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ORIGINAL INVESTIGATION

Predictive factors of the contracture test for diagnosing malignant hyperthermia in a Brazilian population sample: a retrospective observational study

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Anesthesia;
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Abstract

Introduction: Malignant Hyperthermia (MH) is a pharmacogenetic, hereditary and autosomal dominant syndrome triggered by halogenates/succinylcholine. The *In Vitro* Contracture Test (IVCT) is the gold standard diagnostic test for MH, and it evaluates abnormal skeletal muscle reactions of susceptible individuals (earlier/greater contracture) when exposed to caffeine/halothane. MH susceptibility episodes and IVCT seem to be related to individual features.

Objective: To assess variables that correlate with IVCT in Brazilian patients referred for MH investigation due to a history of personal/family MH.

Methods: We examined IVCTs of 80 patients investigated for MH between 2004–2019. We recorded clinical data (age, sex, presence of muscle weakness or myopathy with muscle biopsy showing cores, genetic evaluation, IVCT result) and IVCT features (initial and final maximum contraction, caffeine/halothane concentration triggering contracture of 0.2g, contracture at caffeine concentration of 2 and 32 mmoL and at 2% halothane, and contraction after 100 Hz stimulation).

Abbreviations: CACNA1S, Dihydropyridine receptor voltage-gated calcium channel L subunit alpha 1S; Caf32, Contracture after 32 mM of caffeine; CCD, Central Core Disease; CHCT, Caffeine Halothane Contracture Test; FMC, Final Maximum Contraction; EMHC, European malignant hyperthermia group; FMH, Familial Malignant Hyperthermia; IMC, Initial Maximum Contraction; IVCT, In Vitro Contracture Test; MH, Malignant Hyperthermia; MHN, Malignant Hyperthermia Negative; MHS, Malignant Hyperthermia Susceptible; MHSc, Malignant Hyperthermia Susceptible positive for caffeine only; MHSH, Malignant Hyperthermia Susceptible positive for halothane only; MHShc, Malignant Hyperthermia Susceptible positive for halothane and caffeine; MMD, Multiminicore Disease; PMH, Personal Malignant Hyperthermia; RYR1, Type 1 Ryanodine receptor; STAC3, Protein 3 with cysteine/SH3-rich domain; mM, Millimolar per liter; T100, Contraction after stimulation at 100 Hz.

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Results: Mean age of the sample was 35±13.3 years, and most of the subjects were female (n=43 or 54%) and MH susceptible (60%). Of the 20 subjects undergoing genetic investigation, 65% showed variants in *RYR1*/*CACNA1S* genes. We found no difference between the positive and negative IVCT groups regarding age, sex, number of probands, presence of muscle weakness or myopathy with muscle biopsy showing cores. Regression analysis revealed that the best predictors of positive IVCT were male sex (+12%), absence of muscle weakness (+20%), and personal MH background (+17%).

Conclusions: Positive IVCT results have been correlated to male probands, in accordance with early publications. Furthermore, normal muscle strength has been confirmed as a significant predictor of positive IVCT while investigating suspected MH cases.

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Introduction

Malignant Hyperthermia (MH) is a hypermetabolic hereditary syndrome (autosomal dominant), triggered by halogenated anesthetics and/or succinylcholine, and occurs in 1:10,000 to 1:250,000 anesthetized subjects.[1] MH susceptibility is also associated with idiopathic increase in creatine kinase, exertional/heat hyperthermia, Central Core Disease (CCD), Multiminicore Disease (MMD), and King-Denborough Syndrome.[1] Specific treatment and prompt diagnosis have reduced MH crisis mortality from 70% to less than 5%.[2] MH is caused by mutations in the genes that control intracellular calcium homeostasis and the skeletal muscle excitation-contraction system, such as *RYR1* (type 1 Ryanodine Receptor), *CACNA1S* (voltage-gated Calcium Channel), and *STAC3*.[3]

There are two standard tests for diagnosing MH: the *In Vitro* Contracture Test (IVCT) used by the European Malignant Hyperthermia Group (EMHG), and the Caffeine Halothane Contracture Test (CHCT) used in North America.[4-6] Both are based on abnormal skeletal muscle response, represented by earlier and greater contractures in susceptible individuals after exposure to caffeine/halothane. Additionally, the genetic test is helpful given it detects variants shown to be MH-related.[5] Nevertheless, a negative genetic test does not rule out MH susceptibility.[5] IVCT has 99% sensitivity and 94% specificity,[7] and is associated not only with the intrinsic characteristics of the muscle specimen, but also with individual patient characteristics.[8]

This study aimed to examine which variables were associated with IVCT results in patients referred for malignant hyperthermia diagnosis investigation.

Methods

This descriptive, retrospective, observational study was approved by the ethics committee of the host institution (CAAE: 73681017.9.0000.5505. Proposing Organization: Escola Paulista de Medicina. Number: 4.098.648. Project CEP/UNIFESP: 0979/2017). All participants signed an informed consent form before being submitted to muscle biopsy. Initially, we randomly selected the IVCT graphs of 128 patients among 190 patients who underwent MH investigation from 2004 to 2019.

We only included tests conducted due to a history of personal or family MH and complying with the following feasibility criteria: Initial Maximum Contraction (IMC) ≥1 g or contracture after 32 mMoL.L^{-1} (mM) of Caffeine (Caf32) ≥ 5 g in at least one muscle specimen exposed to caffeine,[5] and IMC ≥ 1 g in at least one specimen exposed to halothane.[5] Tests were kept for 80 of the 128 patients based on these criteria (Supplementary Material Fig. 1). Additionally, we collected data regarding age of participants at the time of muscle biopsy, sex, presence of muscle weakness in a standardized neurological examination (failure to overcome the resistance posed by the examiner during movements assessed), presence of myopathy showing cores (areas with no oxidative activity detected in the muscle histopathologic study), genetic study (*RYR1* and *CACNA1S* gene sequencing), and IVCT description and result (IMC, caffeine and halothane concentration at 0.2 g contracture, contracture at 2 mM caffeine and 2% halothane, Final Maximal Contraction (FMC), contraction after 100 Hz stimulation (T100) and after Caf32 (Fig. 1).

A vastus lateralis muscle biopsy was performed under peripheral nerve block, spinal anesthesia, or general venous anesthesia for IVCT.[4] Two specimens (15-25 × 5 mm) were harvested, subsequently split into four specimens, and kept in carboxygenated Krebs-Ringer solution. The time from specimen harvesting to IVCT completion did not exceed five hours. Each specimen was attached to a silver electrode at one end and to a force transducer on the other, so that the degree of muscle contraction (supramaximal electrical stimulus, 1 ms, 0.2 Hz) and contracture (caffeine/halothane effect) were monitored in real time throughout the test and recorded on a computer for subsequent printing. Contraction was defined as the activation of muscle fibers with shortening under normal conditions.[9] Muscle fiber contracture was defined as a pathological muscle contraction with no recovery to the normal state of muscle fiber relaxation, because of lack of normal redistribution of calcium in the sarcoplasmic reticulum, that occurs in situations such as neuromuscular diseases, tetanus, and MH.[10]

Each patient underwent four static tests: two with caffeine and two with halothane. Every specimen was exposed to halothane or caffeine, separately. The concentration of the substances in the vat containing the test solution was gradually increased (0.5/1.0/1.5/2.0/3.0/4.0/32.0 mM for caffeine and 0.5/1.0/2.0/3.0% for halothane test). Each concentration was administered once the maximum contracture threshold was attained or after three-minute exposure. Test result was defined as the caffeine or halothane threshold, i.e., the lowest concentration that promoted a

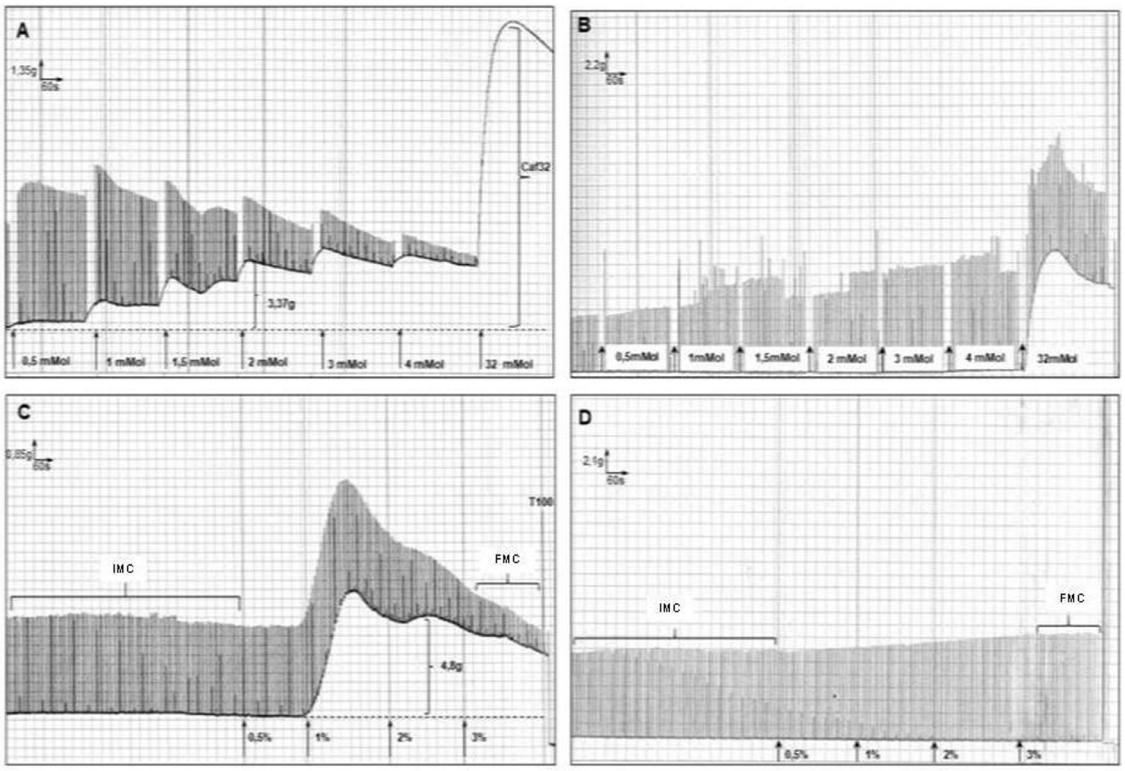

Figure 1 IVCT variables. (A) Positive contracture test for caffeine exposure, (B) Negative contracture test for caffeine exposure, (C) Positive contracture test for halothane exposure, (D) Negative contracture test for halothane exposure. Caf32, Contracture after 32 mM of caffeine; IMC, Initial Maximum Contraction; FMC, Final Maximum Contraction; T100, contraction after stimulation at 100 Hertz.

minimum increase of 0.2 g in baseline muscle tension. We considered as susceptible, patients presenting a contracture of 0.2 g to at least one of the two substances, up to concentrations of 2 mM caffeine or 2% halothane. Consequently, IVCT results were negative – Malignant Hyperthermia Negative (MHN), or positive – Malignant Hyperthermia Susceptible (MHS). Positive tests were subdivided into three categories, according to the substances associated with contractures: MHShc (positive for halothane and caffeine), MHSh (positive only for halothane), and MHSc (positive only for caffeine).[5] At the end of both tests, the specimen was submitted to 100 Hz tetanic stimulation to confirm residual viability of the specimen.

There was no significant difference in contractile parameters between the first and second specimens submitted to halothane tests, and their findings were analyzed together (Supplementary Material Table 1). There was a significant difference for Caf32 between two specimens submitted to caffeine tests, but findings for both specimens were above the 5 g threshold, and all contractile parameters were analyzed together (Supplementary Material Table 2).

Statistical analysis

Comprehensive data available on variables correlated to IVCT results are scarce. Male participants were more often susceptible in a Swedish study, while mutations linked to CCD were associated with more severe contractures in a British study.[8] To examine the relationship between demographic/clinical/laboratory variables and IVCT results, we would require previous studies assessing these variables to perform precise sample calculation. As such data are scarce, in the present study sample size calculation considered an early report referring that 40% of female and 70% of male subjects undergoing IVCT tests presented positive results.[11] To replicate this 30% difference between genders, with a detection power of 80% and a confidence level of 90%, at least 35 patients of each sex would be required.

Data were examined for normal distribution by the Kolmogorov-Smirnov test for distance and expressed as central and scatter values. Groups were compared using unpaired Student's *t*, Mann-Whitney, and Chi-square tests, and differences between groups were depicted in Tables 1 and 2. Tables 1 to 4 in the Supplementary Material describe the analysis by paired Student's *t*, Wilcoxon, Pearson and Spearman tests. We performed regression analysis using the cumulative standard normal distribution function (Probit regression). The model included variables showing probability of significance of up to 0.2 in previous analyses (sex, normal strength, referral reason, presence of cores, and laboratory parameters for the caffeine and halothane tests) and excluded diagnostic variables (concentration of caffeine and halothane in which there was contracture at 0.2 g and contracture at 2 mM caffeine and 2% halothane). Except for the calculations related to Probit regression, $p < 0.05$ was considered significant. Regression used the two specimens exposed to each substance (caffeine/halothane). We used the Prism 3.0 statistical analysis program (GraphPad software, San Diego, CA, USA). Criteria used for classifying correlations/percentages were the following: 0–0.09 (null); 0.10–0.29 (very low); 0.30–0.49 (low); 0.50–0.69 (moderate); 0.70–0.89 (high); 0.90–1.00 (very high).[12]

Table 1 Analysis of clinical and laboratory variables according to the result of the in vitro muscle contracture test.

Variables	MHN (n = 32)	MHS (n = 48)	*p*
Age	33.22 ± 11.36	37.06 ± 14.52	0.21[b]
Gender (male/female)	11/21	26/22	0.08[a]
Referral due to PMH/FMH	6/26	16/32	0.1524[a]
Muscle weakness (yes/no)	7/25	5/43	0.16[a]
Presence of cores in biopsy (yes/no)	2/30	4/44	0.73[a]
Gene variant *RYR1/CACNA1S* (yes/no)	nr	13/7 (65%)	
Variables according to halothane test			
IMC	3.7 (1.82–5.56)	4.6 (2.44–8.59)	**0.02**[c]
FMC	6.47 ± 3.24	7.08 ± 4.06	0.318[b]
T100	27.02 ± 12.64	23.54 ± 13.80	0.109[b]
Concentration at contracture 0.2g (%)	3.00 ± 0.0	1.53 ± 0.65	–[d]
Contracture at 2%	0 (0–0)	0.34 (0.2–1.16)	**<0.0001**[c]
Variables according to caffeine test			
IMC	3.88 (2.46–6.75)	4.16 (2.59–8.3)	0.418[c]
FMC	10.86±5.10	6.68±4.70	**<0.0001**[b]
T100	21.94±12.72	14.11±11.97	**0.0001**[b]
Concentration at contracture 0.2g (%)	18 (4–32)	3 (1.5–4)	**<0.0001**[c]
Contracture at 2 mM	–	0.41±0.73	–[d]
Contracture at 32 mM	8.83 (7.07–11.6)	10 (7.6–13.95)	0.059[c]

MHN, Not susceptible to Malignant Hyperthermia (MH); MHS, Susceptible to MH; PMH, Personal MH; FMH, Familial MH; nr, Not performed; IMC, Initial Maximum Contraction; FMC, Final Maximum Contraction; T100, Tetanus at 100 Hz.
p-values in bold are below 0.05 (5%).
[a] Chi-Square test, absolute values.
[b] Unpaired *t*-test, mean values and standard deviation expressed in g.cm^{-2}.
[c] Mann Whitney test, median values (25th percentile–75th percentile) expressed in g.cm^{-2}.
[d] Number of muscle specimens with contracture of 0.2g in the MHN group was insufficient for statistics.

Results

Mean age ± standard deviation of the 80 patients assessed was 35±13.3 years (range 8–71). Thirty-seven (46%) patients were male and 43 (54%) were female. Forty-eight (60%) patients presented positive IVCT and were diagnosed as MHS, and 32 (40%) as MHN because their IVCT was negative. Positive-IVCT patients comprised four MHSc, 22 MHSh, and 22 MHShc.

Table 2 Analysis of variables according to the reason for referral.

Variable	PMH (n = 22)	FMH (n = 58)	*p*
Age	26.55 ± 9.69	38.64 ± 13.19	**0.0002**[b]
Presence of cores in the biopsy (yes/no)	3/19 (16%)	3/55 (5%)	0.2[a]
Gene variant *RYR1/CACNA1S* (yes/no)	8/4 (40%)	5/3 (25%)	0.84[a]
MHS/MHN	16/6 (73%)	32/26 (55%)	0.152[a]
Variable according to test with halothane			
IMC	6.36 (2.46–8.59)	3.68 (1.98–6.55)	**0.044**[c]
FMC	6.98 ± 4.47	6.78 ± 3.48	0.766[b]
T100	20.90 ± 13.93	26.58 ± 12.85	**0.0161**[b]
Contracture at 2%	1.2 (0.4–2.24)	0 (0–0.2)	**<0.0001**[c]
Concentration at contracture 0.2g (%)	1 (1–2)	2 (2–2)	**<0.0001**[c]
Variable according to test with caffeine			
IMC	4.16 (2.7–6.83)	4.12 (2.4–7.43)	0.975[c]
FMC	7.87 ± 5.70	8.55 ± 5.10	0.47[b]
T100	18.05 ± 13.81	17.03 ± 12.50	0.659[b]
Contracture at 2 mM	0 (0–0.38)	0 (0–0)	0.237[c]
Concentration at contracture 0.2g (%)	3 (1.5–4)	4 (3–32)	**0.014**[c]
Contracture at 32 mM	9.07 (6.9–12.9)	9.6 (7.29–13.2)	0.402[c]

PMH, Personal MH; FMH, Familial MH; MHN, Not Susceptible to Malignant Hyperthermia (MH); MHS, Susceptible to MH; IMC, Initial Maximum Contraction; FMC, Final Maximum Contraction; T100, Tetanus at 100 Hz.
p-values in bold are below 0.05 (5%).
[a] The Chi-Test square, absolute values.
[b] Unpaired *t*-test, mean values and standard deviation expressed in g.cm^{-2}.
[c] Mann- Whitney test, median values (25th percentile–75th percentile) expressed in g.cm^{-2}.

Table 3 Probit regression: model with two specimens.

Dimension	Variable	Two Specimens Mean Marginal Effect (SD) [95% Confidence Interval]
Personal Features	Gender (Male = 1)	+0.12[a] (0.069) [-0.012, 0.256,]
	Normal strength (Yes = 1)	+0.20[b] (0.092) [0.016, 0.376,]
	Reason (Personal = 1)	+0.17[b] (0.081) [0.016, 0.335]
	Cores (Yes = 1)	0.01 (0.126)
Test with caffeine	IMC	0.031[b] (0.013) [-0.006, 0.057]
	FMC	-0.06[c] (0.010) [-0.079, -0.039]
	Caf32	0.001 (0.009)
	T100	0.01[b] (0.004) [0.000, 0.017]
Test with halothane	IMC	0.02 (0.016)
	FMC	0.04[b] (0.019) [0.003, 0.078]
	T100	-0.01[c] (0.004) [-0.022, -0.006]
Prediction agreement percentage	78.29%	
Number of observations	152	

IMC, Initial Maximum Contracture; FMC, Final Maximum Contracture; T100, Tetanus at 100 Hz; Caf32, Contracture after 32 mM of caffeine; SD, Standard Deviation.
[a] Statistically significant at 10%.
[b] Statistically significant at 5%.
[c] Statistically significant at 1%.

We registered 57 families among the total patients studied. Thirty-nine families (68.4%) had an index or solitary case; 14 families had two cases (24.5%), three families had three cases (5.3%), and in one family we registered four cases (1.7%). We detected a total of 18 variants in 13 patients with positive IVCT (16 in *RYR1* and two in *CACNA1S*), as one patient had three variants and three other patients presented two variants each. Variants were classified as benign (two *RYR1*), intronic (one *RYR1*), likely pathogenic (two *CACNA1S*, nine *RYR1*), and pathogenic (four *RYR1*).

When we compared positive with negative IVCT patients, we observed no significant differences between the two groups regarding age at the time of biopsy, sex, muscle weakness, occurrence of cores in muscle biopsy, and number of probands (Table 1). During halothane tests, muscle specimens in the MHS group responded with greater IMC and greater contracture at 2% halothane exposure (Table 1, Supplementary Material Table 3). During caffeine tests, muscle specimens in the MHS group showed smaller Final Maximum Contraction (FMC), lower T100, and required caffeine concentrations three times lower than specimens of the MHN group to achieve contractures ≥ 0.2 g (Table 1, Supplementary Material Table 4).

No differences were found between proband and family subgroups regarding sex, presence of muscle weakness, muscle biopsy cores and proportion of MHN/MHS results (Table 2). The family group was significantly older than the proband group. During the halothane test, muscle specimens in the proband group developed greater IMC than the family group, lower T100, greater contractures during 2% halothane exposure, and required lower halothane concentration to achieve contracture ≥ 0.2 g (Table 2). During the caffeine test, muscle specimens in the proband group required lower concentrations than specimens in the family group to achieve contracture ≥ 0.2 g.[2]

The regression model displayed high rate of correct answers (above 70%) regarding the probability of forecasting positive IVCT. The best model predictors of positive IVCT were male sex (mean marginal effect +12%), absence of muscle weakness (+20%), and history of personal MH (+17%) (Table 3). Regarding the caffeine test, the muscle specimens presenting the highest degree of IMC and T100, and the lowest degree of FMC were predictors of positive IVCT (respectively, mean marginal effect of +3.1%, -6% and +1%) (Table 3). Regarding the halothane test, higher degree of FMC and lower degree of contraction at T100 were predictors of positive IVCT (respectively, mean marginal effect of 4% and -1%) (Table 3). The average marginal effect represented the probability of change in the dependent variable (IVCT changing from negative to positive), defined when the independent variable in question presented in the respective line (sex, muscle strength, reason for MH investigation, biopsy showing cores and other parameters in caffeine and halothane tests) was 1, or increased by one unit without variations in the other variables assessed.

Discussion

IVCT results in patients referred due to personal or family history of MH was mostly associated with clinical variables (sex, muscle strength and reason for the investigation). The intrinsic characteristics of tested muscle specimens contributed less.

Although MH episodes have been reported predominantly in males in their second decade of life,[1] our sample had a mean age within the fourth decade, comprised mostly by female subjects and who were diagnosed as MHS. As our center usually did not perform IVCT in children, MH investigation started with their parents, explaining the older age group of the sample. This would also explain the older age in those investigated due to family MH than due to personal MH. Female sex predominance can be explained because mothers more commonly had the initiative of performing IVCT. The percentage of positive IVCT has ranged from 39% to 45%.[13,14] Our higher incidence of positive IVCT could

possibly result from the fact that only more severe cases were being diagnosed and referred to perform IVCT, as well as a possible higher frequency of mutations occurring in Brazil, as previously indicated by the report of compound heterozygotes.[15] Another explanation could be false positive results, which supposedly would occur in a low percentage of IVCT.[5]

The presence of variants in the *RYR1*/*CACNA1S* genes was similar to the description in other series (47–70%).[16] Patients with positive IVCT and no variants in *RYR1*/*CACNA1S* genes may show variants in the *STAC3* gene or other genes related to the excitation-contraction process that have not been discovered yet. Muscle weakness and incidental cores on muscle biopsy were detected in a minority of cases, similarly to other series, where the frequency ranged respectively, from 2.1% to 10%.[17,18] and from 19% to 22%.[18,19]

In this sample, age did not impact IVCT results, notwithstanding the small positive correlation between age and the 0.2 g halothane concentration in which contracture occurred, that is, muscle specimens harvested from older individuals required exposure to higher halothane concentration to achieve the diagnostic threshold. This finding could indicate depletion of contractile/energy reserves or changes in the RYR1 receptor associated with aging.[20] A British study did not describe age affecting the IVCT phenotype.[21]

Despite the comparable sex distribution in MHS and MHN groups, this variable was able to predict the positive IVCT outcome. This finding was previously attributed to the possible different expression of calcium handling proteins in males.[11] Some authors have suggested that male predominance could indicate a non-Mendelian inheritance pattern.[8] Animal models with mutations in the *RYR1* gene revealed distinct male involvement, with a higher probability of crisis triggered by anesthetics and high temperature scenarios, as well as muscle contractility changes and in vitro release of calcium.[22,23] In previous series, male predominance ranged from 66% to 70%.[10,11]

The absence of muscle weakness as a predictor of positive IVCT outcome has not been previously described, despite the report of muscle hypertrophy in susceptible patients.[24] Conversely, there were proportionately more patients presenting muscle weakness in the MHN group. These patients may have developed atypical reactions to anesthesia other than MH, such as anesthesia-associated rhabdomyolysis, which can develop in neuromuscular disorders due to muscle membrane fragility and/or upregulation of extra junctional acetylcholine receptors.[25]

The proband variable revealed a predictor effect on positive IVCT outcome, indicating that MH diagnosis tends to be confirmed in patients surviving an alleged MH crisis. Alternatively, family members investigated tended to show fewer positive results, because of the 50% likelihood due to the autosomal dominant character. In this sample, detecting cores in the muscle biopsy was not a predictor of positive IVCT, agreeing with an early investigation that did not reveal a relationship between the presence of myopathy/histological abnormalities and IVCT result.[14]

A minority of patients reacted only to the caffeine test. Previous studies have reported this finding ranging from 4.5% to 6%.[26,27] An isolated positive caffeine test is considered less specific for MH and has been associated with the presence of underlying myopathy.[28] This may be because caffeine would activate several metabolic pathways, whereas halothane would specifically bind to the RYR1 receptor.[29] The present study revealed that the groups presented a similar number of patients reacting only to halothane or to caffeine/halothane. In other series using IVCT, the frequency of MHSh results ranged from 16% to 21.4%, while MHShc results occurred in 22.6% to 45.5%.[26,27]

We expected the findings related to diagnostic variables when comparing MHS *versus* MHN group, such as lower caffeine concentration for a 0.2 g contracture and greater contracture at 2% halothane. Regarding the other diagnostic variables, the number of participants in the MHN group was not large enough for calculation, given 0.2g contractures above the 2% halothane concentration threshold occurred in less than five patients and none had contractures at 2 mM of caffeine.

The IVCT protocol recommended actions to maintain muscle integrity and assessment of muscle specimen viability during testing.[27] In the present study, viability was confirmed by the similarity between two specimens tested for each drug. This was also demonstrated by the positive correlations in both groups, between IMC and FMC/T100/Caf32, and between FMC and T100. Moreover, we found a weak positive correlation of IMC with the diagnostic variable contracture level under halothane, but no impact of IMC on the degree of contracture level under caffeine.

There was a greater IMC of MHS muscle specimens during the halothane test, with IMC in the caffeine test as a predictor of positive IVCT outcome, suggesting that the muscle presented greater initial contractile capacity.[21,24] Likewise, the PMH group also revealed greater IMC than the FMH group. However, the resultant average marginal effect of IMC was low in the regression model, revealing little influence, the same being observed for all predictor intrinsic variables of IVCT.

The variables at the end of the test reflected contractile and energy reserves after stimulation with each drug. For the halothane test, there was no difference between groups. However, regarding the caffeine test, the MHS group developed lower FMC/T100 values, indicating larger depletion of contractile and energy reserves, yet allowing the Caf32 value to be similar to the value observed in the MHN group. In addition to caffeine acting non-selectively on the calcium release channel and triggering several additional pathways, patients reacting only to halothane may belong to a distinct group.[30] MHSh patients in the North American protocol probably differed from MHShc patients due to a high cytosolic calcium at rest, indicating that abnormal voltage sensitivity is the main functional defect in this group, leading to a subsequent increase in calcium leakage at rest.[30]

In this sample, greater contractures occurred with lower concentrations of both halothane and caffeine, suggesting results proportional to the severity of mutations linked to calcium release.[21] Conversely, in a sample analyzed with the contracture test complying with the North American protocol, this correlation was absent in the halothane test, indicating an “all or nothing” response pattern and higher non-specificity regarding mutations.[29] Likewise, in the subgroup analysis, the PMH group had higher contractures at 2%

halothane, and lower concentrations of halothane/caffeine at 0.2 g contracture.

This study has some limitations. The scarcity of data from previous investigations focusing on all the variables analyzed in the regression model rendered the estimate of the ideal sample size impossible. The sample size for our entire study was based on the data available for the sex variable. Moreover, due to the exploratory nature of the present study, we used *p*-values of up to 0.2 for Probit regression calculations. However, the findings obtained in the present investigation will be used in the next phase study, enabling the use of multiple variables for sample estimation. The unavailability of genetic studies for the entire sample is another limitation of the present study, especially in relation to patients with the MHN test. Due to economic restrictions, none of the MHN patients and only a portion of positive IVCT patients underwent genetic investigation.

Conclusion

Clinical variables were the major predictors of positive IVCT outcome for the diagnosis of MH susceptibility in Brazilian patients. According to previous studies, a positive IVCT result was related to male probands. Furthermore, absence of muscle weakness was revealed as a significant predictor while investigating a suspected case of MH. Thus, a patient with MH would probably present to the attending anesthesiologist without clinical signs that could alert the practitioner. The fact underlines the need for always having tools for diagnosing and treating MH episodes.

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Declaration of Competing interest

The authors declare no conflicts of interest.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.bjane.2022.06.010.

References

1. Rosenberg H, Pollock N, Schiemann A, Bulger T, Stowell K. Malignant hyperthermia: a review. Orphanet J Rare Dis. 2015;10:1—19.
2. Brady JE, Sun LS, Rosenberg H, Li G. Prevalence of malignant hyperthermia due to anesthesia in New York State, 2001-2005. Anesth Analg. 2009;109:1162—6.
3. Rosenberg H, Sambuughin N, Riazi S, Dirksen R. Malignant hyperthermia susceptibility. 2020. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2020. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1146/.
4. The European Malignant Hyperpyrexia Group. A protocol for the investigation of malignant hyperpyrexia (MH) susceptibility. Br J Anaesth. 1984;56:1267—71.
5. Hopkins PM, Rüffert H, Snoeck MM, Girard T, Glahn KPE, Ellis FR, et al. European Malignant Hyperthermia Group guidelines for investigation of malignant hyperthermia susceptibility. Br J Anaesth. 2015;115:531—9.
6. Larach MG. Standardization of the caffeine halothane muscle contracture test. Anesth Analg. 1989;69:511—5.
7. Ørding H, Brancadoro V, Cozzolino S, Ellis FR, Glauber V, Gonano EF, et al. 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. Acta Anaesthesiol Scand. 1997;41:955—66.
8. Carpenter D, Robinson RL, Quinnell RJ, Ringrose C, Hogg M, Casson F, et al. Genetic variation in RYR1 and malignant hyperthermia phenotypes. Br J Anaesth. 2009;103:538—48.
9. Ferreira AT. Fisiologia da Contração Muscular. Rev Neurociências. 2005;13:60—2.
10. Halsall PJ, Ellis FR. Malignant hyperthermia. Curr Anaesth Crit Care. 1996;7:158—66.
11. Islander G, Rydenfelt K, Ranklev E, Bodelsson M. Male preponderance of patients testing positive for malignant hyperthermia susceptibility. Acta Anaesthesiol Scand. 2007;51:614—20.
12. Witz K, Hinkle DE, Wiersma W, Jurs SG. Applied Statistics for the Behavioral Sciences. J Educ Stat. 1990;15:84.
13. Mezin P, Payen JF, Bosson JL, Brambilla E, Stieglitz P. Histological support for the difference between malignant hyperthermia susceptible (MHS), equivocal (MHE) and negative (MHN) muscle biopsies. Br J Anaesth. 1997;79:327—31.
14. Von Breunig F, Wappler F, Hagel C, Von Richthofen V, Fiege M, Weisshorn R, et al. Histomorphologic examination of skeletal muscle preparations does not differentiate between malignant hyperthermia-susceptible and normal patients. Anesthesiology. 2004;100:789—94.
15. Kossugue PM, Paim JF, Navarro MM, Silva HC, Pavanello RCM, Gurgel-Giannetti J, et al. Central core disease due to recessive mutations in RYR1 gene: Is it more common than described? Muscle and Nerve. 2007;35:670—4.
16. Gillies RL, Bjorksten AR, Du Sart D, Hockey BM. Analysis of the entire ryanodine receptor type 1 and alpha 1 subunit of the dihydropyridine receptor (CACNA1S) coding regions for variants associated with malignant hyperthermia in Australian families. Anaesth Intensive Care. 2015;43:157—66.
17. Larach MG, Gronert GA, Allen GC, Brandom BW, Lehman EB. Clinical presentation, treatment, and complications of malignant hyperthermia in North America from 1987 to 2006. Anesth Analg. 2010;110:498—507.
18. Knuiman GJ, Küsters B, Eshuis L, Snoeck M, Lammens M, Heytens L, et al. The histopathological spectrum of malignant hyperthermia and rhabdomyolysis due to RYR1 mutations. J Neurol. 2019;266:876—87.
19. Monnier N, Kozak-Ribbens G, Krivosic-Horber R, Nivoche Y, Qi D, Kraev N, et al. Correlations between genotype and pharmacological, histological, functional, and clinical phenotypes in malignant hyperthermia susceptibility. Hum Mutat. 2005;26:413—25.
20. Andersson DC, Betzenhauser MJ, Reiken S, Meli AC, Umanskaya A, Xie W, et al. Ryanodine receptor oxidation causes intracellular calcium leak and muscle weakness in aging. Cell Metab. 2011;14:196—207.
21. Robinson RL, Brooks C, Brown SL, Ellis FR, Halsall PJ, Quinnell RJ, et al. RYR1 mutations causing central core disease are associated with more severe malignant hyperthermia in vitro contracture test phenotypes. Hum Mutat. 2002;20:88—97.

22. Yuen B, Boncompagni S, Feng W, Yang T, Lopez JR, Matthaei KI, et al. Mice expressing T4826I-RYR1 are viable but exhibit sex- and genotype-dependent susceptibility to malignant hyperthermia and muscle damage. FASEB J. 2012;26:1311—22.
23. Lopez JR, Kaura V, Diggle CP, Hopkins PM, Allen PD. Malignant hyperthermia, environmental heat stress, and intracellular calcium dysregulation in a mouse model expressing the p.G2435R variant of RYR1. Br J Anaesth. 2018;121:953—61.
24. Dlamini N, Voermans NC, Lillis S, Stewart K, Kamsteeg EJ, Drost G, et al. Mutations in RYR1 are a common cause of exertional myalgia and rhabdomyolysis. Neuromuscul Disord. 2013;23:540—8.
25. da Silva HCA, M Hiray, Vainzof M, Schmidt B, Oliveira ASB, do Amaral JLG. Atypical reaction to anesthesia in Duchenne/Becker muscular dystrophy. Braz J Anesthesiol. 2017;68:4—7.
26. Islander G, Twetman ER. Comparison between the European and North American protocols for diagnosis of malignant hyperthermia susceptibility in humans. Anesth Analg. 1999;88:1155—60.
27. Fletcher JE. Comparison of European and North American Malignant Hyperthermia diagnostic protocol outcomes for use in genetic studies. Anesthesiology. 1999;90:654—61. https://doi.org/10.1097/00000542-199903000-00005. Available from:.
28. Takagi A, Araki M, Kojima S, Ida M, Watanabe T. Caffeine contracture of the skinned muscle fiber in malignant hyperthermia and neuromuscular diseases. In: Morio M, Kikuchi H, Yuge O (eds). Malignant Hyperthermia. Tokyo, Japan: Springer; 1996. p. 67-73. Available from: 10.1007/978-4-431-68346-9_10.
29. Jurkat-Rott K, McCarthy T. Lehmann-Horn F. Genetics and pathogenesis of malignant hyperthermia. Muscle and Nerve. 2000;23:4—17.
30. Figueroa L, Kraeva N, Manno C, Toro S, Ríos E, Riazi S. Abnormal calcium signalling and the caffeine—halothane contracture test. Br J Anaesth. 2019;122:32—41.