Int. J. Morphol.,
41(6):1775-1780, 2023.

Effect of M-wave Amplitude on Inter-evaluator Concordance and Number of Detected Innervation Zones in the Medial Gastrocnemius Using Multichannel Electromyography

Efecto de la Amplitud de la Onda M sobre la Concordancia entre Evaluadores y el Número de Zonas de Inervación Detectadas en el Gastrocnemio Medial Mediante Electromiografía Multicanal

Guzmán-Venegas Rodrigo[1]; Palma-Traro Felipe[1]; Contreras Javier[1] & Caparrós-Manosalva Cristian[2]

GUZMÁN-VENEGAS, R.; PALMA-TRARO, F.; CONTRERAS, J. & CAPARRÓS-MANOSALVA, C. Effect of M-wave amplitude on inter-evaluator concordance and number of detected innervation zones in the medial gastrocnemius using multichannel electromyography. *Int. J. Morphol., 41(6)*:1775-1780, 2023.

SUMMARY: The Innervation Zones (IZ) correspond to clusters of neuromuscular junctions. The traditional method of locating IZs through voluntary muscle contractions may not be feasible in individuals with motor disorders. Imposed contractions by electrostimulation are an alternative. However, there is limited evidence regarding the factors that affect inter-evaluator concordance and the number of localized IZs when using imposed contraction. The main objective of this research was to determine the effect of the amplitude of compound motor action potentials (CMAPs) containing the M-wave on inter-evaluator agreement. As a secondary objective, was investigate the effect on the number of detected IZs. Twenty-four healthy volunteers (age: 21.2 ± 1.5years, weight: 67.4 ± 13.2kg, height: 1.68 ± 0.80m) participated in the study. Electrostimulation was applied to the tibial nerve to induce contraction of the medial gastrocnemius. The IZ were identified based on the M-wave recorded through multichannel electromyography. A receiver operating characteristic (ROC) analysis was conducted to assess sensitivity and specificity in detecting the IZs. Inter-rater agreement was evaluated using a two-way mixed effects test to determine the intraclass correlation coefficients (ICC). A p-value less than 0.05 was considered statistically significant. The ROC analysis revealed that for both evaluators, a specificity of 95% was achieved with an amplitude ≥30 %. The area under the ROC curve was 0.980 [0.964, 0.996], indicating a strong influence of CMAP amplitude on detection of IZs. The highest level of agreement (ICC = 0.788 [0.713, 0.844]) among the evaluators was observed with CMAP amplitudes equal to or greater than 80 % of the maximum M-wave. The findings of this study demonstrate that both the number and the inter-evaluator concordance for detecting IZs using imposed contractions are strongly influenced by the amplitude of the M-wave. Higher M-wave amplitudes were associated with improved concordance and increased IZ detection, making it crucial to standardize amplitude settings for reliable outcomes.

KEY WORDS: Innervation Zones; Imposed Contraction; M-wave; Medial Gastrocnemius; Inter-evaluator concordance.

INTRODUCTION

The Innervation Zones (IZ) correspond to clusters of neuromuscular junctions (Masuda *et al*., 1983; Saitou *et al*., 2000), which can be located using multichannel electromyography, also known as high-density electromyography (HD-EMG) (Merletti *et al*., 2008; Guzmán-Venegas *et al*., 2014). The projection of IZs onto the skin allows for the topographic identification of motor endplates, which proves useful in research settings for defining the placement of surface electrodes and controlling biases associated with the relative electrode position to the IZs, affecting EMG signal amplitude and frequency components EMG (De Luca, 1997; Rainoldi *et al*., 2004). In a more clinical context, the *in vivo* localization of IZs could potentially aid in determining the site of injection for drugs that directly target neuromuscular junctions. One example is the intramuscular injection of botulinum toxin (BoNT) used for the treatment of dystonia and spasticity (Grigoriu *et al*., 2015). BoNT blocks acetylcholine release at the presynaptic terminal of the motor endplate; thus, theoretically, the effectiveness of its application would be

[1] Universidad de los Andes, Facultad de Medicina, Escuela de Kinesiología, Laboratorio LIBFE, Santiago, Chile.
[2] Departamento de Ciencias del Movimiento Humano, Facultad de Ciencias de la Salud, Universidad de Talca, Chile.
FUNDING. This research was funded by Agencia Nacional Investigación y Desarrollo (ANID) Chile, Fondecyt de Iniciación Proyecto 11200521.

Received: 2023-07-31 Accepted: 2023-09-16

associated with proximity to the injection site and IZs (Delnooz *et al*., 2014; Kara *et al*., 2018; Kaymak *et al*., 2018). Experimental evidence in humans demonstrates a decrease in BoNT effect as the injection site moves away from the IZs (Delnooz *et al*., 2014).

A significant percentage of individuals who suffer a stroke develop spasticity (Watkins *et al*., 2002), which can be treated locally through BoNT injections (Dashtipour *et al*., 2016; Royal College of Physicians *et al*., 2018). Consequently, in individuals with stroke sequelae, the *in vivo* localization of IZs becomes relevant as it could serve as a supplementary guide for defining the BoNT injection site. However, the traditional procedure for *in vivo* IZ localization requires the subject to perform voluntary muscle contractions (VC). Both VC performance and the amplitude of the compound motor action potentials (CMAPs) recorded via HD-EMG, which need to be large enough to identify IZs, are clearly affected by a stroke [15]. In this regard, the use of imposed contractions via electrostimulation (IC-ES) presents an alternative for *in vivo* IZ localization, having been demonstrated as effective in both healthy individuals (Guzmán-Venegas *et al*., 2016, 2022) and those with spasticity (Zhang *et al*., 2016, 2017).

Electrical stimulation of a peripheral nerve elicits two distinct responses in EMG recordings: a monosynaptic reflex known as the "H-reflex," and a direct stimulation response known as the "M-wave"(Palmieri *et al*., 2004). The M-wave has been shown to be more useful than the H-reflex for identifying IZs *in vivo* (Guzmán-Venegas *et al*., 2022). The stimulation protocols to elicit the M-wave involve applying electrical pulses of 0.5-1 ms duration with gradually increasing intensities of 1-2 mA until reaching a plateau of CMAP amplitudes. While the detection of IZs is evaluator-dependent and based on observation and experience, it is hypothesized that the amplitude of CMAPs containing the M-wave may also influence the evaluators' capacity to detect IZs and the inter-evaluator agreement. However, to our knowledge, there is currently no evidence on this. Hence, the main objective of this research was to determine the effect of CMAP amplitude containing the M-wave on inter-evaluator agreement. As a secondary objective, the study aimed to investigate the effect on the number of detected IZs.

MATERIAL AND METHOD

Volunteers. For this study, the same population as a previous study was used (Guzmán-Venegas *et al*., 2022). Twenty-four healthy volunteers participated in the study (men: 14; women: 10; age: 21.2 ± 1.5 years, weight: 67.4 ± 13.2 kg, height: 1.68 ± 0.80 m). This sample was recruited according to the following exclusion criteria: obesity (body max index > 30), history of lower limb injuries in the last six months, central or peripheral neurological diseases, injury or infection of the skin in the area of the medial gastrocnemius muscle (MG) and having any contraindication for the use of ES (Rennie, 2010). All volunteers gave their consent in writing. The procedures used in this study were in accordance with the Declaration of Helsinki of 1975 and were approved by the local Bioethical Committee (Ethical Application: CEC202023).

Instrumentation. For the HD-sEMG acquisition, a linear array of 16 electrodes (silver rods of 1 ¥ 5 mm, SA 16/5, OTbiolettronica, Torino, Italy, Fig. 1B) with an inter-electrode distance of 5 mm was used. The EMG signals were amplified in a single differential mode (EMG-USB2, OT Biolettronica, with a sampling frequency of 2048 Hz, 3 dB bandwidth 10–500 Hz, gains of 1000–2000). Electrical stimuli (Single pulses, 1ms duration) were applied with an electrical stimulator (FES-4 OTP, TrainFes, Biomedical Devices Spa., Santiago, Chile). All signals were recorded and stored using data acquisition software (OT Biolab+.V1.5.5.0 OT Biolettronica, Torino, Italy).

Procedure. The procedures used to obtain the contractions imposed by ES and the recording of the EMG signals, were the same used in a previous study (Guzmán-Venegas *et al*., 2022). Each volunteer was positioned on a bench in the prone position with their arms next to their trunk. With the right knee at 20° flexion (0° full extension), the right leg and foot were placed in a custom-made modular wood brace used to fix the ankle joint at 15° of plantar flexion. For EMG recordings, the skin over the area above the MG was prepared by shaving, cleansing with an abrasive paste (Everi, SpesMedica, Italy), and washing with water. An evaluator placed two adhesive electrodes (CDM Medical Spa, Chile) for the ES: The anode (5 ¥ 5 cm) was positioned superior to the patella, and the cathode (radius 15 mm) was fixed on the popliteal fossa. For the M-wave recording, an evaluator placed the linear electrode array on the belly of MG. The evaluator re-positioned the electrode array until an adequate propagation of CMAP was visually recognized. When adequate CMAP propagation was achieved (Fig. 1C), single pulses of progressively increasing intensity (1 mA increments, until a plateau of the maximum amplitude of the M-wave is obtained) were delivered to the tibial nerve. Ten-second rests were given between stimulation at each intensity to avoid potential confounding post-activation depression (Palmieri *et al*., 2004). During the ES, the EMG activity of the MG was recorded and stored for off-line analysis.

Signal processing. All stored EMG signals were filtered offline with a second-order Butterworth digital filter with a bandwidth of 20–400 Hz (OT Biolab+.V1.5.5.0 OT Biolettronica, Torino, Italy). The visualization of the EMG and M-wave processing for IZ identification was done using custom made scripts(Guzmán-Venegas *et al*., 2022) written in a signal processing software (IgroPro 9.0, WaveMetrics Inc., Portland, OR, USA). The amplitude of the M-wave was calculated using peak-to-peak estimations. For the M-wave detection, a window of 20 ms duration was defined as 6 ms after the onset of the artifact produced by the ES (Palmieri *et al*., 2004; Zidan *et al*., 2015) (Fig. 1C). The IZs in the M-wave of each volunteer were identified within a time window of 30ms after the electrical stimulus (Fig. 1C). The identification window for the IZ had 30ms on the time axis and the scale of the vertical axis was adjusted to 100 % of the maximum M-wave. A window was created for each amplitude of the M-wave recorded between 0 and 100 % of the maximum M-wave. The number of windows for each volunteer was variable, depending on the intensity of the ES necessary to reach the plateau at the maximum values of the M-wave. All windows showed an identification number for each volunteer and corresponding percentage amplitude (% maximum M-wave). Two evaluators, in a blind manner with respect to their peer, examined the windows sequentially from 0-100 % of the M-wave, of 24 volunteers. Each evaluator reported with a "1" if an IZ was identified, and with a "0" if it was not, as well as the respective M-wave percentage. Both independent evaluators defined the IZs in those zones where an inversion of CMAP was observed or where a minimum amplitude signal was registered between two inverted CMAP (Fig. 1C) (Masuda *et al*., 1983; Saitou *et al*., 2000; Guzmán-Venegas *et al*., 2016). In order to consider the identification of the IZs valid, the agreement for locating the IZs between both evaluator was previously measured(Guzmán-Venegas *et al*., 2022), reaching an almost perfect level of agreement (kappa coefficient 0,96), according to the agreement scale of Landis & Koch (1977).

Statistical analysis. Initially, to determine the effect of M-wave amplitude, the amplitudes of the CMAPs that allowed or did not allow the detection of IZ were described and then compared using a linear regression model. Observing that signals enabling the detection of IZs exhibited higher amplitudes, a Receiver Operating Characteristic (ROC) analysis was conducted to determine sensitivity and specificity for detecting IZs at different cutoff points, corresponding to different CMAP amplitudes. For this purpose, specificity was the most important statistic as it determined the probability of detecting IZs with amplitudes equal to or greater than the different cutoff points. This analysis was carried out separately for each evaluator and considering whether both evaluators simultaneously detected the IZ. In the second step, considering only those signals where both evaluators detected IZs, inter-evaluator agreement for the detection of channels containing IZs was calculated using a Two-way mixed effects Intraclass Correlation Coefficient (ICC), which was categorized based on the scale proposed by Cicchetti (1994) . To the effect of CMAP amplitude, the ICC was evaluated within different ranges of CMAP amplitudes. All statistical analyses were performed with a confidence level of 95%. A p-value less than 0.05 was considered to indicate a statistically significant difference. The analyses were conducted using statistical software (STATA/IC 14.2, Stata-Corp LP, College Station, USA).

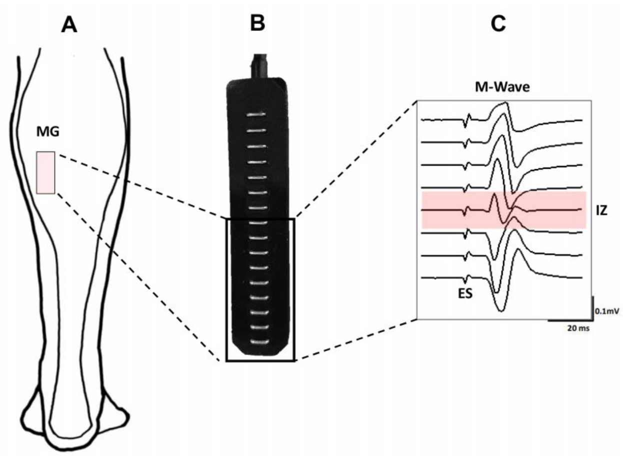

Fig. 1. A. Example of a recording site in the medial gastrocnemius muscle (MG). B. - Multi-electrode recording setup. The eight highlighted channels in the example allow for the identification of the innervation zone (IZ). C. - Multichannel electromyographic recording. ES corresponds to the artifact produced by the electrostimulation. The IZ is highlighted with the colored rectangle.

RESULTS

A total of 306 windows were analyzed, out of which 203 (66.3 %) allowed the identification of Innervation Zones (IZs) by both evaluators, while 103 (33.7 %) could not be identified by at least one investigator. The average amplitude of the CMAPs where IZs were detected by both investigators was 84.9 ± 26.9 %, which was significantly higher than those that were not detected by at least one investigator, with an amplitude of 5.7 ± 13.9 % (p-value <0.001). The ROC analysis revealed that for both evaluators, a specificity of 95% was achieved with an amplitude ≥30 %. This specificity increased to 98.1 % with CMAP amplitudes equivalent to 45 % of the maximum M-wave (Table I). The area under the ROC curve was 0.980 [0.964, 0.996], indicating a strong influence of CMAP amplitude on the investigators' capacity to detect IZs.

Regarding the concordance for detection, the overall ICC between both evaluators was 0.713 [0.631, 0.778]. The ICC values for different amplitude levels were 0.330 [-0.233, 0.716] for amplitudes between 40-60 % (n=15), 0.494 [-0.084, 0.816] for amplitudes between 60-80 % (n=13), and 0.788 [0.713, 0.844] for amplitudes between 80-100 % (n=156).

Table I. Sensitivity and specificity for the detection of innervation zones in M-waves (n=306) by both evaluators together and separately, calculated based on the peak-to-peak amplitude of the M-waves.

Cutoff Point*	Both Evaluators		Evaluator 1		Evaluator 2	
	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
>=0 %	100.0 %	0.0 %	100.0 %	0.0 %	100.0 %	0.0 %
>=5 %	100.0 %	64.1 %	99.1 %	69.6 %	99.5 %	65.7 %
>=10 %	96.6 %	85.4 %	92.1 %	84.8 %	96.1 %	87.9 %
>=15 %	96.1 %	91.3 %	91.1 %	90.2 %	95.7 %	93.9 %
>=20 %	93.6 %	92.2 %	88.8 %	91.3 %	93.2 %	95.0 %
>=25 %	93.1 %	94.2 %	88.3 %	93.5 %	92.8 %	97.0 %
>=30 %	91.6 %	95.2 %	86.9 %	94.6 %	91.3 %	98.0 %
>=35 %	91.1 %	95.2 %	86.5 %	94.6 %	90.8 %	98.0 %
>=40 %	90.6 %	96.1 %	86.0 %	95.7 %	90.3 %	99.0 %
>=45 %	89.7 %	98.1 %	85.1 %	97.8 %	88.9 %	100.0 %

The data is presented only up to 45% of the peak-to-peak amplitude, as beyond that point, the specificity remained constant. *Percentage of the maximum M-wave.

DISCUSSION

The results of this study demonstrate that the amplitude of the CMAP containing the M-wave influences both inter-evaluator concordance and the number of detected Innervation Zones (IZs). The localization of IZs has several applications in the context of basic sciences, sports, and clinical settings. In basic sciences and sports, the localization of IZs can be used to determine the placement of surface electrodes (De Luca, 1997), and to control biases in the recorded data resulting from the effect on EMG signal amplitude and frequency (Rainoldi *et al*., 2004), caused by electrode positioning directly over the IZ. In clinical applications, the localization of IZs could be helpful in defining the site of administration for drugs that act on the neuromuscular junction, such as botulinum toxin injection for spasticity treatment. Particularly in this latter application, it is crucial to understand the psychometric properties of the procedures involved in IZ identification and localization, given their potential impact on the effectiveness of pharmacological treatment.

The use of the M-wave in detecting IZs becomes relevant for patients with motor disorders or impaired consciousness who have difficulties or incapacities in performing voluntary contractions. As voluntary contractions are currently the most common method for locating IZs, employing electrostimulation to obtain M-waves and subsequently locate IZs provides a solution for this specific population, as it eliminates the reliance on the ability to produce voluntary contractions. The influence of amplitude on inter-evaluator concordance and the number of localized IZs can be associated with both neurophysiological factors. Huang *et al*. (2021) demonstrated the impact of motor unit synchronization on the ability to detect IZs. They found that high MU synchronization, caused by a supramaximal electrical stimulus, results in greater interference between CMAPs, making it challenging to locate IZs, especially when the examined motor fibers have double IZs. Therefore, in this study, only records obtained with maximal (equal to 100 % of the M-wave) and submaximal (less than 100 % of the M-wave) stimuli were analyzed, while supra-maximal stimuli were excluded. This likely facilitated both concordance and the number of detected IZs. The results of this investigation indicate that CMAP amplitudes equivalent to 80-100 % of the M-wave were most favorable for both concordance (ICC: 0.79) and the number of detected IZs (156 out of 203). This could be interpreted in two ways. Firstly, such M-wave amplitudes may imply an optimal level of MU synchronization, facilitating IZ identification. Secondly, the amplitude obtained with these magnitudes of stimuli may enhance visual inspection of CMAP, thereby aiding the evaluators in identifying IZs.

One of the limitations identified in this study was the fixed characteristic of the window used to visualize the EMG signals. The window used was adjusted to a fixed width of 30 ms horizontally and 0-100 % vertically. The purpose of using both fixed dimensions was to ensure that both evaluators applied their criteria for identifying the

Innervation Zones under the same conditions, thus controlling potential biases associated with the use of different visualization scales.

CONCLUSION

The findings of this study demonstrate that both the number and the inter-evaluator concordance for detecting Innervation Zones in the medial gastrocnemius using imposed contractions are strongly influenced by the amplitude of the M-wave. The highest number of detected IZs and the highest inter-evaluator concordance were observed when the amplitude of the M-wave exceeded 80 % of its maximum value. These results highlight the importance of considering the M-wave amplitude when using imposed contraction by electrostimulation for IZ detection and underscore the significance of standardizing the amplitude settings to achieve reliable and consistent outcomes.

GUZMÁN-VENEGAS, R.; PALMA-TRARO, F.; CONTRERAS, J. & CAPARRÓS-MANOSALVA, C. Efecto de la amplitud de la onda M sobre la concordancia entre evaluadores y el número de zonas de inervación detectadas en el gastrocnemio medial mediante electromiografía multicanal. *Int. J. Morphol., 41(6)*:1775-1780, 2023.

RESUMEN: Las Zonas de Inervación (IZ) corresponden a grupos de uniones neuromusculares. El método tradicional para localizar IZs mediante contracciones musculares voluntarias puede no ser factible en personas con trastornos motores. Las contracciones impuestas mediante electro estimulación son una alternativa. Sin embargo, existe poca evidencia sobre los factores que afectan la concordancia entre evaluadores y el número de IZs localizadas al usar este tipo de contracciones. El objetivo de esta investigación fue determinar el efecto de la amplitud de los potenciales de acción motores compuestos (PAMCs) que contienen la onda M sobre la concordancia entre evaluadores. Como objetivo secundario, se investigó el efecto sobre el número de IZs detectadas. Veinticuatro voluntarios sanos (edad: 21.2 ± 1.5 años, peso: 67.4 ± 13.2 kg, altura: 1.68 ± 0.80 m) participaron en el estudio. Se aplicó electroestimulación al nervio tibial para inducir la contracción del gastrocnemio medial. Las IZs se identificaron según la onda M registrada mediante electromiografía multicanal. Se realizó un análisis de curva de las característica del receptor (ROC) para evaluar la sensibilidad y especificidad en la detección de las IZs. La concordancia entre evaluadores se evaluó utilizando una prueba de efectos mixtos de dos vías para determinar los coeficientes de correlación intraclase (ICC). Se consideró un valor de p menor que 0.05 como estadísticamente significativo. El análisis ROC reveló que para ambos evaluadores se logró una especificidad del 95% con una amplitud ≥30 %. El área bajo la curva ROC fue de 0.980 [0.964, 0.996], lo que indica una fuerte influencia de la amplitud del CMAP en la detección de las IZs. El nivel más alto de concordancia (ICC = 0.788 [0.713, 0.844]) entre los evaluadores se observó con amplitudes de CMAP iguales o mayores al 80 % de la onda M máxima. Los hallazgos de este estudio demuestran que tanto el número como la concordancia entre evaluadores para detectar IZs mediante contracciones impuestas están fuertemente influenciados por la amplitud de la onda M. Las amplitudes más altas de la onda M se asociaron con una concordancia mejorada y un aumento en la detección de IZs, lo que hace crucial estandarizar los ajustes de amplitud para obtener resultados confiables.

PALABRA CLAVE: Zonas de Inervación; Contracción Impuesta; Onda M; Gastrocnemio Medial; Concordancia entre evaluadores.

REFERENCES

Cicchetti, D. V. Guidelines, criteria, and rules of thumb for evaluating normed and standardized assessment instruments in psychology. *Psychol. Assess., 6(4)*:284-90, 1994.

Dashtipour, K.; Chen, J. J.; Walker, H. W. & Lee, M. Y. Systematic literature review of AbobotulinumtoxinA in clinical trials for lower limb spasticity. *Medicine (Baltimore), 95(2)*:e2468, 2016.

De Luca, C. J. The use of surface electromyography in biomechanics. *J. Appl. Biomech., 13(2)*:135-63, 1997.

Delnooz, C. C. S.; Veugen, L. C.; Pasman, J. W.; Lapatki, B. G.; van Dijk, J. P. & van de Warrenburg, B. P. C. The clinical utility of botulinum toxin injections targeted at the motor endplate zone in cervical dystonia. *Eur. J. Neurol., 21(12)*:1486-e98, 2014.

Grigoriu, A. I.; Dinomais, M.; Rémy-Néris, O. & Brochard, S. Impact of injection-guiding techniques on the effectiveness of botulinum toxin for the treatment of focal spasticity and dystonia: a systematic review. *Arch. Phys. Med. Rehabil., 96(11)*:2067-78.e1, 2015.

Guzmán-Venegas, R. A.; Araneda, O. F. & Silvestre, R. A. Differences between motor point and innervation zone locations in the biceps brachii. An exploratory consideration for the treatment of spasticity with botulinum toxin. *J. Electromyogr. Kinesiol., 24(6)*:923-7, 2014.

Guzmán-Venegas, R. A.; Bralic, M. P.; Cordero, J. J.; Cavada, G. & Araneda, O. F. Concordance of the location of the innervation zone of the tibialis anterior muscle using voluntary and imposed contractions by electrostimulation. *J. Electromyogr. Kinesiol., 27*:18-23, 2016.

Guzmán-Venegas, R. A.; Palma-Traro, F. H.; Valencia, O. D.; Hudson, M. J. & Pincheira, P. A. Location *in vivo* of the innervation zone in the human medial gastrocnemius using imposed contractions: a comparison of the usefulness of the M-wave and H-reflex. *J. Funct. Morphol. Kinesiol., 7(4)*:107, 2022.

Huang, C.; Chen, M.; Li, X.; Zhang, Y.; Li, S. & Zhou, P. Neurophysiological factors affecting muscle innervation zone estimation using surface EMG: a simulation study. *Biosensors (Basel), 11(10)*:356, 2021.

Kara, M.; Kaymak, B.; Ulasli, A. M.; Tok, F.; Öztürk, G. T.; Chang, K. V.; Hsiao, M. Y.; Hung, C. Y.; Yagiz On, A. & Özçakar, L. Sonographic guide for botulinum toxin injections of the upper limb: EUROMUSCULUS/USPRM spasticity approach. *Eur. J. Phys. Rehabil. Med., 54(3)*:469-85, 2018.

Kaymak, B.; Kara, M.; Yagiz On, A.; Soylu, A. R. & Özçakar, L. Innervation zone targeted botulinum toxin injections. *Eur. J. Phys. Rehabil. Med., 54(1)*:100-9, 2018.

Landis, J. R. & Koch, G. G. The measurement of observer agreement for categorical data. *Biometrics, 33(1)*:159-74, 1977.

Masuda, T.; Miyano, H. & Sadoyama, T. The propagation of motor unit action potential and the location of neuromuscular junction investigated by surface electrode arrays. *Electroencephalogr. Clin. Neurophysiol., 55(5)*:594-600, 1983.

Merletti, R.; Holobar, A. & Farina, D. Analysis of motor units with high-density surface electromyography. *J. Electromyogr. Kinesiol., 18(6)*:879-90, 2008.

Palmieri, R. M.; Ingersoll, C. D. & Hoffman, M. A. The Hoffmann Reflex: methodologic considerations and applications for use in sports medicine and athletic training research. *J. Athl. Train., 39(3)*:268-277, 2004.

Rainoldi, A.; Melchiorri, G. & Caruso, I. A method for positioning electrodes during surface EMG recordings in lower limb muscles. *J. Neurosci. Methods*, *134(1)*:37-43, 2004.

Rennie, S. Electrophysical agents - Contraindications and precautions: An evidence-based approach to clinical decision making in physical therapy. Physiother. Can., 62(5):1-80, 2010.

Royal College of Physicians; British Society of Rehabilitation Medicine; The Chartered Society of Physiotherapy & Association of Chartered Physiotherapists in Neurology and the Royal College of Occupational Therapists. Spasticity in Adults: Management using Botulinum Toxin. National Guidelines. London, RCP, 2018.

Saitou, K.; Masuda, T.; Michikami, D.; Kojima, R. & Okada, M. Innervation zones of the upper and lower limb muscles estimated by using multichannel surface EMG. J. Hum. Ergol. (Tokyo), 29(1-2):35-52, 2000.

Watkins, C. L.; Leathley, M. J.; Gregson, J. M.; Moore, A. P.; Smith, T. L. & Sharma, A. K. Prevalence of spasticity post stroke. Clin. Rehabil., 16(5):515-22, 2002.

Zhang, C.; Peng, Y.; Li, S.; Zhou, P.; Munoz, A.; Tang, D. & Zhang, Y. Spatial characterization of innervation zones under electrically elicited M-wave. Annu. Int. Conf. I. E. E. E. Eng. Med. Biol. Soc., 2016:121-4, 2016.

Zhang, C.; Peng, Y.; Liu, Y.; Li, S.; Zhou, P.; Rymer, W. Z. & Zhang, Y. Imaging three-dimensional innervation zone distribution in muscles from M-wave recordings. J. Neural. Eng., 14(3):036011, 2017.

Zidan, M.; Thomas, R. L. & Slovis, T. L. What you need to know about statistics, part II: reliability of diagnostic and screening tests. Pediatr. Radiol., 45(3):317-28, 2015.

Corresponding author:
Guzmán-Venegas Rodrigo
Universidad de Los Andes
Monseñor Álvaro del Portillo 12.455
Las Condes
Santiago
CHILE

E-mail: rguzman@uandes.cl