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A reappraisal of diagnostic tests for myasthenia gravis in a large Asian cohort☆

Yew Long Lo [a,b,*], Raymond P Najjar [a,c], Kelvin Y Teo [c,d], Sharon L Tow [a,c,d,e], Jing Liang Loo [a,c,d,f], Dan Milea [a,c,d]

[a] Duke-NUS Graduate Medical School, Singapore
[b] National Neuroscience Institute, Singapore General Hospital, Singapore
[c] Singapore Eye Research Institute, Singapore
[d] Singapore National Eye Centre, Singapore
[e] National University Hospital, Singapore
[f] Yong Loo Lin School of Medicine, National University of Singapore, Singapore

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ABSTRACT

Background: Myasthenia gravis (MG) is a chronic autoimmune neuromuscular disease characterized by weakness of bodily skeletal muscles. Office-based diagnostic tests such as repetitive nerve stimulation (RNS), single fiber electromyography (SFEMG), and the ice test, are used to refine the differential clinical diagnosis of this disease. Evaluating the clinical sensitivity and specificity of these tests, however, may be confounded by lack of a gold standard, non-blinding, incorporation bias, use of non-representative populations and retrospective data.
Objective: In this study comprising a large Asian cohort of 127 patients recruited from a Neuro-ophthalmology clinic, we minimized aforementioned confounders and tested the diagnostic value of 3 office-based tests against 2 reference standards of MG by virtue of clinical features, antibody assay and response to treatment.
Results: Regardless of the reference standard used, the ice and SFEMG tests displayed a higher sensitivity (86.0 to 97.3%) compared to the RNS test (21.3 to 30.6%). Conversely, the specificity of the ice (31.3%) and SFEMG (21.7% and 17.2%) tests were reduced compared to the RNS test (82.6% and 84.4%). The combined use of the ice test and SFEMG, improved the specificity of MG diagnosis to 63.6% and 64.3%, without affecting the sensitivity of those tests.
Conclusion: Our findings indicate, in an Asian population, high sensitivity of the SFEMG test and suggest the ice test as a valid, affordable and less technically demanding approach to diagnose MG with ocular involvement. Both ice test and SFEMG alone, however, yielded poor specificity. We suggest that the combination of SFEMG and ice test provides a more reliable diagnosis of MG.

1. Introduction

Myasthenia gravis (MG) is a relatively common neurological disorder resulting from an antibody-mediated neuromuscular transmission defect. The majority of patients present initially with ocular symptoms of fatigable ptosis and diplopia (ocular MG or OMG), and some progress to involve extraocular areas (generalized MG or GMG).

The diagnosis of MG has been discussed extensively, both in terms of sensitivity and specificity. Acetylcholine receptor and anti-MUSK antibodies are more often positive in GMG than OMG [1], and this may limit their usage in the latter presentation. For example, up to 70% of patients with OMG may be tested negative for acetylcholine receptor antibody; conversely, at least 70% of patients with GMG are positive for this antibody. Repetitive nerve stimulation (RNS) has a lower sensitivity than single fiber electromyography (SFEMG) [2]. However, SFEMG findings can be influenced by many co-existent factors, including presence of diabetes mellitus, neuropathy, myopathy and previous local trauma and surgery. The edrophonium test is less well-tolerated, but may not be easily interpretable when fatigability is not obvious.

Therefore, clinical information often remains the 'reference or gold standard' utilized in studies of this nature. A detailed critique of previous diagnostic studies by Benatar [3] found that confounding factors

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* Corresponding author at: Department of Neurology, Singapore General Hospital, Outram Road, 169608, Singapore.
E-mail address: lo.yew.long@singhealth.com.sg (Y.L. Lo).

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include non-blinding, incorporation bias, and use of a study population not representative of the actual clinical spectrum of MG.

In patients with ocular involvement, the ice test or ice pack test is a simple and well-tolerated office procedure [4]. However, its clinical value has not been ascertained in large studies. Considering all these elaborated issues, we sought to reappraise the value of electrodiagnostic investigations and the ice test in diagnosing MG in an Asian population.

2. Methods

A total of 127 Asian patients (66 men; mean age: 58.2; age range: 18 to 84 years; 89% ethnic-Chinese; 21 GMG and 106 OMG patients) were consecutively included in this study from a large Neuro-ophthalmological clinic, based on referrals for evaluation of ocular and/or neuromuscular complaints suggestive of MG. All patients had initial ocular symptoms and were followed up for a minimum of 2 years, as conversion to GMG may occur later in some patients. Ethics committee approval was obtained prior to the start of the study.

OMG was suspected in patients with variable, fatiguable ptosis and/or demonstrable variable ophthalmoplegia. Exclusion criteria included prior strabismus surgery as well as any other cause of paralytic strabismus. GMG was defined clinically as OMG patients presenting with or progressing onto having motor weakness, causing one of the following: dysphagia, dysarthria, dyspnea, or remote motor weakness, involving the neck or the extremities. Therefore, the reference standard for "possible" MG was based on presence of clinical features of OMG or GMG in association with at least one of the following clinical investigations: 1) positive acetylcholine receptor antibodies (AChR-Abs), 2) positive clinical response to treatment with pyridostigmine, corticosteroids or other immunomodulation therapy. These are used to form reference standards 1 and 2 below for the purposes of this study, against which all 3 investigations (ice test, RNS and SFEMG) are compared.

2.1. Ice test

The ice pack test is a clinically simple, safe, and affordable procedure, which can be performed in a clinical office or at the bedside, evaluating the effect of ice application on the ptosis. A standardized ice pack test was performed in seventy patients, in procedural agreement with previous studies [4]. The test was performed by an experienced neuro-ophthalmologist, who was blinded to the results of other investigations. After digital suppression of the action of the frontalis muscle, the interpalpebral distance was recorded vertically in both eyes, in the primary gaze, at the level of pupils' centers, using a millimeter ruler. After a baseline measurement of the interpalpebral fissure, the ice pack was applied bilaterally, during 2 min, followed by a new measurement within 10s after removal. An ice-induced enlargement of the palpebral fissure by >2 mm was considered a positive test. Since the pure ocular form of MG is frequently not detected by the traditional paraclinical tests, the ice test is an attractive diagnostic method for OMG, or MG patients with ocular presentation.

2.2. RNS

A health care technician blinded to the clinical features and to the SFEMG findings of each patient performed RNS in 103 patients. This was achieved with a Dantec 9013L0221 bipolar electrode held in place by a fixation strap for surface stimulation. Surface recordings (belly-tendon configuration) were made with disposable adhesive electrodes (Medtronic 9013S0211Medtronic, Skovlunde, Denmark). Studies were performed on a Dantec Keypoint EMG machine with amplifier filter frequencies set at 3 Hz and 5 kHz. Ten single square-wave pulses of 0.3-ms duration were used for each stimulation run at 3 Hz. Surface temperature was kept at 32 °C to 34 °C. Automated decrement calculations of baseline to negative peak amplitude and of negative peak area between the first and fourth supramaximal compound muscle action potentials (CMAP) were obtained. Each patient and control subject underwent five runs testing the abductor digiti minimi muscle aiming to record a mean percentage decrement. After a 5-min period of rest, the patient was instructed to maintain a maximal muscle contraction for 20 s. Immediate postexercise stimulation was performed to exclude the presence of an incremental response, defined as 100% increase in negative peak amplitude. Thereafter, similar 3-Hz stimulations were applied at 1, 2, and 3-min intervals. RNS recordings were made on the abductor digiti minimi muscle. A decremental response above 10% in any muscle recordings was regarded as a positive RNS result [5,6].

2.3. SFEMG

Ninety-nine patients underwent stimulated SFEMG of the orbicularis oculi [7]. This involved the use of disposable adhesive surface electrodes (TECA, Old Woking, United Kingdom) placed 2.5 cm away from the edge of the orbicularis oculi. Stimulation pulses of 0.1 ms at 10 Hz and 5 to 12 mA were administered. A 40-mm 9013K0872 needle electrode (Dantec, Skovlunde, Denmark) was inserted at the edge of the muscle for single-fiber recordings. Filter settings were maintained at 500 kHz to 10 kHz. Single-fiber responses were selected on the basis of short rise times (<300 μs), clear separation from other discharges and stability of waveform. Mean jitter was calculated from 20 accepted single-fiber responses. All SFEMG assessments were performed on a Dantec Keypoint EMG machine. The upper limit for normality for mean jitter was 23 μs. The examination was classified as abnormal if mean jitter exceeded this value and at least 2 of 20 responses had jitter values above 30 μs. The electrophysiologist performing SFEMG was blinded to the clinical findings and result of other tests.

2.4. Workflow

At initial presentation, patients were evaluated by a clinician and routed to separate facilities to undergo the ice test, RNS, and SFEMG. Treatment was initiated based on clinical impression and results of the ice test, given that the outcomes of the other investigations were only available a few weeks after the initial presentation. Patients were also investigated for presence of AChR-Abs. Assays were performed at an offsite facility. These results were available 20 weeks post initial presentation. Each patient had treatment escalation based on clinical response, which was collectively decided by the patient and managing physician. Non-responders were considered on an individual basis by the attending physician after all results were available, at or after 20 weeks. Blinding of personnel performing investigations was ensured in this standardized work flow (Fig. 1).

Patients with suspected OMG were offered a treatment if their fluctuating ocular signs (ptosis, diplopia) were associated with at least one of the following: 1) positive AChR-Abs 2) positive ice test 3) positive RNS or SFEMG, corroborating the clinical diagnosis. In OMG patients with isolated ptosis, the first line treatment was oral pyridostigmine for at least a 4 week period. Over the follow-up period, oral steroids (0.5 to 1 mg/kg/day over 8 weeks) were added if a patient developed additional symptoms and signs of GMG.

2.5. Statistical analysis

Two reference standards were used in this study to reflect the presence of MG in a patient.

2.5.1. Reference standard 1

A patient was considered as having MG if he presented with clinical complaints of OMG and/or GMG, and if he fulfilled at least one of the following two criteria: 1/positive AChR-Abs test or 2/showed improvement in response to treatment. This was to ascertain as accurately as possible a diagnosis of MG in view of a lack of internationally accepted gold standard, by incorporating both antibody positivity and clinical

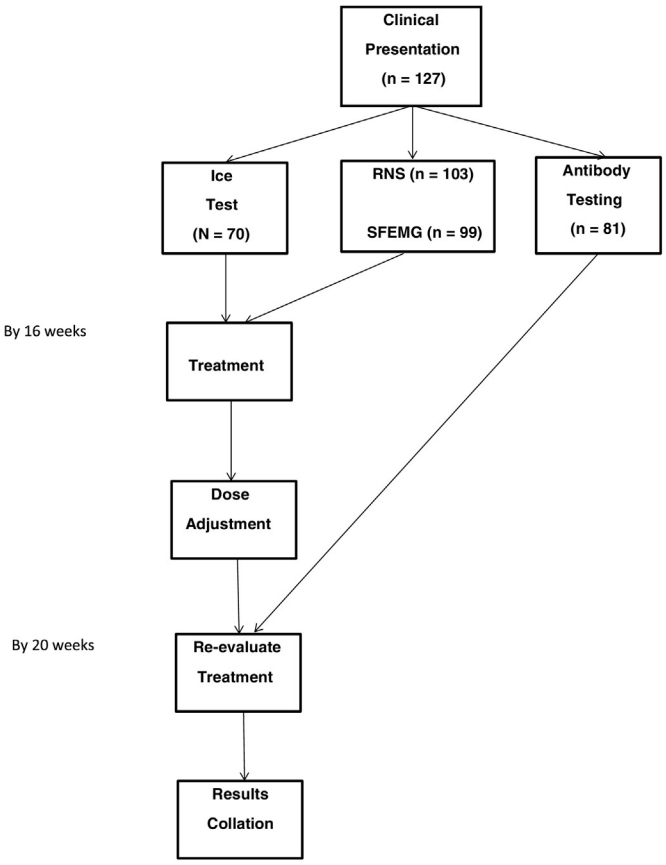

Fig. 1. Workflow, timeline and sample size breakdown of study.

response. As the majority of patients in this study had OMG in which only 50% on average show clinical response to treatment, it was felt that combining antibody status and clinical response best reflected a reference standard diagnosis of MG.

To estimate specificity, we considered non-MG patients as those presenting with clinical complaints as stated, but without antibody positivity or response to treatment.

2.5.2. Reference standard 2

A patient was considered as having MG if he presented with clinical complaints of OMG and/or GMG and had a positive AChR-Abs test. Reference standard 2's criteria did not include response to treatment, which may, rarely, occur in other conditions mimicking myasthenia, and presenting with diplopia/ptosis. Compared to reference standard 1, reference standard 2 was more stringent, in the sense that it took into account only clinical evidence of myasthenia and presence of AchR antibodies. A main drawback of reference standard 2 was that up to 70% of patients with OMG complaints only are known to be antibody negative. We also computed specificity based on the definition of non-MG as patients without antibody positivity, regardless of treatment response.

Sensitivity or true positive rate and specificity or true negative rate, were computed separately for the ice test, RNS and stimulated SFEMG based on reference standards 1 and 2. The specificity and sensitivity of combined testing procedures (i.e. Ice test and SFEMG, Ice test and RNS, SFEMG and RNS and all 3 tests) were also evaluated, and positive predictive value (PPV) and negative predictive value (NPV) were described. The combination of two or more tests was based upon the "and" rule. In brief, for a combination of tests to yield a positive MG diagnosis, all tests taken into consideration in the combination should be positive for MG. Otherwise the combination's outcome will yield a negative diagnosis for MG. Three point-based (0,0; 1-specificity, sensitivity; 1,1) receiver operating characteristic (ROC) curves were plotted using the standard cutoff value adopted for each test. Area under the ROC curve (AUC) of the ice test, RNS, SFEMG, and combination of ice test and SFEMG was extracted using a trapezoid method to assess an estimate of the performance of each procedure. Separate analyses were also performed in sub-groups of patients with GMG or only OMG.

3. Results

3.1. Reference standard 1

Based on this standard, out of 127 patients, 83 patients were classified as MG patients and 44 were non-MG patients. For all MG patients with palpebral involvement, the ice test showed a sensitivity of 86.0% (PPV = 74.1%) and specificity of 31.3% (NPV = 41.7%). The sensitivity for RNS was 21.3% (PPV = 80.9%), while specificity was 82.6% (NPV = 23.2%). SFEMG had a sensitivity of 97.3% (PPV = 80.0%) and specificity of only 21.7% (NPV = 41.7%). The combined use of the ice test and SFEMG in the evaluation of 44 patients, improved the specificity of the diagnosis (specificity = 63.6%, NPV = 50.0%) compared to the ice test alone, without affecting the sensitivity which remained at 78.8% (PPV = 86.7%). On the other hand, the combination of ice and RNS tests in 51 patients improved the specificity of the screening procedure to 92.3% (NPV = 29.3%) but reduced sensitivity to 23.7% (PPV = 90%). The area under the ROC curve was highest for the SFEMG (0.60) compared to the other tests. Combining SFEMG with the ice test increased the AUC to 0.71 (Fig. 2, A). The findings above and additional analyses are summarized in Table 1.

When GMG patients were considered separately, the ice test showed a sensitivity of 90% (95%CI: 55.5–99.8%; PPV = 90%); the sensitivity for RNS was 53.3% (95%CI: 26.6–78.7%; PPV = 80.0%) and SFEMG had a sensitivity of 83.3% (95%l: 51.6–97.9%; PPV = 100%). Specificity was not determinable due to absence of true negative cases. Similarly, when OMG is considered separately, the ice test showed a sensitivity of 85% (95% CI: 70.2–94.3%; PPV = 77.3%) and specificity of 33.3% (96%CI: 11.8–61.6%; NPV = 45.5%). The sensitivity of RNS was 13.8% (95%CI: 6.5–24.7%; PPV = 81.8%), while specificity was 90.5% (95%CI: 69.6–98.8%; NPV = 25.3%). SFEMG had a sensitivity of 100% (95%CI: 94.2–100%; PPV = 77.5%) and specificity of 21.8% (95%CI: 7.5–43.7%; NPV = 100%).

Based on this reference standard, the ice test yielded an extra 23 positive cases whereby RNS was negative. Based on 103 RNS cases performed in total, the ice test yielded an extra 28.8% positive cases. However, it yielded only 1 extra positive case whereby SFEMG was negative.

3.2. Reference standard 2

Based on this standard, out of 81 patients, 47 patients were classified as MG patients and 34 were non-MG patients. For all MG patients, the ice test showed a sensitivity of 96.6% (PPV = 75.7%) and specificity of 31.3% (NPV = 87.5%). The sensitivity for RNS was the lowest compared to the other tests at 30.6% (PPV = 68.8%), while specificity was 84.4% (NPV = 51.9%). SFEMG had a sensitivity of 93.8% (PPV = 55.6%) and specificity of only 17.2% (NPV = 71.4%). The combined use of the ice test and SFEMG in the evaluation of 32 patients, improved the specificity and sensitivity of the screening procedure by 33% and 3.4% respectively (specificity = 64.3%, NPV = 100.0%; sensitivity = 100% (PPV = 78.3%)) compared to the ice test alone. On the other hand, the combination of ice and RNS tests in 39 patients improved the specificity of the screening procedure to 94.1% (NPV = 53.3%) but reduced sensitivity to 36.4% (PPV = 88.9%). The ice test yielded the highest AUC (0.64) compared to SFEMG and RNS. Combining SFEMG with the ice test in this case increased the AUC to 0.82 and improved the accuracy of the diagnosis (Fig. 2, B). The findings above and additional analyses are summarized in Table 2.

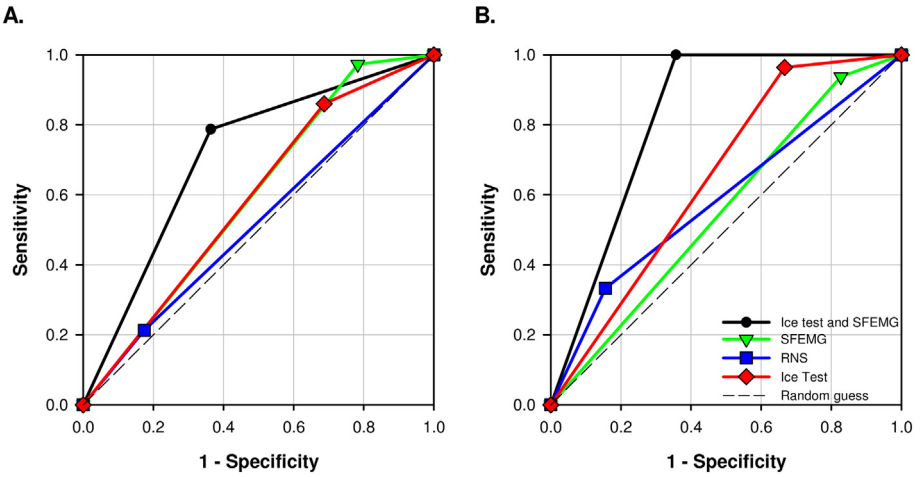

Fig. 2. ROC space of the different diagnostic tests and combination of ice test and SFEMG. A. Using reference standard 1 for MG diagnosis, among the 3 tests the area under the ROC curve was highest for the SFEMG (0.60). The ice pack and RNS tests yielded an AUC of 0.59 and 0.52 respectively. Combining SFEMG with the ice test increased the AUC to 0.71. B. Using reference standard 2 for MG diagnosis, the ice test yielded the highest AUC (0.64). SFEMG and RNS yielded an AUC of 0.56 and 0.58 respectively. Combining SFEMG with the ice test in this case increased the AUC to 0.82 and improved the performance of the diagnosis.

If GMG is considered separately, the ice test showed a sensitivity of 100% (95%CI: 59.0–100%; PPV = 87.5%) and specificity of 50% (95%CI: 1.26–98.8%; NPV = 100%). The sensitivity for RNS was 100% (95%CI: 54.1–100%; PPV = 75%), while specificity was 33.3% (95%CI: 0.8–90.6%; NPV = 100%). SFEMG had a sensitivity of 75% (95%CI: 51.6–96.8%; PPV = 85.7%) and specificity was not determinable due to absence of true negative cases. Similarly, if OMG is considered separately, the ice test showed a sensitivity of 95.5% (95%CI: 77.2–99.9%; PPV = 72.4%) and specificity of 42.9% (95%CI: 17.7–71.1%; NPV = 85.7%). The sensitivity of RNS was 16.7% (95%CI: 5.6–34.7%; PPV = 62.5%), while specificity was 89.7% (95%CI: 72.7–97.8%; NPV = 50.9%). SFEMG had a sensitivity of 100% (95%CI: 86.3–100%; PPV = 52.1%) and specificity of 17.9% (95%CI: 6.1–36.9%; NPV = 100%).

Based on this reference standard, the ice test yielded an extra 13 positive cases whereby RNS was negative. Based on 36 RNS cases performed in total, the ice test yielded an extra 36.1% positive cases. However, it yielded no extra positive case whereby SFEMG was negative. Of the 11 GMG patients with positive RNS, 2 were positive in this muscle only after exercise. In the OMG patients, none of the patients with positive RNS had a positive RNS test for this muscle, even with exercise.

3.3. SFEMG diagnosis

Based on reference standard 1, 17/22 (77.3%) non-MG patients had positive SFEMG and only 5/22 (22.7%) had negative SFEMG.

Based on reference standard 2, 24/29 (82.8%) non-MG patients had positive SFEMG and only 5/29 (17.2%) had negative SFEMG.

Of the false positives patients, 5 patients had diabetes mellitus, 1 with paraneoplastic myopathy, while the remaining patients did not have a definitive diagnosis for the 'false' positive SFEMG result, based on our reference standards.

4. Discussion

Our findings reaffirm a high sensitivity of the SFEMG test and suggest the ice test as valid, affordable and less technically demanding approach to diagnose MG with ocular involvement. Both ice test and SFEMG, however, yielded poor specificity. We also found that combination of SFEMG and ice test provides a more accurate diagnosis of MG. Our study fulfilled the criteria of blinding, lack of incorporation bias applied to a representative population with patients spanning the full spectrum of MG [3].

A major consideration in studies of this nature is the perfect reference standard, which is often not available. Hence, the next best option is to utilize the most appropriate reference which should be clearly defined. We have thus included clinical findings and positive antibody status, and/or treatment response to allow the best 'reference standard', thus incorporating as best the full spectrum of MG patients presenting in an actual clinical situation.

Even though sensitivity of most tests was slightly improved when using reference standard 2, overall, our findings showed that regardless of using reference standard 1 or 2, outcomes were fairly similar for the ice test, RNS and SFEMG. If GMG and OMG are considered separately, caution in interpretation should be exercised for GMG due to small patient numbers compared to OMG. Our results indicate high sensitivity

Table 1
Sensitivity, specificity and accuracy of the diagnostic tests in reference to standard 1.

Reference Standard 1 (n = 127)						
	n	Sensitivity (%)	95%CI (%)	Specificity (%)	95%CI (%)	AUC
Ice test	70	86.0	73.3–94.2	31.3	9.7–53.5	0.59
SFEMG	99	97.3	90.6–99.7	21.7	7.5–43.7	0.60
RNS	103	21.3	12.9–31.8	82.6	61.2–95.1	0.52
Ice test + SFEMG	44	78.8	61.1–91.0	63.6	30.8–89.1	0.71
Ice test + RNS	51	23.7	11.4–40.2	92.3	64.0–99.8	0.58
SFEMG + RNS	94	17.8	9.8–28.5	90.5	69.6–98.8	0.59
Ice test + RNS + SFEMG	44	21.2	9.0–38.9	100	71.5–100	0.61

n: number of patients included in each analysis; AUC: Area under the ROC curve.
Reference standard 1: MG patients (n = 83), non-MG patients: (n = 44).

Table 2
Sensitivity, specificity and accuracy of the diagnostic tests in reference to standard 2.

Reference Standard 2 (n = 81)						
	n	Sensitivity (%)	95%CI (%)	Specificity (%)	95%CI (%)	AUC
Ice test	45	96.6	82.2–99.9	31.3	13.3–59.0	0.64
SFEMG	61	93.8	79.2–99.2	17.2	5.9–35.8	0.56
RNS	68	30.6	16.4–48.1	84.4	67.2–94.7	0.58
Ice test + SFEMG	32	100	81.5–100	64.3	35.1–87.2	0.82
Ice test + RNS	39	36.4	17.2–59.3	94.1	71.3–99.9	0.65
SFEMG + RNS	60	28.1	13.8–46.8	92.9	76.5–99.1	0.59
Ice test + RNS + SFEMG	32	33.3	13.3–59.0	100	76.8–100	0.67

n: number of patients included in each analysis; AUC: Area under the ROC curve.
Reference standard 2: MG patients (n = 47), non-MG patients: (n = 34).

and relatively low specificity of the ice test, low sensitivity but high specificity of RNS, and high sensitivity but low specificity of SFEMG. However, if used alone, none are able to make a diagnosis of MG with high accuracy based on the AUC of the ROC.

Previous studies evaluating the performance of the ice test using a case control method [8] [9] without observer blinding also concluded high sensitivity above 80%, but much higher specificity above 90% compared to ours which is in the region of 30 to 40%. Apart from the above methodological differences, the differences may be related to differing use of reference standards. The ice test can also be difficult to interpret in severe ptosis [4] and standardization of the technique is needed [10]. In all, comparison across studies must be judicious, with awareness of the above confounding factors.

RNS is known to be of lower sensitivity compared to SFEMG, particularly in OMG, compared with GMG [11] [2]. This observation was corroborated by our results. In addition, we show that RNS is of much lower sensitivity than the ice test, but is superior in terms of specificity. This is understandable and is in line with outcomes of many diagnostic or screening tests. However, caution should be exercised when comparing studies, as they differ in the use of muscles, exercise, study group characteristic and again, the reference standard, which is important when ascertaining specificity. RNS testing was only performed in the abductor digiti minimi muscles as consistent patient cooperation was difficult to achieve for the nasalis and trapezius. As this is a distal muscle, sensitivity would expectedly be low, as previously mentioned. We have previously found that 3 min post-exercise was optimal to improve the yield for RNS in a distal muscle [2]. The orbicularis oculi is a useful alternate site for RNS studies in this cohort of patients with predominant ocular complaints. However, both muscles were found to be of similar sensitivities in recent evaluations [12], [13]. In general, most studies, including ours, do indicate that RNS has a superior specificity above 80% [14–16].

Among various electrophysiology methods, we have used SFEMG of the orbicularis oculi, a method which is very operator-dependent and which is not uniformly employed by all researchers. Most studies report a high sensitivity (above 90%), but specificity can range from 66% [17] to 98% [2] based on jitter analysis. This contrasts significantly with our results of between 17.9% and 28.8%. Apart from methodological differences, we had incorporated a large patient cohort whereby referrals were made for investigation of predominantly ocular, and neuromuscular complaints to a lesser extent. Hence, we have encountered a fair number of non-MG patients with other known diagnoses which can account for SFEMG abnormalities, as well as several which were not apparent. This is to be expected for a highly sensitive test when utilized in such a work flow. For previously published studies, which may contain incorporation and spectrum bias, we are conscious that specificity may be overestimated [3]. While the present study is based on the 'best' reference standards possible in this setting, future studies incorporating 'false positive' SFEMG patients, all with known alternate diagnoses apart from MG may help determine more accurately the specificity of SFEMG. However, it is conceivable that even then, MG may also coexist with the alternate diagnosis in a particular patient.

The study however, has a few limitations that should be reported. First, it remains conceivable that a very low percentage of our patients with a negative diagnosis of MG might have had anti-MuSK antibodies. Anti-MUSK testing, however, is not a standard diagnostic procedure in Singapore, and we were not able to explore this in our cohort of patients. We feel, nevertheless, that such an underestimation would have had a low impact on our results, given the fact that anti-MuSK antibodies were found only in 2.5% of a Chinese population with GMG [18]. It is most probable that in our Chinese population with preponderantly ocular myasthenia gravis, this percentage would have been much lower. Second, findings from the Asian cohort (89% ethnic-Chinese) included in this study cannot be generalized to the multiethnic Asian population. This is particularly true in autoimmune conditions in view of inherent differences in diseases susceptible HLA Class II alleles across various ethnic groups. Third, a large proportion of the patients included in this study had an ocular involvement. This finding is not unexpected in a Chinese population [18], but could have affected our results and translated into a smaller percentage of patients with positive RNS (21.4%), compared to series which include a more balanced proportion OMG/GMG [3]. The high number of OMG cases could also explain the high sensitivity of SFEMG (exploring the periocular orbicularis muscles) in our series. Indeed, SFEMG has a high sensitivity in OMG, but relatively low sensitivity in GMG [3]. Finally, the retrospective nature of this study implies a few inherent limitations. For instance, a homogenous testing of all patients, using all three methods would have been ideal, yet impossible in this investigation. Furthermore, evaluating physicians performing the ice-pack test might have been influenced by previous clinical assessments or complaints suggestive of OMG in the patient. Nevertheless, a few parameters aimed to reduce the risk of subjective interpretations. The ice pack test for example, was performed by experienced neuro-ophthalmologists who were completely blinded to the results of other investigations. This test was also well standardized, based on objective, quantitative measurements of the interpalpebral fissure, before and after application of the ice.

Apart from highlighting the importance of methodological requirements in a clinical situation without an ideal reference, we revalidate the value of RNS as a specific test for MG. In addition, as SFEMG is a technically demanding test which is not widely available, a combination of the ice test and RNS can add significantly to the diagnostic yield efficiently. In practice, not all clinical facilities have the full complement of electrodiagnostic and immunological capabilities. Our findings suggest that the ice test may be of contributory value to the low specificity of SFEMG, and the RNS, to a lesser extent.

With improved detection of new autoantibodies, including anti-MUSK, LRP4, and cortactin antibodies [19], further research of a similar nature can be conducted using their titers as reference standards.

To our knowledge, this is the largest study of this nature to date, and our findings have relevant implications for the management and future research of MG.

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