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CAN BE SPHINCTER ELECTROMYOGRAPHY REFERENCE VALUES SHARED BETWEEN LABORATORIES?¹

Simon Podnar, MD, DSc¹ and W. Thomas Gregory, MD²

¹Institute of Clinical Neurophysiology, Division of Neurology, University Medical Center Ljubljana, Slovenia ²Division of Urogynecology and Reconstructive Pelvic Surgery, Department of Obstetrics and Gynecology, Oregon Health and Science University, Portland

Abstract

Aims: Sphincter electromyography (EMG) is an important method in diagnosis of neuropathic sacral lesions. Quantitative EMG analysis increases utility of the test, but requires valid reference values. Although commonly employed, validity of sharing reference data between electrodiagnostic laboratories has not been confirmed. In this study, this approach was assessed by comparing the reproducibility of data sets obtained by the same and different laboratories.

Methods: Confidence intervals and sensitivity of motor unit potential (MUP) parameters in the external anal sphincter (EAS) muscles were calculated using data obtained from 3 different control groups of women (number of women: 41, 48 and 66), examined by the same (the first two groups) and another investigator (the third group). Sensitivities to diagnose neuropathic changes in a known patient group were compared.

Results: When compared to the first reference group, the MUP parameter means of 2/7 (same investigator) vs. 3/7 (different investigator) were significantly different. Similarly, 3/14 vs. 4/14 MUP parameter outliers were different. Finally, 6/14 vs. 7/14 sensitivities (using a combination of MUP parameter means and outliers) were different.

Conclusions: This study demonstrated somewhat larger differences between confidence intervals obtained by different investigators, as compared to those obtained by a single investigator. However, most of these differences can be explained by differences in recruited groups of women, and slight inconsistencies in applied techniques. Presented data suggest that confidence intervals from other laboratories can be used, but only if exact protocols from original normative studies are strictly followed.

Keywords

anal sphincter muscle; neuropathic lesion; reproducibility; sacral nerves; sensitivity

INTRODUCTION

For quantitative electrodiagnostic studies, valid confidence intervals are essential.¹ Ideally, each electrodiagnostic laboratory should obtain its own reference material using standardized technique. However, due to a large number of electrodiagnostically studied anatomical structures (i.e., muscles and nerves), and a large number of electrodiagnostic

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Corresponding author: Simon Podnar, MD, DSc Institute of Clinical Neurophysiology Division of Neurology University Medical Center Ljubljana SI-1525 Ljubljana, Slovenia Tel: + 386 1 522 1500, Fax: + 386 1 522 1533 simon.podnar@kclj.si.

tests, acquisition of a complete set of reference data is an extremely demanding, if not impossible, task. As a consequence none of electrodiagnostic laboratories in the world has a complete set of its own, valid normative values. Due to more difficult recruitment of asymptomatic subjects, sacral region tests are particularly demanding in this respect.

It would be much easier if electrodiagnostic laboratories could share their reference data. Although practiced widely, the validity of this approach has not been proven. For optimal chance of success, all investigators would need to adhere to the same rigorously standardized technique.

In the present study, we propose to determine if reference data sharing is appropriate in several ways by using motor unit potential (MUP) parameters obtained in the external anal sphincter (EAS) muscle. First, using 3 different putative control (normal) groups, we will compare the reproducibility of confidence intervals obtained by the same investigator (or laboratory), to those obtained by another investigator (or laboratory). Second, the clinical impact of observed differences in confidence intervals will be assessed by calculation of sensitivity obtained by these 3 sets of reference data to diagnose neuropathic abnormalities in a large group of patients with chronic cauda equina or conus medullaris lesion.

MATERIALS AND METHODS

Selection of Subjects

In this study, 3 groups of control women, and a single group of patients with chronic cauda equina or conus medullaris lesion were studied. The first 2 groups of control women (SP-1 and SP-2) were defined and examined by the first author (SP) in Ljubljana, Slovenia, and the third group (WTG-1) by the second author (WTG) in Portland, OR, USA. In the first control group (SP-1), 41 women, aged 46 ± 16 years were recruited mainly from gynecological and urological wards while awaiting operations unrelated to the pelvic floor or its innervation. All of them were without major uro-neurologic or proctologic disorders, and without genitourinary prolapse.² The second control group (SP-2) comprised 48 women, aged 55 ± 16 years, referred consecutively for uro-neurophysiologic investigation for minor pelvic floor dysfunction.³ We excluded those women with: (A) a history suggestive of possible focal sacral neuropathic lesion (sciatica, lumbosacral spine or pelvic fracture or surgery), peripheral neuropathy (diabetes, renal failure, alcohol abuse) or extrapyramidal disorders; (B) perineal numbness or paresthesias; (C) abnormalities on clinical neurologic examination. In the third control group (WTG-1), 66 women, aged 27 ± 6 years, included in a previous study on effect of delivery on the EAS innervation,⁴ were studied. Of these, 23 were nulliparous, and 43 had recently delivered their first child (examined 12 to 24 weeks after delivery) and had no or very minor postpartum urinary or bowel incontinence.⁴

The patient group consisted of 69 subjects (29 women) with clinical, radiologic, and electrodiagnostic findings supportive of a cauda equina or conus medullaris lesion.^{3,5} The original studies were approved by the National Ethics Committee of Slovenia and Institutional Review Board at Oregon Health and Science University. All subjects provided informed consent.

EMG Technique

Both investigators used a standard concentric EMG needle and the same EMG system (Keypoint; Alpine Biomed Neurodiagnostics, Skovlunde, Denmark) with standard settings (filters, 5 Hz – 10 kHz). The first two control groups (SP-1 and SP-2) and a patient group were examined by a standardized electrode placement in the middle of individual EAS muscle quadrants.⁶ After examination of the original site the needle tip was turned anteriorly and posteriorly, so that in each individual half of the EAS muscle 4-6 positions were

sampled (performed by SP). The third control group (WTG-1) was examined by insertion of the needle electrode in the middle of EAS halves (right at 9 o'clock, and left at 3 o'clock). The needle was advanced until sharp and crisp MUPs were observed. After MUP sampling the needle was advanced for ~ 5 mm deeper, 2-4 times on each side (performed by WTG). Both investigators tried to obtain 20 different MUPs on each side using the multi-MUP technique.^{5,7} They later reviewed the selected MUPs, discarded duplicates, and those with poor baselines. Both investigators did not move the cursors pointing to the onset and termination of the MUPs. The first investigator (SP) also specifically looked for MUPs that, in his opinion, had inappropriately prolonged MUP duration as suggested by the EMG system, and deleted them. Only muscles with at least 20 MUPs sampled were included in further analyses.

Calculation of Parameter Means and Outliers

MUP parameters of amplitude, duration, area, thickness,⁸ size index,⁹ number of phases, and number of turns were measured.^{7,10} Several values were calculated for each parameter. Means and their confidence intervals (mean $\pm$ 2SD) were initially calculated. Then, outliers for each parameter were determined. This was done by defining the lower limit outlier as the 5th percentile of the 3rd/20 MUPs and the upper limit as the 95th percentile of the 18th/20 MUPs.^{11,12}

In addition, the confidence intervals of parametric reference limits were determined in the first control group (SP-1). First, standard error (SE) was calculated as:

$$SE = \sqrt{\frac{SD^2}{n} + \frac{1,96^2 SD^2}{2n}} \quad (1)$$

where *SD* is the standard deviation of the mean values for MUP parameters obtained in individual subjects (muscles), and *n* is sample size [i.e., number of included subjects (muscles)].¹³

Comparison Among Subject Groups

Reference limits obtained in other two control groups (SP-2 and WTG-1) were then compared to SP-1 confidence intervals of parametric reference limits. In addition, MUP parameter means, outlier lower and outlier upper confidence limits obtained in individual subjects from group SP-1 were compared to group SP-2, and WTG-1 using non-paired t-test ($p < 0.05$ was regarded significant).

Determination of Sensitivities

Each MUP parameter from patients' muscles was then compared to sets of confidence limits obtained in all 3 groups of control women. To declare the EAS muscle neuropathic for an individual MUP parameter, the mean value or at least 3 (out of 20) individual MUPs (outliers) had to be above the reference range.¹²

Initially, we used the confidence limits to calculate sensitivity (%) for each of the individual MUP parameter values. Sensitivity (%) was calculated as [number of patients with abnormal test/number of evaluated patients $\times$ 100)].¹³

In addition, 4 alternate combinations of MUP parameters were used to calculate sensitivity in detecting neuropathic changes: (A) MUP amplitude, duration and number of phases ("classic neuropathy set"); (B) MUP area, duration and number of turns ("empirical neuropathy set");¹⁴ (C) MUP area, duration, number of phases, and number of turns

(“alternative neuropathy set”);¹⁵ (D) all 7 MUP parameters. For these MUP parameter combinations sensitivity, when at least 1, at least 2, and at least 3 diagnostic criteria (mean values and outliers for MUP parameters) were above the appropriate confidence limits,³ were calculated then separately for individual EAS muscles. Significance of observed differences in sensitivities obtained by data sets SP-2 and WTG-1 as compared to sensitivities obtained by data set SP-1 was calculated by the z-test for two paired proportions (significance level, $p < 0.05$).^{13,16}

RESULTS

MUP Parameter Means

Compared to group SP-1, the means of MUP parameters phases and turns were statistically different in group SP-2, and the means of amplitude, area and size index were different in group WTG-1 on t-test (underlined in Table I). Furthermore, the upper confidence limits for MUP amplitude, phases and turns, as well as the lower confidence limits for MUP thickness for group SP-2 were outside the 95% confidence interval of group SP-1 appropriate confidence limits (see * in Table I). Likewise, the upper confidence limits for MUP amplitude, duration, area, thickness and phases as well as the lower confidence limits for MUP duration, size index and turns for group WTG-1 were outside the 95% confidence interval of SP-1 group confidence limits (see * in Table I).

MUP Outliers

Comparing outliers between groups revealed the following. The lower outlier limit for thickness and the upper outlier limit for phases and turns were statistically different between groups SP-2 and SP-1. In contrast, the lower outlier limit for amplitude and the upper outlier limit for amplitude, area and size index were statistically different between groups WTG-1 and SP-1 on t-test (underlined in the last two columns of Table I).

Sensitivities

Except for duration and thickness, the highest sensitivities to diagnose neuropathic changes were demonstrated by the MUP confidence limits in group WTG-1 (Fig. 2). For mean values and outliers combined, sensitivities obtained by confidence intervals calculated from groups SP-2 and WTG-1 were significantly different compared to sensitivities obtained by confidence intervals calculated from group SP-1, 6 and 7 times, respectively (see * in Fig. 2 for 4 of 7 MUP parameters). On analysis of MUP parameter combinations, sensitivities of SP-1 and WTG-1 sets obtained from different investigators were much more similar than sensitivities of SP-1 and SP-2 sets obtained from the same investigator (Table II).

DISCUSSION

The present study demonstrated moderate variability in MUP parameter confidence intervals obtained in 3 populations of control women (Table I, Fig. 1). No large difference in reproducibility of confidence limits obtained by the same as compared to different investigators was observed. In general, confidence limits were slightly more reproducible between data obtained by the same investigator, but sensitivities were more reproducible on comparison of data obtained by different investigators (Table II). This, somewhat contradictory, finding can be explained by direction of observed differences; confidence intervals obtained in group SP-2 (the second group examined by the same investigator) being in general slightly larger (i.e., more “neuropathic”), and confidence intervals obtained in group WTG-1 (examined by another investigator) being in general slightly smaller (i.e., less “neuropathic”) than confidence intervals obtained in group SP-1 (Table I, Fig. 1). As a result, sensitivity to diagnose neuropathic changes using confidence intervals obtained in

SP-2 group were much lower than sensitivity using confidence intervals obtained in other two groups (Table II).

Observed differences were therefore probably mainly due to differences in groups of studied women. In group WTG-1 probably the most strictly recruited control women were included, as the investigator (WTG) examining this group is a uro-gynecologist with easier access to asymptomatic women. Although about a third of WTG-1 group had mild sacral symptoms, remaining two thirds were completely asymptomatic women. By contrast, in spite of rather strict exclusion criteria, group SP-2 probably included some women with slight neuropathic changes in the EAS muscles, as suggested by in general larger MUP parameters. This is not surprising as these women were actually referred to uro-neurophysiologic exam due to some sort of sacral dysfunction. Group SP-1, which served as a standard for comparison, was clinically and electrodiagnostically between the other two groups (SP-2 and WTG-1, Fig. 1). In addition, women in group WTG-1 were much younger than women in other two groups, which might also incline the latter two groups to slightly more neuropathic findings, although in our previous study age effects on MUPs in the EAS muscle were not prominent.²

Differences between confidence limits obtained in groups SP-1 and SP-2 were probably caused almost exclusively by differences in included groups of subjects, as they were examined by the same investigator, using identical technique in all subjects. By contrast, at least part of observed differences in confidence limits obtained in group WTG-1 was due to differences in EMG technique used by both investigators. The disproportionately long MUP duration in WTG-1 group (Table I, Fig. 1) was probably caused by the first author's specific attention to MUPs with, in his opinion, prolongation of MUP duration due to inappropriate automatic setting of duration cursors. Such MUPs were deleted. This approach is probably more sensitive for inadequacy of the EMG signals that just observation of baseline before and after the MUP. Different levels of activity during MUP sampling might be another source of differences. MUPs sampled during higher level of activity are, in general, larger than those obtained at low level of activity.¹⁷ However, in continuously active sphincter muscles, level of MUP activity is often difficult to control. By contrast, more superficial sampling employed by the first author probably did not introduce much difference, as no significant difference in MUP parameters was observed between the subcutaneous and deeper EAS muscles.¹⁸ Nevertheless, differences in employed EMG technique between individual investigators could be easily diminished in a prospectively designed study. Our data analysis was retrospective, and authors could not try to reduce differences in their approach.

Good inter-examiner reproducibility of multi-MUP analysis has been demonstrated previously.¹⁹ In that study, no significant inter-examiner variability was found between 4 different investigators that consecutively examined deltoid muscles of 5 control subjects. The inter-examiner variability they observed was similar to intra-examiner variability, which they found by single investigator repeating investigations of the deltoid muscles in 5 controls 5 times.¹⁹ Results of that study thus also support sharing reference data between different investigators. The main difference with regard to our study is that all investigators included in that study worked at that time in the same laboratory, which ensured high similarity of EMG technique.

The potential limitations of this study are differences between evaluated three groups of control subjects and slight differences in techniques of MUP sampling and analysis used by both investigators. However, these differences present a typical situation when laboratories use published reference data from other laboratories, and was the main focus of this report. One author (WTG) is a urogynecologist whose focus is on childbirth and potentially subtle

neurologic alterations leading to bowel and bladder dysfunction.⁴ Another author (SP) is a neurophysiologist who has a large clinical practice comprised of patients with well-described neurologic diseases and injuries, including major cauda equina and conus medullaris lesions.²⁰ Profound injuries in this region would be expected to result in MUP parameter values well outside a “normal” range (once defined). Given these two clinical experiences, which group of subjects to use as a “normal” group is a potentially important question. As mentioned above, this question was partially explored in this study by comparing several groups of women with varying degrees of reproductive and pelvic floor exposures. No large differences were observed.

Despite the fact that the 2 authors in this study used nearly similar techniques, the other important question is technique and post-processing analysis. Although multi-MUP algorithms on today's advanced systems minimize the extent of user intervention, some user analysis is still required. Those interventions, as well as subtle differences in software among different EMG manufacturers could lead to minor differences in normative data.²¹ Comparison of the present data with data obtained in a prospectively organized study could probably provide information on potential of rigorous standardization to improve validity of sharing reference material.

It has been previously shown that applying three MUP sampling techniques (manual-MUP, single-MUP and multi-MUP) on the same EMG signal obtained in the control subjects' EAS muscles results in significantly different MUP parameter confidence intervals.⁵ Therefore, confidence intervals obtained in the same muscle by the same MUP sampling technique needs to be used. Furthermore, different commercially available EMG systems use different varieties of nowadays most commonly used template operated MUP sampling technique (i.e., multi-MUP analysis). For EAS muscles in addition to confidence intervals for EMG system used in this study (Keypoint; Alpine Biomed Neurodiagnostics, Skovlunde, Denmark), confidence intervals for another EMG system (Medelec/TECA Synergy, CareFusion, Old Woking, UK) were published.²² Unfortunately, utility of data from that study is limited by inclusion of only 15 nulliparous women (mean age $\pm$ SD, 29 ± 8 years), and average of only 6 MUPs sampled from the EAS muscle of each. Resulting confidence intervals were as a consequence wide, and furthermore provided SD's imprecise, which hampers comparisons. Nevertheless, MUP area (mean $\pm$ SD, 360 ± 200 μ Vms), amplitude (370 ± 130 μ V) and thickness (0.91 ± 0.4 ms) reported in that study were similar to those obtained in the present study (respective SP-1 values were: 339 ± 126 μ Vms, 383 ± 118 μ V, 0.86 ± 0.12 ms). Such degree of similarity suggests that for these 3 MUP parameters confidence limits obtained by Keypoint EMG system presented here and before,² could be probably used also on Medelec/TECA Synergy EMG system. By contrast, differences were much more pronounced for other 3 available MUP parameters (duration: 8.27 ± 2.5 vs. 5.45 ± 1.02 ; phases: 3.77 ± 0.9 vs. 2.97 ± 0.39 ; turns: 2.28 ± 0.4 vs. 2.81 ± 0.50). Therefore, our confidence intervals for these MUP parameters should not be used also on Medelec/TECA Synergy EMG system. Differences in these 3 parameters are caused by different algorithms for their measurement used by both EMG systems. Similar patterns of findings are also expected for other versions of template operated MUP analysis techniques found on some other EMG systems, as found previously for a limb muscle.²¹ However, direct comparison of confidence limits obtained by different template operated EMG systems would be needed to test validity of these speculations.

Based on data from our study we believe that continuing effort to standardize examination techniques and populations of studied subjects is needed in order to obtain confidence intervals useful for all interested laboratories. In the interim, reference data from other laboratories can be used only if exact protocols from original normative studies are

followed. Moreover, we strongly suggest standardization of software for MUP sampling in equipment produced by different manufacturers.

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Abbreviations

EAS	external anal sphincter
EMG	electromyography
MUP	motor unit potentials

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Fig. 1.

Comparison of mean values (horizontal line within the shaded area), confidence intervals for mean values (T and inverted T within the shaded area), and confidence intervals for outliers (lower and upper limit of the shaded area) for motor unit potential (MUP) parameters amplitude, duration, area, and number of turns obtained in the external anal sphincter (EAS) muscles by a single (data SP-1 and data SP-2), and another (data WTG-1) investigator.

Fig. 2.

Comparison of sensitivity (%) of 3 sets of confidence limits obtained by the same (SP-1 and SP-2), and another (WTG-1) investigators. Data for mean values, outliers and their combination of 4 motor unit potential (MUP) parameters were calculated in 105 subcutaneous EAS muscles from 69 patients with chronic cauda equina or conus medullaris lesion.³ Upper limits of columns demonstrate sensitivity above (i.e., typical neuropathic changes), and lower limit of columns sensitivity below confidence intervals (i.e., myopathy or incomplete reinnervation after severe denervation). Asterixes (*) show significant ($p < 0.05$) difference in sensitivity using confidence intervals SP-2 or WTG-1 compared to confidence intervals SP-1 using paired z-test.¹⁶ Asterixes above columns denote differences in sensitivity to diagnose abnormalities above confidence limit, and asterixes below to diagnose abnormalities below confidence limit.

Confidence intervals for motor unit potential (MUP) parameters obtained in the external anal sphincter (EAS) muscles by a single (SP-1 and SP-2), and another (WTG-1) investigator.

Table 1

	Confidence intervals for means			Confidence intervals for outliers		
	SP-1	SP-2	WTG-1	SP-1	SP-2	WTG-1
Amplitude (μ V)	148 - 618	156 - 709*	<u>127 - 457*</u>	83 - 1123	89 - 1414	<u>84 - 909</u>
Duration (ms)	3.42 - 7.48	3.51 - 7.73	<u>2.78* - 9.05*</u>	1.80 - 11.14	1.66 - 12.37	1.78 - 15.42
Area (μ Vms)	86 - 591	68 - 654	<u>49 - 467*</u>	62 - 1289	47 - 1331	<u>48 - 1001</u>
Thickness (ms)	0.61 - 1.10	0.54* - 1.09	0.57 - 1.17*	0.44 - 1.76	<u>0.39 - 1.75</u>	0.39 - 2.06
Size index	-0.74 - 0.27	-0.76 - 0.40	<u>-1.01* - 0.24</u>	-1.40 - 1.49	-1.60 - 1.49	-1.52 - <u>1.38</u>
Phases	2.19 - 3.76	<u>2.37 - 3.99*</u>	1.90 - 4.00*	1.90 - 6.10	1.94 - <u>6.69</u>	1.9 - 6.08
Turns	1.80 - 3.81	<u>1.92 - 4.16*</u>	1.29* - 3.98	1.00 - 6.10	0.94 - <u>7.69</u>	0.9 - 6.1

Data was obtained in 41 (SP-1), ² 48 (SP-2)³ and 66 (WTG-1)⁴ control women using multi-MUP analysis. Confidence intervals for means were calculated as mean $\pm$ 2SD and for outliers as 5th percentile of the 3rd/20 MUPs (lower limit), and 95th percentile of the 18th/20 MUPs (upper limit). Underlined are confidence limits obtained in groups SP-2 and WTG-1 that are significantly ($p < 0.05$) different from confidence limits obtained in group SP-1 on t-test.

* mean value confidence limits of SP-2 and WTG-1 data that fall outside of 95% confidence interval of SP-1 reference limits.

Sensitivity (%) of motor unit potential (MUP) analysis for detection of neuropathic abnormalities using four sets of MUP parameters calculated by the three sets of confidence limits obtained by the same (SP-1 and SP-2), and another (WTG-1) investigators.

Table II

PARAMETERS	1 CRITERION			2 CRITERIA			3 CRITERIA		
	SP-1	SP-2	WTG-1	SP-1	SP-2	WTG-1	SP-1	SP-2	WTG-1
Amplitude, duration, number of phases ²³	55.2	39.0*	60.0	35.2	20.9*	43.8	24.8	8.6*	12.4*
Duration, area, number of turns ¹⁴	57.1	36.2*	61.9	43.8	26.7*	42.8	31.4	19.0*	21.9
Duration, area, number of phases and turns ¹⁵	58.1	41.9*	63.8	46.7	29.5*	45.7	36.2	21.9*	24.8*
All 7 MUP parameters	64.8	48.6*	68.6	51.4	36.2*	55.2	40.9	28.6*	50.5

Results for different number (1-3) of diagnostic criteria to declare muscle abnormal are shown.³ Data was obtained in 105 subcutaneous EAS muscles of 69 patients with chronic cauda equina or conus medullaris lesion.³

* sensitivity significantly ($p < 0.05$) different using confidence intervals obtained in groups SP-2 or WTG-1 compared to confidence intervals obtained in group SP-1 using paired z-test.¹⁶