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ORIGINAL ARTICLE

# Non-invasive evaluation of sudomotor function in patients with myasthenia gravis

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**Summary**
*Objectives.* — Myasthenia gravis (MG) is an autoimmune disease associated with antibodies against the nicotinic muscle acetylcholine receptor (AChR) at the neuromuscular junction. Dysautonomia has been previously described in MG. Electrochemical skin conductance (ESC), assessed by Sudoscan®, is a non-invasive method that allows evaluation of sudomotor function. Since sweat glands are innervated by sudomotor, postganglionic, cholinergic sympathetic C-fibers, we hypothesized that ESC could be a reliable method for assessing autonomic dysfunction in MG.
*Methods.* — ESC measurements were prospectively assessed in patients with generalized MG and in healthy controls. Patients with diabetes mellitus, anticholinergic medication or electrophysiological findings of peripheral neuropathy were excluded. Data regarding demographic and disease features were collected. Presence of autonomic symptoms in patients with MG was assessed by Compass-31. For statistical analysis we performed student *t*-test and Chi$^2$ test for comparison between both groups.
*Results.* — We included 24 patients (mean age of 46.4 ± 10.6, 75% women, mean disease duration 12.5 years, 62.5% positive for AChR antibodies) and 37 controls. We found no difference in either foot ($P = 0.13$) or hand ($P = 0.83$) ESC measurements between patients and controls, even after correcting for age.

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*Conclusion.* — We could not prove the presence of autonomic sympathetic dysfunction in our cohort of MG patients when assessed by Sudoscan®. In addition, Compass-31 was not a useful questionnaire in this clinical context.


## Introduction

Myasthenia gravis (MG) is a chronic autoimmune disease, characterized by fluctuating muscular weakness and fatigability, in most cases resulting from binding of antibodies specific to the nicotinic muscle acetylcholine receptor (AChR antibodies) at the neuromuscular junction.

Autonomic dysfunction is not a commonly recognized feature of the disorder, unlike Lambert Eaton Myasthenic Syndrome (LEMS) [7]. However, several reports have indicated autonomic symptoms in MG, usually associated with thymoma [8,10,15].

Studies on autonomic function in MG patients have yielded conflicting results. Elsais et al. found abnormal questionnaire-reported autonomic symptoms [4]. Some studies evaluating heart rate and blood pressure variability suggested parasympathetic dysfunction [6,11], while others have found no significant differences compared with healthy controls [12]. Sympathetic hyperexcitability was demonstrated in a study on sympatho-vagal reflex tests [6], while another on the norepinephrine and epinephrine response to forearm ischemia has suggested a sympathetic deficiency [14]. However, the majority of these studies have not taken into account the possibility of cholinergic hyperactivity due to anticholinesterase medication.

The literature is ambiguous regarding the precise pathophysiology of autonomic function in patients with MG. The pentameric muscle AChR has extracellular domains of two alpha-1 subunits, which are major antigenic targets for antibodies. Neuronal AChR in the autonomic ganglia are structurally similar to muscle AChR. The extracellular domain of the alpha-3 subunits in the ganglia AChR is 60% identical in amino acid sequence to the muscle alpha-1 subunit. There is a possibility that a subset of antibodies can bind to AChR in autonomic ganglia, as occurs in autoimmune autonomic ganglionopathy [17]. Balestra et al., have found neuronal AChR antibodies in a proportion of patients with seropositive MG [1]. A more diffuse involvement of cholinergic fibers could be possible, as supported by the observation of ultrastructural changes in sweat gland innervation [5].

Sweat glands are innervated by the sudomotor, postganglionic, unmyelinated cholinergic sympathetic C-fibers. Sudoscan® is a non-invasive, fast, recently developed method that provides an accurate evaluation of sweat gland function. It is a medical device for galvanic skin response assessment. Patients place their hand and foot volar surface, a skin region with a high density of sweat glands, on nickel electrodes in which an incremental low direct voltage (lower than 4V) is applied during two minutes. Electrochemical skin conductance (ESC) is then calculated from the resulting voltage and the generated current, which is expressed in microSiemens (μS) for the hands and feet [9]. It has been identified as a reliable method for screening for diabetic neuropathy, cardiovascular autonomic neuropathy and hereditary amyloidosis related to transthyretin (hATTR) V30M, and has been used in a longitudinal study for risk assessment of diabetes mellitus [9]. Furthermore, a decreased ESC measurement seems to correlate to changes in conventional neurocardiological tests such as heart rate variability and Ewing tests reflecting sympathetic dysfunction in patients with diabetes mellitus type 2. These findings suggest that Sudoscan® may be also a useful screening test to detect sympathetic autonomic dysfunction [2,8].

Therefore, Sudoscan® might be a reliable method for evaluating the presence of sudomotor dysfunction in patients with MG.

We aimed to ascertain whether MG patients have sudomotor dysfunction, compared with healthy controls.

## Materials and methods

### Patients

ESC measurements were prospectively assessed in patients with generalized MG followed at a neuromuscular outpatient clinic and in healthy controls. The diagnosis of MG was based on conventional diagnostic criteria: patients with typical exertional muscular weakness, and at least two supportive results (positive clinical response to cholinesterase inhibitor/steroids, and/or the presence of AChR/anti-MuSK antibodies in serum, and/or neurophysiological findings consistent with MG). The latter were: decrement > 8% at 2—3 Hz on repetitive motor nerve stimulation of proximal or facial muscles; and/or increased jitter on single-fiber electromyogram. Patients with any of the following possible confounding factors were excluded: diabetes mellitus; neurodegenerative disorders involving dysautonomia; anticholinergic medication use; electrophysiological findings of peripheral neuropathy. Selected patients had good or excellent clinical control, in order to tolerate anticholinesterase therapy (i.e. pyridostigmine) wash-out > 8 hours before sudomotor function assessment. The healthy controls had no known medical comorbidities, no current medication and no signs of peripheral neuropathy on clinical assessment.

Data regarding demographic and disease features: age at onset of disease, disease duration, disease stage (Myasthenia Gravis Foundation of America Staging — MGFA), seropositivity to AChR antibodies and histopathologic

changes in thymectomized patients were retrospectively collected, using medical records.

The protocol was approved by the local Ethics Committee, and an informed consent was signed prior to the neurophysiological evaluation.

### Sudomotor function

ESC measurement, as assessed by Sudoscan®, was performed in all patients with generalized MG and in healthy controls. Subjects were asked to place their hands and feet on the electrodes and to stand still for 3 min. Individual right and left hands and feet were studied, but a final mean score of both hands (hands ESC) and both feet (feet ESC) was given automatically and considered for analysis. The subject's evaluation was performed in the same room with a stable temperature (range 20—25 °C) and in a stress-free environment.

### Compass-31

The presence of autonomic symptoms in patients with MG was assessed by Compass-31, which is a self-assessment instrument of autonomic symptoms and function tool to assess and grade symptoms relevant to autonomic function. It was designed to provide a global autonomic severity score and domain scores, ranging from 0 to 100 [13].

### Statistical analysis

Data analysis was performed with SPSS 23.0 software. The Student *t*-test or the Mann-Whitney U test were used as appropriate to compare the ESC results in hands and feet between the experimental and control groups. The effect of potential confounding on ESC measurements was assessed using multivariate linear regression, by adding the confounding factor to the disease vs. control variable. Model assumptions were examined through residual vs. fitted plots, to exclude heteroscedasticity, and overall model fit was obtained through $R^2$ calculation. A $P$ value $< 0.05$ was considered statistically significant.

## Results

We included 24 patients with generalized MG with a mean age of $46.4 \pm 10.6$ years and with female sex predominance (75%). The mean duration of disease was 12.5 years. Most patients were classified as stage II in MGFA classification, were seropositive to AChR antibodies and thymectomized at some point of their disease. The main histological change was hyperplasia. A single patient had the diagnosis of thymoma. Only two patients were not treated with anticholinesterase therapy, only taking a small dose of prednisolone. In about half of patients, additional immunosuppressive therapy was given. Presence of autonomic symptoms in MG patients was assessed by Compass-31 with a mean total score of $17.04 \pm 14.5$ (median 14.5). Gastrointestinal, pupillomotor and orthostatic intolerance were the domains with the highest scores. The baseline characteristics are presented in Table 1.

Healthy controls ($n = 37$) were slightly younger (mean age 39.6 years) than patients (mean age 46.4 years, $P = 0.020$), but there was no difference regarding sex and body mass index (BMI) (Table 1). Feet and hands ESC measurements in patients with MG were within the normal range ($76.8 \pm 7.9$ μS and $70.7 \pm 14.6$ μS, respectively), suggesting absence of sudomotor dysfunction when assessed by Sudoscan® (Fig. 1).

Comparing to healthy controls, no difference in foot ($P = 0.126$) or hand ($P = 0.83$) ESC measurements was found; even after correcting for age ($P = 0.496$ for hands and $P = 0.119$ for feet ESC). In patients, there was no significant association between either hands ($P = 0.961$) or feet ($P = 0.591$) ESC and documented presence of AChR antibodies.

Additionally, no difference was found when comparing patients with different disease severity, as defined by MGFA class, seropositivity to AChR antibodies and treated either by immunosupressor or prednisolone (Table 2). No significant correlation between ESC measurements and Compass-31 was found (Table 2).

Considering the age difference between both groups, a sub-analysis was performed including younger patients ($n = 24$) in the control group ($45.4 \pm 10.3$ vs. $46.4 \pm 10.6$; $P = 0.732$). Nevertheless, no difference was found regarding ESC measurement in both feet and hands between both groups ($76.8 \pm 7.9$ vs. $80.1 \pm 4.9$; $P = 0.091$; $70.8 \pm 14.6$ vs. $68.3 \pm 13.7$; $P = 0.557$, respectively).

## Discussion

This is the first study assessing sudomotor function in patients with generalized MG using Sudoscan®, a non-invasive and reliable method for detection of autonomic dysfunction [9]. Since sweat glands are innervated by post-ganglionic, unmyelinated cholinergic sympathetic C-fibers, it could be hypothesized that patients with MG and seropositivity for AChR antibodies would have some degree of sudomotor dysfunction. The possible cross-reactivity of muscular anti-AChR antibodies with ganglionic AChR would be a plausible explanation for the development of dysautonomia in these patients.

Previous studies on autonomic function in patients with MG have not taken into account possible confounders such as the current use of anticholinesterase medication at inclusion. In our study, all patients discontinued anticholinesterase agents before sudomotor assessment. Likewise, patients treated with drugs with anticholinergic properties were excluded. Hence, possible selection bias for our results was minimized.

Although our MG patients significantly complained of autonomic symptoms, as ascertained by Compass-31, we could not show the existence of autonomic dysfunction by ESC measurement. Compass-31 is a self-questionnaire that addresses autonomic symptoms present during the last years, which increases the hazard of confounders [13]. Considering the domains of Compass-31 with the highest scores (i.e. gastrointestinal and pupillomotor) we suspect that anticholinesterase medication and blurred vision due to ocular symptoms are related causes.

**Table 1** Comparison of ESC measurement assessed by Sudoscan® between patients with generalized MG and controls.

| | MG patients ($n = 24$) | Healthy controls ($n = 37$) | *P* value |
|---|---|---|---|
| Age, y, mean ± SD | 46.4 ± 10.6 | 39.6 ± 11.6 | 0.02[a] |
| Female sex, *n* (%) | 18 (75%) | 26 (70%) | 0.687[b] |
| BMI, mean ± SD | 26.5 ± 5.1 | 24.2 ± 3.7 | 0.07[a] |
| Feet ESC, µS, mean ± SD | 76.8 ± 7.9 | 79.7 ± 5.1 | 0.126[a] |
| Hands ESC, µS, mean ± SD | 70.7 ± 14.6 | 70.0 ± 11.9 | 0.83[a] |
| Duration of disease, y, mean ± SD | 12.5 ± 8.9 | | |
| Disease stage (MGFA), *n* (%) | | | |
| Class I | 0 (0%) | | |
| Class IIa | 10 (41.7%) | | |
| Class IIb | 4 (16.7%) | | |
| Class IIIa | 7 (29.2%) | | |
| Class IIIb | 3 (12.5%) | | |
| Class IV | 0 (0%) | | |
| Seropositivity to AChR antibodies, *n* (%) | 15 (62.5%) | | |
| Thymectomy, *n* (%) | 15 (62.5%) | | |
| Histologic changes | | | |
| Hyperplasia | 7 (46.7%) | | |
| Thymoma | 1 (6.6%) | | |
| Unknown | 7 (46.7%) | | |
| Therapy, mg, mean ± SD | | | |
| Pyridostigmine ($n = 22$) | 234.5 ± 109.4 | | |
| Prednisone ($n = 18$) | 17.6 ± 17.4 | | |
| Azathioprine ($n = 12$) | 150 ± 42.6 | | |
| Mycophenolate mofetil ($n = 3$) | 1000 | | |
| Compass-31, mean ± SD | | | |
| Orthostatic intolerance | 2.46 ± 2.69 | | |
| Vasomotor | 0.71 ± 1.46 | | |
| Secretomotor | 1.29 ± 1.30 | | |
| Gastrointestinal | 5.04 ± 3.54 | | |
| Bladder | 0.83 ± 1.05 | | |
| Pupillomotor | 6.71 ± 3.47 | | |
| Total compass | 17.04 ± 9.72 | | |

ESC: electrochemical skin conductance; MG: myasthenia gravis; MGFA: myasthenia gravis foundation of America staging.
[a] Student *t*-test.
[b] $Chi^2$ test.

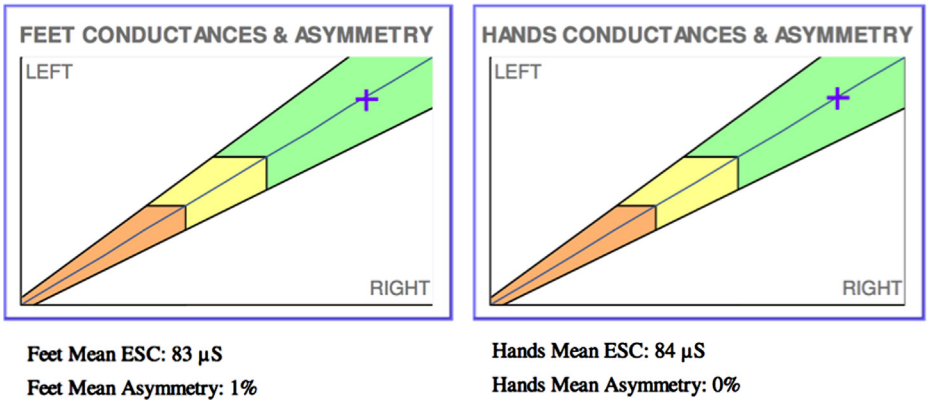


**Figure 1** Electrochemical skin conductance measurement in a myasthenia gravis patient.

**Table 2** Comparison of ESC measurement between different subgroups of MG patients and correlation between Compass-31 and ESC measurement.

| | Hands ESC (μS, mean ± SD); *P* value | Feet ESC (μS, mean ± SD); *P* value |
|---|---|---|
| Disease stage (MGFA), class II vs. III | 71.8 ± 13.2 vs. 69.3 ± 16.9; 0.690[a] | 77.8 ± 5.9 vs. 75.5 ± 10.4; 0.501[a] |
| Seropositivity to AChR antibodies, y vs. n | 70.9 ± 14.6 vs. 70.6 ± 15.3; 0.961[a] | 77.5 ± 7.2 vs. 75.7 ± 9.5; 0.619[a] |
| Therapy, y vs. n | | |
| Prednisone | 68.5 ± 15.9 vs. 76.1 ± 9.7; 0.254[a] | 76.3 ± 9.4 vs. 78.0 ± 2.9; 0.520[a] |
| Immunosuppressants (AZA or MMF) | 67.7 ± 16.0 vs. 76.7 ± 9.5; 0.159[a] | 75.9 ± 8.6 vs. 78.7 ± 6.7; 0.418[a] |
| Compass-31 | $r_s$ = −0,160; *P* = 0.455[b] | $r_s$ = −0.052; *P* = 0.810[b] |

AZA: azathioprine; ESC: electrochemical skin conductance; MG: myasthenia gravis; MGFA: myasthenia gravis foundation of America staging; MMF: mycophenolate mofetil.
[a] Student *t*-test.
[b] Spearman rank correlation test.

We cannot exclude that patients with thymoma [8,15,16] or with more severe disease could present abnormal findings. However, ESC measurement has been proved to have a high sensitivity for detection of autonomic dysfunction in both hATTR and diabetes patients (76 and 78% respectively) [3,18]. Additionally, foot ESC measurement has been shown to be a significant and independent predictor of autonomic dysfunction in hATTR patients [3]. Considering these data, we believe that ESC measurement would be useful method even to detect mild autonomic dysfunction as hypothesized in MG patients with mild to moderate disease.

Our study has some limitations. The group of patients with MG is small, since we excluded patients with diabetes and those taking other medication that could interfere with the results, and we did not perform longitudinal assessment. In addition, healthy control subjects were significantly younger than MG patients, although the absence of an association remained after controlling for age. Despite these limitations, our protocol was strict.

In summary, we could not prove the presence of autonomic sympathetic dysfunction in our cohort of MG patients when assessed by Sudoscan® as reported by other authors. Moreover, our results do not support the use of Compass-31 as a screening tool for evaluating autonomic symptoms in patients with MG.

## Author contributions

Catarina Falcão de Campos and Pedro Viana have contributed equally to the work.

## Disclosure of interest

The authors declare that they have no competing interest.

## References

[1] Balestra B, Moretti M, Longhi R, Mantegazza R, Clementi F, Gotti C. Antibodies against neuronal nicotinic receptor subtypes in neurological disorders. J Neuroimmunol 2000;102:89—97.
[2] Calvet JH, Dupin J, Deslypere JP. Screening of cardiovascular autonomic neuropathy in patients with diabetes by quick and simple assessment of sudomotor function. Diabetes Metab 2012;3:192.
[3] Castro J, Miranda B, Castro I, de Carvalho M, Conceição I. The diagnostic accuracy of Sudoscan in transthyretin familial amyloid polyneuropathy. Clin Neurophysiol 2016;127:2222—7.
[4] Elsais A, Wyller VB, Loge JH, Kerty E. Fatigue in myasthenia gravis: is it more than muscular weakness? BMC Neurol 2013;13:132.
[5] Kimura S, Nezu A. Peripheral nerve involvement in myasthenia gravis. Brain Dev 1989;11:429—32.
[6] Kocabas ZU, Kizilay F, Basarici I, Uysal H. Evaluation of cardiac autonomic functions in myasthenia gravis. Neurol Res 2018;2:1—7.
[7] McEvoy KM. Diagnosis and treatment of Lambert-Eaton myasthenic syndrome. Neurol Clin 1994;12:387—99.
[8] Nikolic A, Peric S, Nisic T, Popovic S, Ilic M, Stojanovic VR, et al. The presence of dysautonomia in different subgroups of myasthenia gravis patients. J Neurol 2014;261:2119—27.
[9] Novak P. Electrochemical skin conductance: a systematic review. Clin Auton Res 2017, http://dx.doi.org/10.1007/s10286-017-0467-x [Epub ahead of print].
[10] Peltier AC, Black BK, Raj SR, Donofrio P, Robertson D, Biaggioni I. Coexistent autoimmune autonomic ganglionopathy and myasthenia gravis associated with non-small-cell lung cancer. Muscle Nerve 2010;41:416—9.
[11] Peric S, Rakocevic-Stojanovic V, Nisic T, Pavlovic S, Basta I, Popovic S, et al. Cardiac autonomic control in patients with myasthenia gravis and thymoma. J Neurol Sci 2011;307:30—3.
[12] Shukla G, Gupta S, Goyal V, Singh S, Srivastava A, Behari M. Abnormal sympathetic hyper-reactivity in patients with myasthenia gravis: a prospective study. Clin Neurol Neurosurg 2013;115:179—86.
[13] Sletten DM, Suarez GA, Low PA, Mandrekar J, Singer W. COMPASS-31: a refined and abbreviated composite autonomic symptom score. Mayo Clin Proc 2012;87:1196—201.
[14] Stoica E, Enulescu O. Deficiency of sympathetic nervous system function in myasthenia. J Auton Nerv Syst 1992;38:69—76.

[15] Vernino S, Cheshire WP, Lennon VA. Myasthenia gravis with autoimmune autonomic neuropathy. Auton Neurosci 2001;88:187—92.
[16] Vernino S, Lennon V. Autoantibody profiles and neurological correlations of thymoma. Clin Cancer Res 2004;10: 7270—5.
[17] Vernino S, Sandroni P, Singer W, Invited Article:. Autonomic ganglia: target and novel therapeutic tool. Neurology 2008;70:1926—32.
[18] Yajnik C, Kantikar V, Pande AJ, Deslypere J. Quick and simple evaluation of sudomotor function for screening of diabetic neuropathy. ISRN Endocrinol 2012:103714.