the number of individuals who may thus benefit from IVCT is large compared to the number of patients who have themselves developed signs of MH. Another purpose is to establish a definite diagnosis in those individuals who do have the MH phenotype and to inform the patients and their attending doctors of this disease. A research purpose is to establish a link between the clinical phenotype, the IVCT result, and the presence or absence of the mutations associated with MH. For this purpose, a systematic analysis of the clinical signs of MH present during a crisis and the results of the IVCT must be performed.

There has been no formal assessment of the Clinical Grading Scale which was developed by an international panel of MH experts by the Delphi method (17).

It represents an attempt at providing objective criteria for classification of clinical MH. For each individual process involved in MH, points are awarded according to the occurrence of specific signs, realising that several variables may be signs of the same process. Thus, double-counting is avoided although not completely. Points may be given for both hypercarbia, low pH, and negative base excess, although these parameters are related. Another problem is that since none of the symptoms or signs of MH are specific, it follows that the Clinical Grading Scale cannot be specific either. Thus, severe rhabdomyolysis following cardiac arrest during anaesthesia may give rise to high scores classifying the episode as highly likely to be MH although nothing points to MH (14, 23). The 3 patients with other neuromuscular diseases whom we excluded from the analysis of IVCT data represent patients with such non-specific high scores. We find the exclusion of these patients appropriate because it is well known that patients with other neuromuscular diseases may develop signs similar to MH without having the MH phenotype (14).

The result of scoring is dependent on the quality of documentation of the case. In this study, some cases of real and severe MH, mostly occurring many years ago, were not well documented at the time of the crisis and thus did not obtain a score high enough to be included. Other cases were well documented and considered to represent real, fulminant MH in the investigation centre but were excluded because they did not obtain a score of at least 50. Such cases are considered representative of less severe MH, often due to early diagnosis and treatment. The fact that we observed a significant correlation between the score and the proportion of MHS responses seems to validate the usefulness of the Clinical Grading Scale for comparison of groups of patients, though it should not be used for diagnostic purposes for an individual.

The sensitivity of 99% observed in this study is satisfactory for a diagnostic test, the more so because the clinical adverse event in the single MHN patient scoring above 50 points may well be due to other factors than MH. This case illustrates the fact that a definite diagnosis may not always be established even with use of the invasive IVCT. The observed sensitivity of the IVCT is comparable to that obtained by the North American MH Group (24).

The specificity of the IVCT observed in the present study is also satisfactory, given the high sensitivity, and also compares with the observations made by the North American MH Group (25). However, for other purposes than patient safety, it seems wise not to include patients with the MHE response into the group of MH-susceptible patients. This suggested guideline is supported by the fact that in families with known chromosome 19 mutations related to MH, MHE individuals rarely do have the mutation in question (26–28). Thus the original statement concerning the MHE group, that this group is under permanent review, is still valid (2).

Should the critical contracture size be changed from the present 2 mN value? Based on our present rather large data sample, there is no justification for change. As shown in Table 9, only a change from 2 mN to 5 mN significantly increases specificity – and this occurs at a cost of a significantly reduced sensitivity, which would be unacceptable. The reason why our halothane threshold contracture is smaller than that used by the North American MH Group is that our dose-response curve has cumulative increments in concentration to 2%, whereas the NAMHG uses a single addition of 3% halothane. The single dose has previously been shown to result in larger contractures (4).

For clinical decision-making it is not enough to know the sensitivity and specificity of the IVCT. What really counts is the predictive value of a positive or negative response, which depends on the prevalence of the disease (in this case MH) in the population tested (4, 6). With a very high sensitivity of the test and a somewhat lower specificity, as presently observed for the IVCT, the predictive value of a negative test result is around 99%. The predictive value of a negative result is not much influenced by the prevalence of the disease when the specificity is as high as that found for the IVCT, and it increases with decreasing disease prevalence (4, 6). On the other hand, the predictive value of a positive test result is heavily influenced by the disease prevalence in the test population. The *a priori* risk of a first-degree relative of a patient surviving fulminant MH having inherited the disease is 50%, and in such a patient the predictive value of a positive test result is about 90% (4). However, if instead a cousin with an *a priori* risk of 12.5% is investigated the predictive value of a positive result of the IVCT is reduced to approximately 50% (4). The predictive value of a negative result in the same patient is close to 100% (4). An abnormal result in a member of the general population who has a very low *a priori* risk of MH is therefore much more likely to be a false positive result than indicating MH susceptibility. These considerations are important when selecting patients for IVCT. The greatest confidence in the results is obtained in those with the highest *a priori* risk of susceptibility.

Although data are insufficient to prove that results vary between centres, it is obvious from Tables 6 and

7 that individual test procedures probably could be more standardised. Since data were collected for this study steps have been taken to standardise muscle length and weight as well as the size of the preload and the method to obtain this preload. These guidelines are presented in the updated current protocol (Appendix).

In conclusion, the observed sensitivity of 99% and specificity of 93.6% in the IVCT which we have obtained in this joint Europan study is considered satisfactory for patient safety. For research purposes, it is recommended to increase the specificity to 98% by not regarding MHE patients as susceptible.

References

1. European MH Group. A protocol for the investigation of malignant hyperpyrexia (MH) susceptibility. *Br J Anaesth* 1984: **56**: 1267–1269.
2. European MH Group. Laboratory diagnosis of malignant hyperpyrexia susceptibility (MHS). *Br J Anaesth* 1985: **57**: 1038.
3. Larach MG. Standardization of the halothane caffeine muscle contracture test. *Anesth Analg* 1989: **69**: 511–515.
4. Ørding H. Investigation of malignant hyperthermia in Denmark. *Dan Med Bull* 1996: **43**: 111–125.
5. Hartung E, Sold M, Preis I. Diagnostik der Malignen Hyperthermie: Ein Vergleich des Halothan-Muskel-Kontrakturtestes nach dem Europäischen und dem Nordamerikanischen Protokoll. *Anaesthesist* 1990: **39**: 635.
6. Riegelman RK, Hirsch RP. *Studying a study and testing a test. How to read the medical literature*, 2nd edn. Boston: Little, Brown and Company, 1989: 151–163.
7. Ørding H. The European MH Group: protocol for *in vitro* diagnosis of susceptibility to MH and preliminary results. In: Britt BA, ed. *Malignant hyperthermia.* Boston: Martinus Nijhoff Publishing, 1987: 269–277.
8. Hartung E. *Die Bedeutung des Pflanzenalkaloids Ryanodin für die Diagnostik der Malignen Hyperthermie.* Würzburg: University Press, 1996: 55–61.
9. Isaacs H, Badenhorst M. False-negative results with muscle caffeine halothane contracture testing for malignant hyperthermia. *Anesthesiology* 1993: **79**: 5–9.
10. Wedel DJ, Nelson TE. Malignant hyperthermia – diagnostic dilemma: false-negative contracture responses with halothane and caffeine alone. *Anesth Analg* 1994: **78**: 787–792.
11. Ørding H, Hedengran AM, Skovgaard LT. Evaluation of 119 anaesthetics received after investigation for susceptibility to malignant hyperthermia. *Acta Anaesthesiol Scand* 1991: **35**: 711–716.
12. Allen GC, Rosenberg H, Fletcher JE. Safety of general anesthesia in patients previously tested negative for malignant hyperthermia susceptibility. *Anesthesiology* 1990: **72**: 619–622.
13. Islander G, Ranklev-Twetman E. Evaluation of anaesthesias in malignant hyperthermia negative patients. *Acta Anaesthesiol Scand* 1995: **39**: 819–821.
14. Heytens L, Martin JJ, van de Kelft E, Bossaert LL. *In vitro* contracture tests in patients with various neuromuscular diseases. *Br J Anaesth* 1992: **68**: 72–75.
15. Halsall PJ, Cain PA, Ellis FR. Retrospective analysis of anaesthetics received by patients before susceptibility to malignant hyperpyrexia was recognized. *Br J Anaesth* 1979: **51**: 949–954.
16. Bendixen D, Skovgaard LT, Ørding H. Analysis of anaesthesia in patients suspected to be susceptible to malignant hyperthermia before diagnostic *in vitro* contracture test. *Acta Anaesthesiol Scand* 1997: **41**: 480–484.
17. Larach MG, Localio AR, Allen GC, Denborough MA, Ellis FR, Gronert GA et al. A clinical grading scale to predict malignant hyperthermia susceptibility. *Anesthesiology* 1994: **80**: 771–779.
18. Ellis FR, Harriman DGF. A new screening test for susceptibility to malignant hyperpyrexia. *Br J Anaesth* 1973: **45**: 638–639.
19. Ranklev E, Fletcher R, Blomquist S. Static versus dynamic *in vitro* tests in the diagnosis of malignant hyperthermia susceptibility. *Br J Anaesth* 1986: **58**: 646–648.
20. Ørding H, Skovgaard LT. *In vitro* diagnosis of susceptibility to malignant hyperthermia: comparison between dynamic and static halothane and caffeine tests. *Acta Anaesthesiol Scand* 1987: **31**: 458–461.
21. Ricker K, Moxley III RT, Heine R, Lehmann-Horn F. Myotonia fluctuans: A third type of muscle sodium channel disease. *Arch Neurol* 1994: **51**: 1095–1102.
22. Britt BA, Kalow W. Malignant hyperthermia: a statistical review. *Can Anaesth Soc J* 1970: **17**: 293–315.
23. Bendixen D, Poulsen TD, Knudsen F, Nielsen J, Ørding H. Clinical variability of possible malignant hyperthermia as revealed by two cases. *Acta Anaesthesiol Belg* 1994: **45**: 93–98.
24. Larach MG, Landis JR, Shirk SJ, Diaz M, The North American Malignant Hyperthermia Registry. Prediction of malignant hyperthermia susceptibility in man: improving sensitivity of the caffeine halothane contracture test. *Anesthesiology* 1992: **77**: A1052.
25. Larach MG, Landis JR, Bunn JS, Diaz M, The North American Malignant Hyperthermia Registry. Prediction of malignant hyperthermia susceptibility in low-risk subjects. *Anesthesiology* 1992: **76**: 16–27.
26. Quane KA, Keating KE, Manning BM, Healy JMS, Monsieurs K, Heffron JJA et al. Detection of a novel common mutation in the ryanodine receptor gene in malignant hyperthermia: implications for diagnosis and heterogeneity studies. *Hum Mol Genet* 1994: **3**: 471–476.
27. Keating KE, Quane KA, Manning BM, Lehane M, Hartung E, Censier K et al. Detection of a novel RYR1 mutation in four malignant hyperthermia pedigrees. *Hum Mol Genet* 1994: **3**: 1855–1858.
28. Healy JMS, Quane KA, Keating KE, Lehane M, Heffron JJA, McCarthy TV. Diagnosis of malignant hyperthermia: a comparison of the *in vitro* contracture test with the molecular genetic diagnosis in a large pedigree. *J Med Genet* 1996: **33**: 18–24.

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Appendix

The European Group protocol for investigation of malignant hyperthermia susceptibility

1. The biopsy should be performed on the quadriceps muscle (either vastus medialis or vastus lateralis), using regional (avoiding local infiltration) or general anaesthetic techniques.
2. The muscle samples can be dissected *in vivo* or removed as a block for dissection in the laboratory within 15 min.
3. The excised muscle should be placed immediately in precarboxygenated Krebs-Ringer solution with a composition of:

NaCl	118.1 mmol l^{-1}
KCl	3.4 mmol l^{-1}
$MgSO_4$	0.8 mmol l^{-1}
KH_2PO_4	1.2 mmol l^{-1}
Glucose	11.1 mmol l^{-1}
$NaHCO_3$	25.0 mmol l^{-1}
$CaCl_2$	2.5 mmol l^{-1}
pH	7.4

   The ion concentration of the Krebs-Ringer solution should be as stated with a maximal deviation of±10%, and should be checked at least every month. pH should be in the range 7.35–7.45 at 37°C.
4. The muscle should be transported to the laboratory in Krebs-Ringer solution at ambient temperature. In the laboratory it should be kept at room temperature and carboxygenated.
5. The time from biopsy to completion of the tests should not exceed 5 h.
6. The tests should be performed at 37°C in a tissue bath perfused either intermittently or continuously with Krebs-Ringer solution and carboxygenated continuously. At least four tests should be performed, each on a fresh specimen. These include two static caffeine tests and two halothane tests. The halothane test could consist of either one static and one dynamic test or two static tests. Each laboratory should be consistent in the method employed. Separate tissue baths should be used for different agents.
7. **Muscle specimen dimensions.** Muscle specimens suitable for *in vitro* investigation should measure 15–25 mm in length between ties with a thickness of 2–3 mm. For measurement of length, see 8 below. The weight of the specimens should be 100–200 mg. The specimens are blotted and weighed after the test, between sutures.
8. **Determination of specimen length and predrug force.** The static tests are performed at optimal length (l_0) which is determined 5 min after suspension of the specimen in the tissue bath by slowly stretching the muscle to force of 2mN (0.2g). The length between sutures is measured (initial length). Leave the muscle for another 5 min at initial length, then commence electrical stimulation (see 9 below) and stretch the muscle slowly to 150±10% of initial length. This new length is considered to be optimal length (l_0) and is recorded. Gassing of the Krebs-Ringer solution is stopped temporarily during measurement of length.
   The muscle is left at optimal length (l_0) to stabilise for at least 15 min and until baseline force does not vary more than 2.0 mN (0.2 g) within a 10-min period. Then drugs may be added. The baseline force immediately before addition of drug is recorded as the predrug force.
9. **Electrical stimulation.** To demonstrate viability, the muscle specimen should be electrically stimulated with a 1–2 ms supramaximal stimulus at a frequency of 0.2 Hz. Following suspension of the muscle in the tissue bath and obtainment of initial length, current or voltage is slowly increased until twitch height does not increase any more (initial stimulus intensity). For the supramaximal stimulation, the current or voltage is increased to 120% of initial stimulus intensity.
10. **The static cumulative caffeine test and measurement of the caffeine threshold.** The concentrations of caffeine (as free base, analytical grade) in the tissue bath should be increased stepwise as follows: 0.5; 1.0; 1.5; 2.0; 3.0; 4.0; and 32 mmol l^{-1}. Each successive concentration of caffeine should be administered as soon as the maximum contracture plateau induced by the previous concentration of caffeine has been reached, or after exposure of the muscle to the caffeine concentration for 3 min if no contracture occurs. The muscle is not washed with fresh Krebs-Ringer solution between successive concentrations of caffeine.
    Caffeine should be added to the tissue bath either as a bolus by injection, or with low-volume baths in the Krebs-Ringer perfusate. A rapid change of caffeine concentration must be achieved.
    The result of this test will be reported as the threshold concentration which is the lowest concentration of caffeine which produces a sustained increase of at least 2 mN (0.2 g) in baseline force

from the lowest force reached. In addition, the maximum contracture achieved at 2 mM caffeine should be reported.

Please note that the lowest force is not necessarily the same as the predrug force.

11. **The static halothane test and measurement of static halothane threshold.** The halothane threshold is obtained using the halothane concentrations 0.11; 0.22; 0.44 and 0.66 mmol l^{-1} as equivalent to 0.5; 1.0; 2.0 and 3.0% v/v, respectively. The specimen should be exposed to each halothane concentration for 3 min. The measurement of halothane threshold is similar to 10 above. The maximal contracture achieved with 0.44 mmol l^{-1} halothane should also be reported. For determination of halothane concentration see 14 below.

    The flowrate of gas should be set to maintain the correct halothane concentration in the tissue bath. The gasflow into the tissue bath should be controlled using a low-flow rotameter or similar device, situated close to the inlet port of the tissue bath. The time to reach equilibration of the halothane concentration in the bath should be determined in order to ensure that the muscle sample is exposed to the test drug for the required period. The equilibration time will depend on bath volume, gas and perfusion flowrate and aerodynamics of the system.

12. **The dynamic halothane test and measurement of dynamic halothane threshold.** This test is dependent on a motor. Initially, the muscle is stretched at a constant rate of 4 mm min^{-1} to achieve a force of approximately 30 mN (3 g) and held at this new length for 1 min. The stretching process is then reversed for 1.5 min. The movement of the transducer from the end of the 1-min rest period to the low force is measured accurately using a vernier scale. This measurement is then used to achieve all subsequent length/tension curves, i.e. the muscle is stretched and shortened 6 mm in each cycle. The muscle is allowed to rest for 3 min. The process is then repeated to obtain 3 control curves with 1 min rest at high force and 3 min rest at low force. At the end of the descent of the third control curve, the muscle is exposed to 0.11 mmol l^{-1} halothane (0.5%) for 3 min and the stretch process is repeated. The procedure is repeated for 0.22 and 0.44 l^{-1} halothane (1 and 2%).

    The force is measured at the end of the 1-min rest after stretching and the dynamic halothane threshold is the lowest concentration increasing force 2 mN (0.2 g). The maximal contracture at 0.44 mmol l^{-1} is also recorded.

13. **Diagnostic criteria**

    MHS

    A caffeine threshold (as defined earlier) at a caffeine concentration of 2.0 mmol l^{-1} or less, *and* a halothane threshold concentration at 0.44 mmol l^{-1} or less.

    MHN

    A caffeine threshold at a caffeine concentration of 3 mmol l^{-1} or more *and* a halothane threshold concentration above 0.44 mmol l^{-1}.

    MHE

    All other results are deemed equivocal but designated MHEh if reacting to halothane only or MHEc if reacting to caffeine only.

    It is envisaged that most MHE patients will be regarded clinically as MH susceptible. MHE results must be considered to be under permanent review pending the acquisition of further control and mutation data.

    MHE results should be treated separately in research studies.

14. **Quality control**

    Viability in any specimen used should be demonstrated by twitches ≥10 mN (1 g) at the beginning of a test, or for the caffeine test a response to 32 mmol l^{-1} ≥50 mN (5 g) at the end.

    The concentrations of halothane and caffeine in the tissue bath should be checked at least every 3 months. The samples should be taken directly from the tissue bath under the same dynamic conditions as when testing. Samples for determination of halothane concentrations should be taken immediately after the gas flow has been stopped to avoid sampling from the gas phase. Halothane concentrations can be measured using GLC or HPLC and caffeine using UV spectroscopy.

    Halothane 0.44 mmol l^{-1} and caffeine 2 and 32 mmol l^{-1} should be checked.

    Accepted maximal deviation from the desired concentrations are ±10%. Lambda halothane (air/Krebs-Ringer) is taken to be 0.72 at 37°C.

    The vaporizer should be serviced and calibrated at yearly intervals.

    It is recommended that halothane concentrations in the gas phase are monitored close to the gas inlet port to the tissue bath.

    Temperature of the tissue bath should be checked.

15. **Control biopsies.** All MH units are asked to investigate control muscle biopsies according to this protocol. For control biopsies, the following groups of patients are considered suitable: healthy

volunteers, patients having amputations for localized disease (not systemic or vascular disease), patients with varicose veins, brain-dead patients within the first 24 h, patients with fractures within the first 24 h.

16. **Optional tests.** Tests with other drugs may be performed on an optional basis. Results of optional tests are not used for diagnosis. However, to allow for comparison of results between centres it is recommended that optional tests are performed in a uniform way, agreed upon by the EMHG Board of Directors. At present, protocols exist for tests with ryanodine and 4-chloro-m-cresol. These protocols may be obtained from the group.
17. **Protocol revision.** The EMHG protocol for investigation of MH susceptibility is regularly revised, latest in May 1997.