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An update on malignant hyperthermia diagnostics and anaesthetic machine preparation for patients at risk in Africa

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The EMHG first published its protocol for an in vitro contracture test (IVCT) in 1984[1] and this has ever since formed the bedrock of clinical diagnosis and phenotyping. In 2015[2] the EMHG published the updated guidelines for investigation of malignant hyperthermia susceptibility. Evidently the rigour of the IVCT stood the test of time and was used in more than 10 000 individuals worldwide to define their MH risk, representing an example of preventative medicine and improved patient safety.

The IVCT is a clinically useful and robust test[3] that is sensitive and specific. The initial point estimate for specificity of 94% could possibly be explained by the use of atrophied muscle, because muscle sampled during ipsilateral joint arthroplasty was included.[3] The reported point estimate for sensitivity of the IVCT was 99%. On closer examination of the single patient (case 4) who reduced the sensitivity from 100% to 99%, it was found that the authors misapplied the Larach Clinical Grading Scale (LCGS)[4] which specifically excludes rigidity on emergence of general anaesthesia as an indicator of MH. The LCGS score for this patient should have been 35, therefore excluding the patient from the category of "almost certain" MH used to determine the sensitivity of the test. This was possibly a case of iatrogenic hyperthermia and febrile convulsion in an infant who had received atropine before surgery and overly enthusiastic warming during surgery.

The robustness of the IVCT was further evident from its use to phenotype members of MH families in molecular genetic studies, firstly with the linkage analysis that identified *RYR1* as the major locus implicated in MH[5 6], and later with the second locus *CACNA1S*[7 8] providing further evidence for the involvement of interacting gene products. *STAC3* is the most recent gene associated with MH.[9] A homozygous *STAC3* mutation has been linked to Native American myopathy and MH susceptibility in one Native American family.[10]

The complex nature of the genetics of MH, which would not have been realized without the IVCT, has been confirmed further by early results of next-generation sequencing in MH families.[11 12] Once an MH-causative mutation has been identified in an MH index patient, the family can be screened for this mutation and all the relatives carrying this mutation will be considered MH susceptible. Unfortunately 50% of MHS individuals do not carry potentially pathogenic variants in the known MH-associated genes, resulting in a rather low sensitivity of MH genetic testing.[13]

Currently the presence of rare variants in *RYR1*, *CACNA1S* and *STAC3* also has a low specificity. While there are more than 200 reported *RYR1* variants associated with MH, only 42 *RYR1* and 2 *CACNA1S* variants are accepted as MH causative (www.emhg.org) and can be used in diagnostic genetic testing for MH. It is also important to note that these causative mutations are population specific and the patients from African origin are most likely to be negative for these causative mutations described in Caucasian patients. If the specific variants in the African population have been identified, it needs to be shown to produce functional changes compatible with a pathogenic role in appropriate model cell systems.[14] Currently we do not have a laboratory in South Africa that does functional studies and calcium handling.

Because of the complex nature of MH genetics, a negative genetic test result cannot be used to rule out MH susceptibility and patients with negative genetic results should be offered *in vitro* contracture testing to confirm their MH-negative status.

Before the establishment of *in vitro* contracture testing[15], persistently raised serum creatine kinase (CK) concentration was documented in association with MH susceptibility and proposed as a diagnostic test. The utility of resting CK concentration is, however, limited because of a lack of sensitivity and specificity.[16] However, in patients with so-called idiopathic hyperCKaemia, investigation of MH susceptibility may be warranted after other causes have been excluded by a full neurological examination.[17] As with patients with rhabdomyolysis, the neurologist should liaise with an MH testing centre, where any muscle biopsy should be carried out to also do IVCT and histology.

Criteria for patient referral

The most common reasons for referral for investigation of MH susceptibility[2] are listed in Table 1.

Table 1. Patients to be referred to the MH diagnostic centre for investigation of MH susceptibility.

	Reasons for referral for investigation of MH susceptibility
1.	Family history of MH.
2.	Adverse reaction to general anaesthesia where a trigger agent has been used, involving any combination of signs of increased metabolism (unexplained increase in carbon dioxide production, tachycardia, temperature increase), muscle rigidity, rhabdomyolysis, disseminated intravascular coagulation or death. Initial signs should be evident within 60 minutes of discontinuation of anaesthesia.
3.	When MH cannot be excluded after LCGS calculation of adverse anaesthetic event.
4.	Family history of unexplained perioperative death.
5.	Postoperative rhabdomyolysis after exclusion of other myopathies.
6.	Exertional rhabdomyolysis, recurrent rhabdomyolysis or persistently raised serum creatine kinase concentration where no cause has been identified after neurological work-up (idiopathic hyperCKaemia).
7.	Exertional heatstroke requiring hospital admission, where known predisposing factors have been excluded.
8.	Myopathy and detection of an uncharacterised, rare, potentially pathogenic *RYR1* variant like central core disease, multiminicore disease, congenital centronuclear myopathy, nemaline myopathy, congenital fibre-type disproportion, King-Denborough syndrome, benign Samaritan congenital myopathy, atypical periodic paralysis and statin myopathies.[9]

The diagnostic pathway for MH susceptibility

The diagnostic pathway for investigation of MH susceptibility is explained in the following flow chart published by the EMHG.[2]

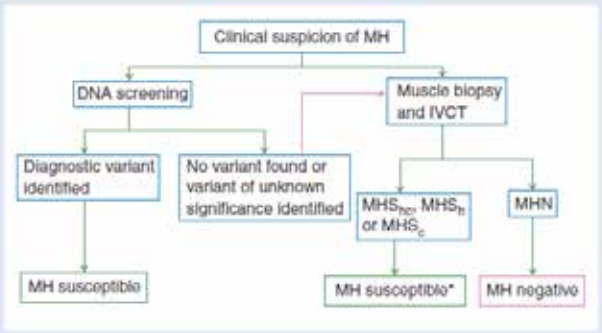

Fig 1 Diagnostic pathway for investigation of MH susceptibility. The decision to pursue either DNA screening or muscle biopsy and IVCT in the first instance will be made on a patient-by-patient basis by the MH diagnostic centre in consultation with the patient and their health-care funder. Factors that may be taken into consideration will include the availability of the respective tests (including turnaround time), the urgency of the test (for example, if the patient is awaiting surgery), the prior probability of a positive diagnosis, and the costs of the tests in the relevant laboratory. The prior probability of a positive diagnosis will be estimated by the clinical director of the MH centre based on clinical judgement, published data,[3-16] or a combination of these. *These patients should be invited to take part in research studies of the genetic basis of malignant hyperthermia. IVCT, *in vitro* contracture test; MH, malignant hyperthermia; MHN, *in vitro* contracture test laboratory classification applied when all contracture tests are negative; MHS$_{hc}$, MHS$_h$, and MHS$_c$, IVCT laboratory classifications applied when contracture responses to both halothane and caffeine are abnormal, response to halothane alone is abnormal, or response to caffeine alone is abnormal, respectively.

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The EMHG recommendations for minimum age and weight for children undergoing the IVCT is 10 years and 30 kg.[2] The biopsy should be performed on the quadriceps muscle, either the *vastus medialis* or *vastus lateralis,* using regional or trigger-free general anaesthetic techniques. Avoid any local anaesthetic infiltration of the muscle tissue. As the IVCT is a biophysical test and physical conditions around the muscle biopsy directly impact on the viability of the muscle specimen, muscle biopsy should be done at the MH centre where the muscle can be transported in carboxygenated Krebs-Ringers solution and the time from biopsy to completion of the test should not exceed five hours. The requirements for muscle specimen dimensions are 20–25 mm in length between ties, with a thickness of 2–3 mm. The weight of the specimen should be 100–200 mg.

The updated laboratory diagnostic classification is as follows:

- **MHS$_{hc}$:** a caffeine threshold at the caffeine concentration of 2.0 mmol litre^{-1} or less in at least one caffeine test, and a halothane threshold concentration at 0.44 mmol litre^{-1} or less in at least one halothane test.
- **MHS$_h$** : a halothane threshold concentration at 0.44 mmol litre^{-1} or less in at least one halothane test and a caffeine threshold at a caffeine concentration of 3 mmol litre^{-1} or more in all caffeine tests.
- **MHS$_c$** : a caffeine threshold at a caffeine concentration of 2.0 mmol litre^{-1} or less and a halothane threshold concentration above 0.44 mmol litre^{-1} in all halothane tests.
- **MHN**: a caffeine threshold at a caffeine concentration of 3 mmol litr^{-1} or more in all caffeine tests and a halothane threshold concentration above 0.44 mmol litre^{-1} in all halothane tests.

The **MHE** (Malignant Hyperthermia Equivocal) classification is no longer used and MH susceptibility is indicated by **MHS$_{hc}$, MHS$_h$** and **MHS$_c$,** and patients not susceptible to MH by **MHN.**

The most fundamental role of MH diagnostics is to be conservative in applying the MHN diagnosis because a false-negative diagnosis is most likely to have disastrous consequences. The evidence is that a laboratory diagnosis of MHN by IVCT provides a high degree of security if carried out in an EMHG-accredited laboratory. The EMHG quality assurance program assures that an accredited laboratory conducts the tests according to the EMHG published protocol, assures that specimens fulfill the viability criteria, and guarantees control of the test bath constituents and conditions, including concentration measurements by a reference laboratory. While the offspring of a patient who tested MHN cannot inherit MH susceptibility from that parent, it is still possible that they may be susceptible by transmission from the other untested parent or through a *de novo* genetic mutation. The probability of either of these events is less than the population prevalence of MH susceptibility, which is 1:15 000.

Activated charcoal filters

Along with the development of the newer anaesthetic machines came more challenges in preparation of the theatre for malignant hyperthermia susceptible patients[18]. Increased amounts of plastic and rubber parts on the internal circuit of new anaesthetic workstations that adsorb anaesthetic vapour serve as a reservoir of anaesthetic vapour, and after lengthy purging

it is evident that because of the rebound phenomenon, residual emission of anaesthetic vapour still occurs. It is also impossible to give a fixed time for purging to create a safe, clean machine as the amount of vapour absorbing parts in each make and model of anaesthetic machine differs. Therefore, the time it takes to purge the machine to get the vapour concentration in the fresh gas system to less than 5 ppm differs as well. It is recommended that after lengthy purging, fresh gas flow remains above 10 L/min throughout the anaesthetic.

Alternatively, activated charcoal filters (ACFs) can be used to prepare the anaesthetic machine to deliver a trigger-free general anaesthetic to MH susceptible patients or to patients with increased risk of developing an MH crisis.[19] ACFs can clean the anaesthetic machine in 90 seconds, irrespective of the specific make or model.

Recommendations on the use of activated charcoal filters [20] are summarised in Table 2.

Table 2. Recommendations on the use of activated charcoal filters in preparing an anaesthetic machine for a malignant hyperthermia susceptible patient.

	Recommendations
1.	Remove vaporisers from the anaesthetic machine.
2.	Flush the circuit for 90s with oxygen or air @ 10 l/min using the ventilator with a 2 litre test lung attached.
3.	Change the full breathing circuit and soda lime while maintaining flushing @ 10 l/min (the ventilator is left unchanged).
4.	Insert ACFs on both inspiratory and expiratory ports of the breathing circuit.
5.	Maintain FGF of 10 l/min for 90 minutes from the beginning of the anaesthetic.
6.	After 90 minutes it is safe to reduce the FGF to 3 l/min.
7.	Then ACFs can be used at 3 l/min until a total of 12 hours has elapsed from the commencement of the anaesthetic.
8.	After 12 hours ACFs need to be replaced.
9.	ACFs are single-use items.
10.	Single ACF can be placed in only the inspiratory limb but if misplaced accidentally in expiratory limb, it will be completely ineffective. Because of this potentially dangerous situation it is recommended that ACFs be used as a pair, one in each limb.

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Conclusion

Being able to determine potential malignant hyperthermia patients' real risk after so many years without an IVCT laboratory definitely improves the standard of care in South Africa. Additionally, the *in vitro* contracture testing facility could also be contributing to the DNA mapping of population groups with undefined causative MH variants. The availability of activated charcoal filters gives anaesthesiologists an option of expediting and improving anaesthetic machine preparation for malignant hyperthermia susceptible patients.[9 20]

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