SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Incidence of postoperative residual neuromuscular blockade in the Central European region and steps taken to prevent it: the development of a new electromyography-based neuromuscular monitor

by Réka Nemes, MD

Supervisor: Béla Fülesdi, MD, PhD, DSci

UNIVERSITY OF DEBRECEN
DOCTORAL SCHOOL OF NEUROSCIENCES

DEBRECEN, 2021

Incidence of postoperative residual neuromuscular blockade in the Central European region and steps taken to prevent it: the development of a new electromyography-based neuromuscular monitor

By Réka Nemes

Supervisor: Béla Fülesdi, PhD DSc

Doctoral School of Neurosciences, University of Debrecen

Head of the **Examination Committee**:	Miklós Antal, PhD, DSc
Members of the Examination Committee:	Katalin Darvas, PhD
	Balázs Pál, PhD

The Examination (online format) takes place at 11:00 AM, July 19, 2021.

Head of the **Defense Committee**:	Miklós Antal, PhD, DSc
Reviewers:	Lajos Bogár, PhD, DSc
	Judit Boczán, PhD
Members of the Defense Committee:	Katalin Darvas, PhD
	Balázs Pál, PhD

The PhD Defense (online format) takes place at 13:00 PM, July 19, 2021.

Publicity is guaranteed during the online Defense. If you are willing to participate, please send an e-mail to nemes.reka@med.unideb.hu before 16:00 PM, July 18, 2021. Due to technical reasons later sign-ups are not possible and you will not be able to join the online Defense.

1. INTRODUCTION

Neuromuscular blocking agents (NMBA) has been used by anesthesiologists since the 1940s. In the last 80 years we have gathered much of experience with them, and we have gradually started to discover their advantages, however their dangerous properties still seem to be neglected. Postoperative residual neuromuscular blockade (PORNB) can be encountered in 10-70 % of patients and more and more pieces of evidence have been gathered over the years showing PORNB's adverse effect of postoperative outcome. The number of critical respiratory events and intrahospital mortality has been observed in higher number of those patients who received intermediate acting NMBAs compared to those who did not. We are talking about a worldwide problem. Several experts have tried to explore its causes and published recommendations and guidelines to prevent the problem. However, change takes long.

In the first half of my doctoral thesis, I am first investigating the incidence of PORNB in the Department of Anesthesiology and Intensive Care, University of Debrecen, then I am trying to explore the possible causes through a survey conducted among Hungarian and Romania anesthesiologists. The survey investigated the current practice and knowledge of the anesthesiologist colleagues regarding safe neuromuscular blockade management. In the second half of my thesis, I am presenting a possible solution, a new neuromuscular monitor in whose development we have actively participated over the years.

2. OBJECTIVES

2.1. Impact of reversal strategies on the incidence of postoperative residual paralysis after rocuronium relaxation without neuromuscular monitoring. A partially randomized placebo controlled trial

We conducted this prospective, partially-randomized, double blind, placebo controlled study in our institution to observe the outcome of current routine practice (absence of routine neuromuscular blockade monitoring and omittance of reversal agents whenever possible. We aimed to determine the effectiveness of different reversal strategies (sugammadex reversal, neostigmine reversal, spontaneous recovery) to prevent PORNB.
The primary endpoint of the study was the incidence of normalized train-of-four ratio (nTOFR) in the four study groups (sugammadex reversal, neostigmine reversal, spontaneous recovery, placebo control) at post anesthesia care unit (PACU) admission. The secondary endpoint of the study was the incidence rescue medication administration in the study groups.

2.2. International survey of neuromuscular monitoring in two European countries: a questionnaire study among Hungarian and Romanian anesthesiologists

The aim of this electronic survey was to map the monitoring and reversal habits of Hungarian and Romanian anesthesiologists. We aimed to identify of those gaps in knowledge and infrastructure whose amelioration would help to improve surgical outcomes. For sake of comparability, we constructed a similar questionnaire to previous surveys.

2.3. The development of a new electromyography-based neuromuscular monitor

Although experts have long been calling for the adoption of routine neuromuscular blockade monitoring the lack of reliable monitors have hindered this process. We joined the development process of a new electromyography-based neuromuscular monitor in 2013 and

we have performed several investigations to test the different prototypes and validate the measurements. The development process is still ongoing. My thesis presents the most significant steps of the development process.

2.3.1. Testing of the first prototype of a new electromyography-based neuromuscular monitor in the operating theater

The aim of the investigation was to test the first software prototype in the clinical setting. We wanted to:
- test if the stimulation and recording parameters are working
- test the quality of the stimulating current and the electrical signals from the stimulated muscle
- see if erythema appears at the site of electrode placement
- test if other OR electrical devices interfere with the measurements
- test the effect of background electrical noise on the device
- evaluate the general usability of the device

2.3.2. Performance Assessment of a New Electromyography-based Neuromuscular Monitor and Subjective Discomfort in Unmedicated Volunteers

The aim of the investigation was to test the reproducibility of the measurements and assess the discomfort associated with neurostimulation. We wanted to:
- assess the discomfort associated with different levels of neurostimulation using the visual analog scale anchored at 0 and10
- identify the threshold current, which can elicit clinically palpable muscle contractions ($I_{Th\text{-}clinical}$) and recordable muscle action potentials ($I_{Th\text{-}EMG}$).
-identify the connection between strength of stimulating current intensity and amplitude of compound muscle action potentials (CMAPs) and to identify the current intensity which can elicit maximal CMAPs (I_{max}).
- examine the repeatability of ST stimulations at different current intensities
- examine the repeatability of TOF stimulations at different current intensities
- discover any possible side effects related to stimulations

2.3.3 Awake Volunteer Pain Scores During Neuromuscular Monitoring

Three clinical centers participated in the volunteer study conducted in 2017 and 2018, which enrolled 135 participants. The study compared the preproduction prototype of TetraGraph to TOF-Watch neuromuscular monitor. The preproduction prototype used TetraGraph's own strip electrode (TetraSens)., whose stimulating surface are is twice as big as that of standard ECG electrodes.

The primary aim of the study was to compare the discomfort associated with neurostimulation elicited with the two devices. We hypothesized that the larger stimulating surface area of the TetraSens electrode array and its elongated shape would decrease the discomfort compared to ECG electrodes. Verbal numerical rating scale (VNRS) was used to assess the level of discomfort anchored at 0 (no discomfort) and 10 (worst imaginable pain / discomfort) points.

Secondary aim of the study was to investigate the repeatability of the TOFRs obtained with the two devices. We hypothesized that TetraGraph measurements would show higher repeatability as electromyography is no subject to reverse fade phenomenon.

2.3.4. Comparative investigation of acceleromyography-based IntelliVue NMT and electromyography-based TetraGraph quantitative neuromuscular monitors: a pilot study.

The study was conducted at Mayo Clinic, Jacksonville, Florida in 2018. The aim of the clinical investigation was to compare the preproduction prototype of TetraGraph to the acceleromyogrphy-based Philips IntelliVue NMT modul. The preproduction prototpye employed a decreased sensing threshold (noise filter set at 1 mV) compared to previous prototypes. We wanted to test the efficacy of the new noise filter and the last software version. The two devices ran parallelly on the two arms of the patients from induction of anesthesia until extubation and analyzed neuromuscular function of the adductor pollicis muscle. Bland-Altman analysis was used to compare AMG and EMG derived TOFRs.

2.3.5. Ipsilateral and simultaneous comparison of responses from acceleromyography- and electromyography-based neuromuscular monitors

The aim of this clinical study was to compare the neuromuscular responses obtained with the last marketed version of TetraGraph and AMG-based TOF-Watch SX recorded in a unique simultaneous, ipsilateral, same nerve/muscle configuration. The two devices were connected and synchronized via fiber optic cable link . The TOF-Watch SX was used to stimulate the ulnar nerve and both devices analyzed the function of the same adductor pollicis muscle. This setting allowed us to exclude arm-to arm variation factors.

The primary endpoint of the study was the agreement of AMG- and EMG-derived TOFRs in the recovery range of ≥80% (near-recovery). Secondary endpoints of the study were (i) the agreement of AMG- and EMG-derived TOFRs in the <80% range, and (ii) the agreement of measurements of the baseline TOFR between the two devices. Because the 80% boundary can be judged based on either the AMG or the EMG measurement, we performed analyses of the primary and secondary endpoints using data obtained with both devices to assess the sensitivity of the AMG and EMG monitors. Additional endpoints of the study were the agreement of AMG and EMG devices in measurements of (i) post-tetanic counts (PTC) during deep block, (ii) TOF counts during deep and moderate block, and (iii) the first recovery of the TOF ratio (reappearance of the 4th twitch of the TOF).

3. METHODS

3.1 Impact of reversal strategies on the incidence of postoperative residual paralysis after rocuronium relaxation without neuromuscular monitoring. A partially randomized placebo controlled trial

This single-center, prospective, partially randomized, placebo-controlled, double-blind, four-group parallel arm study was approved by the Ethics Committee of the University of Debrecen (RKEB/IKEB 3923–2013) and by the National Institute of Pharmaceutics, Budapest, Hungary. The study was registered under EUDRACT number 2013–001965–17. The study was conducted between October 2013 and December 2015. The trial included 128 patients.

Interventions and neuromuscular monitoring

TOF-Watch-SX acceleromyograph was used for objective measurement of neuromuscular function, and was undertaken by one of the study anesthesiologist not involved in the routine care of the patients.

After induction of anesthesia the monitor was calibrated and a train-of- four (TOF) stimulus was applied every 15 s. Once the neuromuscular recording was stable, the TOF ratio was noted (the control TOF ratio) and rocuronium (0.6 mg/kg) was injected intravenously. The trachea was intubated at the discretion of the attending anesthetist. After the intubation of the trachea, the NMM was suspended and the acceleromyograph was put on standby mode. Muscle relaxation was maintained with bolus doses of rocuronium, as required for the surgery. The acceleromyography data were recorded and stored on a computer using the TOF-Watch SX software version 2.2 INT (Organon Ireland Ltd. Dublin, Ireland) for offline

analysis. At the end of surgery, the ventilator was stopped and the attending anesthetist decided whether pharmacological reversal was necessary. This decision was based on the cumulative dose of rocuronium, the time elapsed from the last rocuronium administration, respiratory tidal volume, respiratory rate, end-tidal CO2, the shape of the CO2 curve and, if the endotracheal tube was tolerated, head lift and handgrip.

In those patients for whom reversal was deemed necessary, a study anesthetist prepared and injected the reversal agent so that the attending anesthetist was unaware of the contents of the syringe. The reversal agent was either a) 2.0 mg/kg sugammadex in 15 ml of saline, b) 0.05 mg/kg neostigmine with 0.015 mg/kg atropine in 15 ml of saline or c) 15 ml saline (placebo). Patients who were deemed not to require reversal had no treatment and they comprised the spontaneous recovery patient group. Although NMM was suspended for the duration of surgery, it was recommenced before extubation of the trachea. The study anesthetist recorded the degree of NMB when the decision was made about reversal or spontaneous recovery, and then again immediately after tracheal extubation. At no point were the attending anesthetists or anesthetic nurses aware of the result of the acceleromyography.

Postoperative assessment

After extubation of the trachea, patients were transferred to the postanesthesia care unit (PACU). During transportation, the acceleromyograph was set on standby mode and the hand adapter's position was secured. In the PACU, a second independent study anesthetist, who was not aware of the reversal strategy, monitored the patients. On arrival in the recovery room, oxygen saturation, noninvasive blood pressure and ECG monitoring were recommenced and 4 l min_1 of oxygen was administered via nasal cannulae. Acceleromyographic monitoring was recommenced without further recalibration and three consecutive TOF measurements with 15 s between them were carried out (time 0). These three TOF ratios were then averaged and normalized, as were those taken 20, 40 and 60 min later. As the control TOF ratios were usually greater than 1, normalization was required and this consisted of the division of subsequent TOF ratios by the control TOF ratio. Muscle strength was evaluated at each time point.

In cases of respiratory depression diagnosed by the attending anesthetist in the OR immediately after extubation of the trachea, bag–mask ventilation and supplementary oxygen administration was given. If this failed to resolve breathing insufficiency or to improve oxygen saturation to more than 95%, the attending anesthetist requested the study anesthetist to administer the rescue medication – sugammadex (2.0mg kg_1) – to ensure safe transport of the patient to the PACU. These cases were included in the primary outcome measure. In the PACU, the study anesthetist responsible for the postoperative monitoring of the patient could also administer the same rescue medication.

Outcome measures

The primary outcome was the incidence of normalized TOFR (nTOFR) less than 0.9 on arrival in the PACU. The secondary outcome was the number of patients who needed rescue medication because of clinical signs of residual paralysis (i.e. if a patient complained about muscle weakness or oxygen desaturation <95% occurred). An additional outcome variable was the value of nTOFR throughout the study.

Sample size calculation

We assumed a reduction in the incidence of PORNB from 40% in the spontaneous recovery group to 10% following pharmacological reversal of NMB. We hypothesized that the attending anesthetist would allow spontaneous recovery in 50% of the cases. We further assumed two groups of equal size, the spontaneous recovery group and those assigned to reversal of the NMB (which also included the placebo subgroup) and, with the a-error set at 0.05 and a power of 90%, we calculated 49 study participants per group as a minimum sample size. To allow for dropouts, we aimed to recruit 50 patients in these two groups. The study

was designed to end when 50 patients had been enrolled into the spontaneous recovery group, irrespective of the number recruited to the other arm of the study.

Randomization and blinding

A sealed envelope method was used to assign participants to the three treatment groups: sugammadex, neostigmine and placebo.

Statistics

When the parametric assumptions of normality and equal variance were met by the nTOFR data, we used one-way ANOVA to compare means in the four groups. We used Kruskal–Wallis tests when these assumptions were not met. Post hoc testing was undertaken using Dunn's tests. Odds ratios (OR) and 95% confidence intervals (CI) were used for comparing the incidence of PORNB between the treatment groups, based on the z statistic. For comparing proportions among the study groups, we used Chi^2 tests and Cramer's V statistic of association when the assumptions required for Chi^2 tests were not met. Sigma-Plot for Windows Version 11.0 (Systat Software Inc., San Jose, California, USA) and PAST version 3.11 (Hammer and Harper, Oslo, Norway) were used for calculations.

3.2. International survey of neuromuscular monitoring in two European countries: a questionnaire study among Hungarian and Romanian anesthesiologists

In order to obtain the opinion of Hungarian and Romanian anesthesiologists on the incidence of PORNB, on the necessity of neuromuscular monitoring and on their habits of reversal of neuromuscular block, we edited a ten-point questionnaire in this respect. We collected data on the professional background (experience) of the participants, on the type of workplace and on the availability of neuromuscular monitors in their institutions. In order to compare our data with those of international surveys we asked similar questions, but also we included new questions specific to regional circumstances.

The questionnaire was available on the website via *SurveyMonkey* software (https://www.surveymonkey.com). The link was forwarded to all members of the Hungarian and Romanian Society of Anesthesiology and Intensive Care via e-mail. The questionnaire was open between June 27, 2016 and January 4, 2017 in Hungary and September 1, 2017 and February 20, 2018 in Romania.

1. What is your estimate? What is the incidence of PORNB at your institution?	
A) <1% B) 1-5% C) 6-20% D) >20%	
2. How often do you monitor neuromuscular function in those patients who received NMBA during surgery?	
A) Never B) Rarely (1-10%) C) Sometimes (11-30%) D) Often (31-60%) E) Regularly (61-90%) F) Almost always (91-100%)	
3.Do you have access to neuromuscular monitors at your work place?	
Quantitative TOF monitor	A) Yes, it's available in every OR B) Yes, two ORs share one device C) Yes, three or more ORs share one device D) No, they are not available
Qualitative monitor	A) Yes, it's available in every OR

	B) Yes, two ORs share one device C) Yes, three or more ORs share one device D) No, they are not available
Both types of devices are available	A) Yes, it's available in every OR B) Yes, two ORs share one device C) Yes, three or more ORs share one device D) No, they are not available
4. Clinical test and signs (like 5 sec head-lift test, hand grip strength, ability to smile) are often used to examine neuromuscular function. How much do you agree with these statements?	
Clinical signs are absolutely reliable, TOF monitors are absolutely unnecessary.	A) Strongly agree. B) Agree. C) No opinion. D) Disagree. D) Strongly disagree.
Clinical signs are usually reliable therefore neuromuscular blockade should be monitored only in certain cases (like high risk patients).	A) Strongly agree. B) Agree. C) No opinion. D) Disagree. D) Strongly disagree.
Clinical signs are not reliable at all to exclude PORNB. The use of TOF monitors is absolutely required.	A) Strongly agree. B) Agree. C) No opinion. D) Disagree. D) Strongly disagree.
5. When NMBA was used, how often do you administer reversal agent at the end of the surgery?	
A) 1-25% B) 26-50% C) 51-75% D) 76-95% E) Reversal agent is always given. F) Reversal agent is never given.	
6. If you chose not to give reversal agents, what parameters do you consider to make this decision?	
Total dose of ND-NMBA	Yes – No
Clinical duration of ND-NMBA	Yes – No
Elapsed time from last dose of ND-NMBA	Yes – No
Number of top-up doses of ND-NMBA	Yes – No
No fade is present with qualitative monitoring	Yes – No
TOFR $\geq$0,9 with quantitative monitoring	Yes – No
Patient can sustain a 5-sec had lift test	Yes – No
Adequate spontaneous tidal volumes	Yes – No
Other parameters:	Yes – No
7. What is your opinion how much time should pass after neostigmine administration before the patient can be extubated safely is neuromucular function is not monitored?	
A) <2 min B) 3-5 min C) 6-10 min	

D) >10 min	
8.How much do you agree with these statements?	
PORNB is a significant problem whose importance is underestimated by many anesthesiologists.	A) Strongly agree. B) Agree. C) No opinion. D) Disagree. D) Strongly disagree.
The significance of PORNB is overestimated.	A) Strongly agree. B) Agree. C) No opinion. D) Disagree. D) Strongly disagree.
PORNB, which can bot be recognized based on clinical signs is not dangerous.	A) Strongly agree. B) Agree. C) No opinion. D) Disagree. D) Strongly disagree.
9. What is your opinion about TOF monitoring?	
Complicated	Yes – No
Not reliable	Yes – No
Unnecessary	Yes – No
I would use it, if it was less complicated	Yes – No
I would use, but I do not have the knowledge	Yes – No
Should be part of daily routine	Yes – No
10. Your workplace and clinical experience:	
A) Intern (1-2 years of experience) B) Resident (3-4 years of experience) C) Consultant for 1-5 years D) Consultant for 6-10 years E) Consultant for >10 years	
A) University clinic B) County hospital C) City hospital	

Descriptive statistics were used for data analysis. Two generations of participants were distinguished according to their professional experience: more than 10 years for senior staff, less than 10 years for junior staff including residents. The Chi-square test was used to compare the monitoring and reversal habits of senior and junior staff.

3.3. The development of a new electromyography-based neuromuscular monitor

3.3.1. Testing of the first prototype of a new electromyography-based neuromuscular monitor in the operating theater

The first clinical trial with the first prototype (NEAT device, Acacia Designs BV, Amsterdam, the Netherlands) was conducted in 2014 (RKEB/IKEB 4170-2014, EEKH 028605-010/2014/OTIG, NCT02241304).

After proper cleaning and degreasing of the skin standard ECG electrodes were applied along the course of the ulnar nerve or stimulation, and above the muscle belly of the abductor digiti

minimi muscle (mADM) for recording. The reference electrode was placed above the first interphalangeal joint of the little finger. TOF stimulation was used with 20 sec interval time and 30 mA current intensity from induction of anesthesia until tracheal extubation.
Throughout the surgeries the number of elicited muscle twitches were recorded every 20 sec along with drug administration (name, dose, administration time) skin temperature and the time of surgical cautery use.
Data stored on the SD card of the device were compared to the clinical notes.

3.3.2. Performance Assessment of a New Electromyography-based Neuromuscular Monitor and Subjective Discomfort in Unmedicated Volunteers

This prospective, unblinded, randomized, single-center study was approved by the Hungarian Office for Health Authorization and Administrative Procedures (028605-010/2014/OTIG) and registered at clinicaltrials.gov (NCT02630576).
The study population consisted of normal, healthy volunteers of age 18 years and older. Ten male and ten female volunteers were enrolled in the study. All subjects were required to provide written informed consent prior to inclusion in the study. Exclusion criteria were presence of an underlying neuromuscular disease, use of medications known to interfere with neuromuscular transmission (e.g., antiepileptics, anticholinesterases and magnesium sulphate), presence of renal or hepatic disease, or presence of open sores at the skin sites needed for electrode application.
Neuromuscular testing was performed at two separate stimulation/ recording sites: ulnar nerve stimulation and abductor digiti minimi muscle (mADM) recording; as well as ulnar nerve stimulation and adductor pollicis muscle (mAP) recording. The side of testing (right or left hand) was determined a priori via envelope randomization, to ensure that 10 volunteers (5 male and 5 female) each were tested on the right and 10 on the left hands.
The stimulation protocols (ST and TOF) were identical for the two muscles. The stimulating parameters of ST stimulation were 1 Hz frequency, 0.2 msec pulse width and 10-60 mA current intensity that was increased in 10 mA steps. The stimulating parameters of TOF stimulations were 0.2 msec pulse width, 20 sec interval time between TOF sequences, and 10-60 mA current intensity levels, increased in 10 mA steps. All ST and TOF stimulations were repeated three times at each current intensity level. All measurements were saved to the built-in SD card of the device for off-line analysis.
The lowest current intensity of any stimulation mode that elicited a repeatable visible muscle contraction (twitch) in the fingers was considered the clinical threshold current intensity ($I_{Th\text{-}clinical}$) and was recorded on the data sheet. This was compared to the lowest current intensity that could elicit a measurable CMAP ($I_{Th\text{-}EMG}$). The current intensities that elicited the highest CMAP amplitudes (I_{Max}) were noted. The volunteers were asked to rate the discomfort of neurostimulation at every current intensity of each stimulation mode on a 0-10 visual analogue scoring (VAS) scale. Zero represented “no discomfort” and 10 represented “worst pain ever experienced” elicited by the stimulation.
When the parametric assumptions of normality and equal variance were met, paired T-test was used to compare data pairs from the same volunteer and Student’s T-test and one-way ANOVA to compare study groups. When the above assumptions were not met, paired Signed Rank test, Mann-Whitney U-test and One-way ANOVA on Ranks were used. For normally distributed variables, mean ± standard deviation (SD) and for non-normally distributed variables, median and the interquartile range (IQR) are presented. The predetermined level of significance was $p<0.05$. Sigma-Plot for Windows Version 11.0 (Systat Software Inc., San Jose, California, USA) was used for calculations.

3.3.3. Awake Volunteer Pain Scores During Neuromuscular Monitoring

This study was approved by the universities' institutional review board (IRB) (Mayo Clinic: IRB #16-005022; NorthShore University Health System: IRB #EH16-251; University of Debrecen: DE RKEB/IKEB 494–2018). The trial was registered before patient enrollment at clinicaltrials.gov (NCT02912039).

The study aimed to enroll 135 volunteers from the 3 centers, 45 volunteers from each. Hospital staff was asked to volunteer in this preclinical investigation. Written informed consent was obtained from all subjects participating in the trial before enrollment.

Setup of TOF-Watch neuromuscular monitor

Single-use ECG electrodes (Red Dot; 3M Health Care, St Paul, MN) were placed along the ulnar nerve at the wrist 3 cm apart from each other, with the distal electrode 1 cm proximal to the wrist crease. The stimulating surface area of 1 electrode is 113 mm2. The piezoelectric probe of the device was attached to the thumb. A hand adapter (Organon BV, Boxtel, the Netherlands) was used to provide a preload to the thumb muscles.

Setup of TetraGraph neuromuscular monitor

The TetraGraph was not approved in the United States. at the time of investigation, but it had gained the CE mark previously. The design and electronics of the investigational device used for the study were identical to the production device marketed outside the United States. The TetraGraph used the proprietary, single-use surface electrodes (TetraSens) for nerve stimulation and recording of compound muscle action potentials (CMAPs). The peak-to-peak amplitudes of CMAPs are used to calculate the TOFR.

The surface area of the stimulating electrodes is roughly twice as large (228.5 mm^2) as that of commercially available adult ECG electrodes. These electrodes also have an elongated shape and are applied perpendicularly to the course of the ulnar nerve to increase the likelihood of nerve stimulation. The stimulating electrodes are placed in similar position and with the same polarity as the AMG ECG electrodes (negative electrode placed distally). Similarly, the TetraSens uses silver chloride gel to reduce the impedance and improve electrical conductivity of the skin. The active sensing electrode was attached to the thenar eminence (muscle belly), while the referential electrode was attached to the interphalangeal joint of the thumb (tendon insertion site).

Neurostimulation

The order of the neuromuscular devices and the applied current intensities were random, at the recorder's discretion.

At first, 1 moderate intensity (30 mA) single twitch stimulation was delivered to prepare the volunteers for forthcoming stimulations and obtain an anchor (baseline) VNRS score. Afterward, TOF stimulations were delivered with 20 to 30 to 40 to 50 mA current intensity and 0.2 ms pulse width in random order with both devices. Three stimulations were delivered at each current intensity for 1 device. After these TOF stimulations at each current, the other device was utilized in a similar fashion. The order of each device utilized was random. The time interval between the individual stimulations was 15 s with the TOF-Watch and 20 s with the TetraGraph. The volunteers were asked to rate the discomfort associated to TOF stimulation at each current intensity with both devices on a 0–10 VNRS, anchored by 0 (representing no discomfort) and 10 (representing the worst imaginable discomfort).

Statistical Methods

Age and pain scores were reported as mean ± standard deviation (SD) and median (range), while sex was reported as frequency. Mean + SD and median (range) of all pain scores were first calculated by device and current level for descriptive purpose without taking into consideration within-subject correlation.

We evaluated the difference in pain scores between devices using a linear mixed-effects model with unstructured correlation matrix, which accounted for the within subject correlation across repeated measurements from the 3 stimulations per patients for each device

at each current level. Baseline pain scores, current intensity level, age, gender, current level, and center were adjusted in the model.
All tests were 2-sided with α level set at 0.05 for statistical significance. SAS 9.4 (SAS Institute Inc, Cary, NC) was used for statistical analysis.
After an initial pilot study was completed on 10 volunteers, we determined that a mean VNRS score difference of 0.5 was clinically meaningful. We needed a total of 128 volunteers to have 80% power at the 0.05 significance level to detect a mean difference of ≥0.5 on VNRS, assuming an SD of 2 for the difference between methods, based on paired *t*-test. We recruited a total of 135 volunteers to account for the expected dropouts and technical failures.

3.3.4. Comparative investigation of acceleromyography-based IntelliVue NMT and electromyography-based TetraGraph quantitative neuromuscular monitors: a pilot study.

The study was conducted at Mayo Clinic, Jacksonville, Florida in 2018 after the approval of the Institutional Review Board: IRB#17-006680. The study enrolled 50 patients, whose surgery necessitated muscle relaxation.
The two neuromuscular monitors were placed on the two arms of the patients according to the manufacturers' instructions. We could not apply preload to the thumb for acceleromyography monitoring as the piezoelectric probe of the Philips IntelliVue module is not compatible with the Organon Hand Adapter.
After induction of anesthesia but before NMBA administration both devices were calibrated, then the TOF stimulations were started simultaneously and left to run until extubation. 2-3 baseline TOF measurements were obtained before NMBA administration. AMG and EMG data pairs were collected continuously through along the operations.
During the operations patients were under the supervision of trained anesthesia nurses who also decided about top-up doses of NMBAs. To guide the decision, the Philips device was used. TetraGraph measurements were kept hidden from them so that they could not influence patient care.
After the conclusion of surgeries patients received sugammadex routinely for reversal of neuromuscular blockade. Unfortunately, the rapid effect of sugammadex adversely affected the number of recordable data pairs.
Paired T-test was used to compare baseline TOFRs obtained with the two devices. Bland-Altman analysis was used to describe the bias between the two techniques regarding recovery TOFRs.

3.3.5. Ipsilateral and simultaneous comparison of responses from acceleromyography- and electromyography-based neuromuscular monitors

<u>Study population and perioperative management</u>
The study was conducted at the Department of Anesthesiology and Intensive Care of the University of Debrecen Medical Center from June 26, 2019 to December 18, 2019 (ClinicalTrials.gov Identifier: NCT03987607). The study had been previously approved by the Ethical Board of the National Institute of Pharmacy and Nutrition (approval number: OGYÉI2690/2018). The study enrolled 50 patients undergoing elective surgery requiring muscle relaxation.
In order to synchronize nerve stimulation and neuromuscular monitoring, a fiber optic link was constructed to link the two neuromuscular monitors. In this configuration the ulnar nerve was stimulated exclusively by the AMG-based device, while the two connected monitors recorded simultaneous acceleromyographic (TOF-Watch SX) and electromyographic (TetraGraph) responses. This setup avoided cross-interference of stimulating currents between the two devices.

Management of neuromuscular blockade monitoring

After proper cleansing of the skin along the ulnar nerve at the wrist, the thenar eminence and the thumb, two single-use electrocardiography electrodes 3 cm apart were applied to the volar forearm along the ulnar nerve 2 cm proximal to the wrist crease to provide stimulation to the ulnar nerve. The two sensing (distal) electrodes of the TetraGraph (TetraSens electrodes) were applied according to manufacturer's instructions on the thenar eminence and the interphalangeal joint of the thumb. The stimulating electrode pair of TetraSens was not used for neurostimulation and was electrically isolated by leaving in place the packaging plastic cover. After affixing the TetraSens electrodes, a Hand Adapter (Organon Teknika B.V., Boxtel, The Netherlands) was applied to the thumb and the fingers were strapped to the arm board. The piezoelectric probe of AMG monitor was secured to the thumb via the Hand Adapter. Then the stimulating leads of the AMG monitor cable were connected to the electrocardiography stimulating electrodes, with the negative electrode placed distally.

After turning on both devices, the EMG monitor was set to manual mode and single twitch (ST) stimulation option was chosen for calibration and to measure baseline CMAP amplitudes. The predetermined current intensity for each patient was 60 mA with 0.2 msec pulse duration and the predetermined calibration mode of the AMG monitor was set to CAL1 to determine the gain. After stabilization of the signals, between 2 and 5 baseline TOFRs were recorded, then the intermediate-duration neuromuscular blocking agent was administered intravenously. The type and dose of neuromuscular blocking agent were determined by the anesthesiologist responsible for the patient and tailored to the estimated length of surgery and individual patient characteristics. The anesthesiologist also decided the time of intubation and extubation of the trachea as per usual clinical practice based on the measurements obtained with the AMG monitor. Additional neuromuscular blocking agent boluses during surgery were also at the discretion of the attending anesthesiologist, but all effort was made to achieve spontaneous recovery from neuromuscular block at the end of surgery.

Intraoperatively, TOF measurements were performed every 15 sec according to the cycle time of the AMG monitor. When there was no response to TOF stimulation (TOF count 0) by acceleromyography, post tetanic count (PTC) stimulations were performed every 3-5 min to measure the exact level of deep (TOFC=0; PTC $\geq$1) or complete (PTC=0) neuromuscular block until the TOF count (TOFC) returned to 1.

We aimed to provide spontaneous recovery curves recorded by the two monitors; however, when surgery ended earlier than anticipated, neostigmine was used for reversal of neuromuscular block. Data collection was continued until tracheal extubation or return of both AMG- and EMG-derived TOFRs to baseline values.

Sample size calculation

To estimate sample size, we used data from the pilot study conducted with the preproduction prototype of TetraGraph at Mayo Clinic (Jacksonville, FL) in 2018 and also used estimates from the pilot study and formulae from Liang et al.

With consideration to the primary endpoint, we required at least 100 comparisons each from at least 30 patients so that at least 20 comparisons would be available from the TOF range of primary interest for each patient. Expecting dropouts due to technical difficulties with the set-up caused by electrical disturbances of OR equipment, motion artefacts caused by patient positioning, and pharmacological protocol violations we decided to enroll 50 patients and monitor the entire neuromuscular block period.

General statistical methods

As a general approach, we used the Bland-Altman analysis to assess the agreement of simultaneous measurements by the two devices. We applied the Bland-Altman method in the analysis of the primary, secondary, and additional endpoints. In all analyses, we considered a difference less than 10% as acceptable agreement (bias: <10, limits of agreement: -5 to 5). For

these calculations, original AMG measurements were normalized to the mean of baseline TOFR, as recommended by Murphy. Because normalization is unfortunately often neglected in clinical practice, raw data are probably more meaningful to clinicians; therefore, we also chose to present results of the primary and secondary endpoints based on non-normalized, raw data.

Specific analyses

To analyze differences in the baseline measurements of TOFRs recorded by the two devices, we calculated mean baseline TOFR values, standard deviations, and coefficients of variation for each patient and then used paired t-test to analyze the differences in means and F-test to analyze the difference in variance between AMG and EMG readings. We also performed a random-effects one-way analysis of variance to calculate the repeatability of non-matched baseline TOFR measurements.

We analyzed the number of post-tetanic counts (PTCs) detected by the two devices during deep block (TOFC = 0, PTC ≥1) by Bland-Altman analysis to study bias, limits of agreement and repeatability. We also used a linear mixed-effects model with device (AMG or EMG) as fixed effect and patient identity as a random effect to calculate adjusted means to compare the number of PTCs by the two devices and to control for the repeated measurements.

We also used the Bland-Altman analysis to compare TOFCs during deep and moderate block. During the transition period from moderate to shallow neuromuscular block (TOFC =4, TOFR <40%), often one device indicated moderate block (TOFC 1-4) while the other device indicated shallow block (TOFR). These data pairs were not included in the analysis to avoid comparing TOFCs to TOFRs.

4. RESULTS

4.1 Impact of reversal strategies on the incidence of postoperative residual paralysis after rocuronium relaxation without neuromuscular monitoring. A partially randomized placebo controlled trial

The data of 125 patients were analyzed. When 50 patients had been recruited to the spontaneous recovery group, 75 patients had been assigned to the treatment group. Thus, the proportion of spontaneous recovery patients was 40%. Of the 75 patients assigned to the treatment groups, 27 (36%) received sugammadex, 26 (34.7%) neostigmine and 22 (29.3%) placebo.

Patient characteristics were similar among the groups. In general, the decision to allow spontaneous recovery from the NMB was selected in cases with a shorter duration where a smaller total dose of rocuronium had been administered. The time intervals between decision-making and extubation were similar in each group as were the time intervals from tracheal extubation to arrival in the PACU.

Primary endpoint

Overall, 28 (22.4%) cases of PORNB occurred: one (3.7%), four (15.3%), 13 (26%) and 10 (45.4%) after sugammadex, neostigmine, spontaneous recovery and placebo, respectively. Compared with placebo, both sugammadex and neostigmine were more effective in preventing PORNB (OR: 0.05, 95% CI: 0.005 to 0.403, PĽ0.005; and OR: 0.218, 95% CI: 0.05 to 0.847, P=0.028, respectively). The incidence rate of PORNB after spontaneous recovery was not significantly different from the placebo group (OR: 0.57, 95% CI: 0.21 to 1.50, P=0.25). Although sugammadex was more effective than spontaneous recovery (OR: 0.11, 95% CI: 0.014 to 0.889, P=0.039), neostigmine was not (OR: 0.518, 95% CI: 0.15 to 1.786, P=0.297). Although the difference between sugammadex and neostigmine seemed apparent, it was not statistically significant (OR: 0.212, 95% CI: 0.022 to 2.0347, P=0.1786).

Pharmacological reversal (sugammadex and neostigmine groups pooled) was more effective than spontaneous recovery (OR: 0.3, 95% CI: 0.097 to 0.906, P=0.03).

Secondary endpoint

Among the 28 patients with PORNB, 16 had a nTOFR 0.7 or less. Of these 16 patients, 11 received rescue treatment (three, four and four after neostigmine, spontaneous recovery and placebo, respectively). The TOF values are presented in Table 3. After sugammadex reversal, rescue treatment was not necessary. Of the 11 rescue treatments, six occurred in the operating room before the transfer to PACU, and five were given in the PACU. In these cases, irrespective of the fact that six were still in theatre when the rescue treatment was given, the primary endpoint was regarded as having been attained when the rescue treatment was given, and the timing of further postoperative monitoring was counted from this point.

Additional endpoint

At the time of decision-making about reversal, the average nTOFRs were lower in those patients assigned for reversal than in those where reversal was deemed unnecessary (P=0.001). At the time of extubation those patients who had received a reversal agent had higher TOFRs than those who received placebo (P=0.002).

By definition, PORNB occurred in one patient in the sugammadex group whose nTOFR was 0.86, but as there were no signs of residual paralysis no rescue treatment was given.

After 20 min in the PACU, there were no longer any differences in TOF ratios between the groups. The average scores for postoperative muscle strength assessment showed no differences among the groups at any of the postoperative time points.

4.2. International survey of neuromuscular monitoring in two European countries: a questionnaire study among Hungarian and Romanian anesthesiologists

The Hungarian Society of Anesthesiology and Intensive Care has 1328 registered members. 124 of the 1328 members (9.34%) answered the questions. 423 of the 878 members of the Romanian Society of Anesthesiology and Intensive Care received our questionnaire and 153 (17.4%) filled it in. The majority of our respondents were residents and young consultants (n = 189, 62,5%) and the larger proportion came from non-academic centers (55%).

The incidence and significance of PORNB

The respondents equivocally underestimated the incidence of PORNB. Only 2.2% of them answered correctly that the incidence of PORNB is >20%.

74.8 % of the respondents did not feel PORNB to be an insignificant problem.

18.9 % thought that PORNB is not a risk factor.

Clinical signs of PORNB

26.3 % of the respondent thought that neuromuscular monitoring was unnecessary and PORNB could be excluded with the help of clinical tests.

Accordingly, 63.6 % of the respondents thought that neuromuscular monitoring was necessary for only high-risk patients.

Only 48.9% of the respondents agreed with current evidence showing that clinical signs and testing are not reliable indicators of neuromuscular function and all patients receiving NMBA should be monitored.

Neuromuscular monitoring habits

Only 7.7% of the respondents use neuromuscular monitors routinely. In this respect, there was no difference between young and more experienced colleagues. 27% of them never monitor neuromuscular function, 35% rarely (in less than 10% of cases).

Availability of neuromuscular monitors

14.6% of the hospitals had no neuromuscular monitoring devices. Those institutions who had them mainly had quantitative monitors (74.1%). Both Hungarian and Romanian anesthesiologists prefer quantitative monitoring against qualitative monitoring.

General opinion on neuromuscular monitors

26.3% of the clinicians thought that the use neuromuscular monitors were complicated. 13.6% felt them to be unreliable, 44.7% would use them if they were more user friendly, and 9.3% if they had the proper knowledge to operate them. 65.5% of the respondents stated that neuromuscular monitoring should be part of daily routine.

Reversal of neuromuscular blockade

One third of the respondents (22.7%) rarely or never administers reversal agent at the end of the operation, 45.8% does it often (in >50% of the cases).

Most of the respondents (83.2%) overestimate the efficacy of neostigmine as they believe that 10 min is enough for neostigmine to reverse the neuromuscular block. Only 16.7% of the respondents knew it correctly that neostigmine needed more than 10 min to exert its effect.

For the timing of extubation the clinicians use the following parameters:

- elapsed time from last dose of NMBA (95.9%)
- clinical duration of NMBA (91.9%)
- number of top-up doses (76.3%)
- total dose of NMBA (68.7%)
- return of adequate spontaneous breathing (82.1%)
- ability to sustain a 5 sec head-lift (76.8%)

4.3. The development of a new electromyography-based neuromuscular monitor

4.3.1. Testing of the first prototype of a new electromyography-based neuromuscular monitor in the operating theater

Fifty patients were enrolled in the study (age ≥18 years), ASA I-III) who needed muscle relaxation for their elective surgery. Mean age ± SD was 52 ± 14 years, male : female ratio was 10 : 40, mean BMI ± SD was 25 ± 5. 28 out of 50 patients received succinyl-choline, and 22 patients received ND-NMBA for intubation. To maintain muscle relaxation 33 patients received atracurium, 11 patients received cisatracurium and 6 patients received rocuronium.

TetraAnalyzerViewer2014a64 software (Applied Biomedical Systems, Maastricht, Hollandia) was used to analyze the data saved on the SD card of the device. In two cases the device did not store intraoperative data.

1) Display of CMAPs

The device proved able to stimulate the abductor digiti minimi muscle through the ulnar nerve and register the elicited CMAPs.

2) Baseline measurements – consistency of measurements

We could register 3-10 baseline TOF measurements in 20 patients after the induction of anesthesia but before NMBA administration. The man ± Sd of the measurements was 103,75 ± 3,3% (range 98,0 – 111,1%). We did not experience reverse fade phenomenon.

3) Concordance of data saved on the SD card and clinical notes

We compared the TOF recordings of the device with our clinical notes on subjective TOF monitoring (number of muscle twitches). We wanted to see if the recordings showed the onset and offset neuromuscular blockade. Full concordance was achieved if the individual phases of

muscle relaxation based on clinical twitch recordings could be identified in the device's recordings (onset and offset of depolarising and non-depolarising NMBA, effect of reversal agent administration).

Full concordance was seen in 23 / 50 patients. In these cases the diminution of CMAP amplitudes (T1-T4), TOF fade and CMAP recovery could be identified in the recordings.

Our post hoc analysis identified two factors that could explain the low success rate:

- Our clinical twitch recording was probably too sensitive. All tiny twitches were registered, which were too low in amplitude and probably did not reach the sensing threshold (2 mV) of the device. 2 mV sensing threshold was the a priori set noise filter to exclude electrical noise.

- The current intensity used for nerve stimulation was 30 mA, which was probably too low in many cases to evoke CMAPs higher than 2 mV.

4) Effect of environmental electric noise

The signal-noise ratio (SNR) was stable over the course of surgeries except for 7 cases. Nevertheless, the fluctuation of SNR did not affect the quality of recordings.

Monopolar cautery use caused SNR fluctuation but did not affect the quality of recordings. Bipolar cautery devices or ultrasonic devices did not influence the performance of the device.

4.3.2. Performance Assessment of a New Electromyography-based Neuromuscular Monitor and Subjective Discomfort in Unmedicated Volunteers

There was no statistical difference in age (p=0.592), body mass index (p=0.231) and handedness (p=1.0) among the four groups. All volunteers were right-handed, therefore the terms dominant and nondominant side refer to all volunteers. The female and male groups had similar body weight (p=0.145), height (p=0.684) and wrist circumference (p=0.247).

1) VAS scores

The level of discomfort and the corresponding VAS scores increased as a function of increasing stimulating current intensity for both ST and TOF stimulation. Women had higher VAS scores than men at the same current intensities. The largest recorded difference in VAS scores was 5 (3.75-6) vs. 3 (3-4), respectively, at 60 mA TOF stimulation, (p=0.01).
Two female volunteers refused ST and TOF stimulation at 50 and 60 mA, due to discomfort at 40 mA. The other eighteen volunteers tolerated all stimulation current intensities well. I_{Max} stimulating current intensity was associated with a median VAS score of 3 (2-5) in the pooled cohort.

2) Threshold stimulating current intensity

The threshold current intensity that could elicit visible muscle twitches ($I_{Th\text{-}clinical}$) and recordable CMAPs ($I_{Th\text{-}EMG}$) was found to be 20 (20-30) mA {median (IQR)}. The clinical examination ($I_{Th\text{-}clinical}$) and monitor ($I_{Th\text{-}EMG}$) recordings were congruent (p=0.854) in 64 out of 80 stimulation protocols (80%). Women needed significantly lower current intensity than men to elicit recordable CMAPs {20 (20-20) vs. 30 (20-30) mA, p=0.002}. The abductor digiti minimi muscle had lower $I_{Th\text{-}EMG}$ than the adductor pollicis {23.75 ± 7.0 vs. 25.5 ± 6.78 mA (mean ± SD), p=0.039)}. There was a moderate correlation between wrist circumference and $I_{Th\text{-}EMG}$ (Pearson's r=0.51, p=0.0217) and between BMI and $I_{Th\text{-}EMG}$ (Pearson's r=0.505, p=0.023).

3) CMAP amplitude tendencies at increasing intensity ST stimulation

Increasing current intensities resulted in the increase of elicited CMAP amplitudes. The current intensity that induced the highest CMAPs (I_{Max}) was significantly different between the two muscles. The mADM needed lower current intensity than mAP to reach maximal stimulation {30 (20-40) mA vs. 50 (50-60) mA, respectively, p<0.001}. During maximal stimulation, the highest elicited CMAP amplitudes were moderately lower in the mADM (10.40 ± 1.9 mV) than in the mAP (12.84 ± 5.1 mV), p=0.031.

Female volunteers had higher CMAP amplitudes than men at the same stimulating current intensity, especially in the dominant mAP. Also, the female volunteers required lower stimulating current intensity to elicit maximal CMAP amplitudes than men {40 (20-50) vs. 45 (30-60) mA, respectively, p=0.042}.

4) Consistency of CMAP amplitudes (intracurrent differences) in ST protocols at increasing stimulating current intensities

The consistency of CMAP amplitudes was examined in 240 ST stimulation protocols (20 volunteers × 2 muscles × 6 current intensities). Of these, 59 stimulation protocols were excluded from the analysis because the current was under the threshold to evoke measurable CMAP. Another 4 protocols were missed because two female volunteers wished to discontinue the testing at higher (50-60 mA) current intensity. Altogether, CMAP amplitude consistency was examined in 177 cases (73.75%). The overall intracurrent difference, when the results of all stimulating intensities were pooled, was 0.42 (0.21-0.87) mV. In 145/177 (81.92%) of cases, the intracurrent difference was <1.0 mV. There was no statistical difference in the consistency of CMAP amplitudes between sexes {men: 0.37 (0.18-0.84) mV vs. women: 0.47 (0.25-0.87 mV, p=0.285} or between the two muscles {mADM: 0.42 (0.24-0.90) vs. mAP: 0.44 (0.19-0.87) mV, respectively, p=0.511}. The stimulating current intensity (20, 30, 40, 50, 60 mA) did not influence the level of intracurrent CMAP amplitude variability during ST stimulation {0.36 (0.24-1.13), 0.28 (0.20-1.01), 0.33 (0.17-0.61), 0.67 (0.21-0.89), 0.30 (0.15-0.83), respectively, p=0.688}, if it was high enough to evoke reproducible CMAPs.

5) Consistency of TOF measurements

Seven hundred-twenty TOF measurements were performed in the study (20 volunteers × 2 muscles × 6 current intensities × 3 stimulations at each current intensity). TOF analysis could be performed in 532 (74%) cases, those in which the stimulating current intensity was sufficient to elicit CMAPs and produce TOF ratios. Two female volunteers declined TOF stimulation at 50 mA and 60 mA. In 4 cases, false detection was experienced, as only a TOF count was determined instead of TOF ratio. The overall consistency of elicited TOF ratios was 1.02 (0.98-1.06). In 445/532 (83.64%) of cases, the TOF ratios were in the range of 0.9-1.1. The TOF ratios were closer to the expected 1.0 in men than in women {1.01 (0.97-1.06) vs. 1.02 (0.99-1.05), respectively, p=0.029} and in mADM than in mAP {1.01 (0.98-1.05) vs. 1.02 (0.99-1.06), respectively, p=0.031}. The stimulating current intensity (20, 30, 40, 50, 60 mA) did not influence the precision of TOF ratio measurement {1.03 (0.99-1.06), 1.02 (0.99-1.07), 1.01 (0.97-1.05), 1.01 (0.98-1.06), 1.02 (0.99-1.05), respectively, p=0.256}.

6) Skin irritation

Two volunteers presented mild, painless hyperemia at the stimulation site after removing the ECG electrodes. The hyperemia disappeared in a few minutes and no further irritation or adverse events were reported.

4.3.3. Awake Volunteer Pain Scores During Neuromuscular Monitoring

One hundred thirty-five subjects (age: 38.3 ± 11.7 years; 62 women and 73 men) were analyzed; each received nerve stimulations with 4 different current amplitudes (20, 30, 40, and 50 mA) in random order with each of the 2 monitors. After completion, 1620 VNRS measurements were obtained among all subjects and currents, and utilizing both devices.

In the linear mixed-effects model, there were no statistically significant differences in VNRS scores between devices at any of the stimulating current intensities, $P = 0.38$. The VNRS scores at individual current intensities showed significantly higher VNRS scores at higher intensity stimulation ($P < 0.001$). Higher anchor VNRS scores were associated with higher pain scores on both devices ($P < 0.001$). However, no device effect was observed after adjusting for volunteer age ($P = 0.46$) and sex ($P = 0.427$).
TOFRs were also collected with the TetraGraph and TOFWatch devices at the 3 sites. However, there were difficulties with 1 site's ability to consistently obtain TOFRs with the TOF-Watch SX (74% of all readings were TOFCs instead of TOFRs), and the data were excluded from analysis. There were successful TOFR measurements with the TetraGraph in 91% of the 1620 stimulations. The number of false TOFC readings (when the device gave only a TOFC instead of a TOFR approximating 100%) with TetraGraph was 73 of 405 stimulations (18.0%) at 20 mA stimulating current intensity, 36 (8.9%) at 30 mA stimulating current intensity, and 21 (5.2%) at both 40 and 50 mA stimulating current intensity ($P < 0.001$).
The mean ± SD of the 1469 TOFRs obtained with TetraGraph was 100.43% ± 7.74% (95% CI: 100.04–100.83). A total of 64.7% of measurements were in the range of TOFRs 95%–105%, and 87.1% of the measurements fell within the range of TOF ratios 90%–110%.
None of the volunteers reported any adverse events related to the study.

4.3.4. Comparative investigation of acceleromyography-based IntelliVue NMT and electromyography-based TetraGraph quantitative neuromuscular monitors: a pilot study.

50 patients were enrolled in the study, but due to technical difficulties the results of 37 patients could be analyzed. Demographic parameters were as follows: age 50 ± 16 years, male : female ration 17 :20, BMI: 27 ± 5.
Baseline TOF ratios obtained with TetraGraph fell closer to the ideal 100% than the AMG values: EMG TOFR median (range) = 10 (90-109), AMG TOFR median (range) = 106 (83-168), p=0.06.
574 AMG-EMG recovery TOFR data pairs could be registered in 37 patients. Based on Bland-Altman analysis, there was a moderate agreement between the two techniques. The bias ± SE was –8.41 ± 1.4, which means that AMG "overestimated" EMG TOFRs by 8.41. The limits of agreement were wide (-38,96 - +22,14). The normalization of recovery TOFRs decreased the bias to (-3,66 ± 2,33 SE), but the limits of agreement remained to be wide (-38,82 - +31,49). The wide limits of agreement are explained by technical factors, eg. the lack of preload, and the perfect positioning of the arm could not be achieved in every cases.

4.3.5. Ipsilateral and simultaneous comparison of responses from acceleromyography- and electromyography-based neuromuscular monitors

A total of 50 patients were enrolled in this study. Two patients were excluded due to succinyl-choline and sugammadex administration.
Primary endpoint
We recorded 5731 pairs of simultaneous measurements of TOFRs with AMG and EMG during recovery in the 48 patients (mean 119.4 ± SD 50.6 data-pairs per patient; range: 39-221), of which at least one measurement showed TOFR ≥80% in 2977 data-pairs. Of these 2977 data pairs, both AMG and EMG recordings showed a TOFR ≥80% in 2236 (75.1%) pairs. In 693 (23.3%) of the data-pairs, the AMG-measured TOFR was ≥80% while the EMG TOFR was <80%; in the remaining 48 (1.6%) data-pairs, the EMG-measured TOFR was ≥80% while AMG TOFR was <80%. AMG more frequently indicated recovery earlier than

EMG, resulting in more AMG measurements of TOFR ≥80% than with the EMG monitor. The Bland-Altman analysis of normalized data showed that the bias when the AMG TOFR reading was ≥80% (N = 2929 data-pairs) was 1.3 ± SE 1.0 with limits of agreement -14.0 to 16.6. The bias was less (-0.5 ± 0.9), and the limits of agreement were similar (-14.7 to 13.6) when the EMG reading was ≥80% (N = 2284 data pairs).

The between-subject variance was greater than the within-subject variance, and smaller repeatability coefficients indicated that repeatability and precision were higher for AMG (6.3) than for EMG (8.4) when the AMG reading was above 80%, likely as a result of more measurements and larger sample size from the AMG monitor than from the EMG monitor in this range (see above). When the EMG reading was above 80%, repeatability was slightly higher for EMG than for AMG (4.8 vs. 5.1, respectively).

Secondary endpoints

In the TOFR <80% range based on AMG measurement (N = 2802 data-pairs), the Bland Altman analysis of normalized data showed that the bias was 2.1 ± 1.1 with limits of agreement -16.1 to 20.2. In the TOFR <80% range based on EMG measurement (N = 3447 data-pairs), the bias and limits of agreement were similar (2.6 ± 1.0; -14.4 to 19.6, respectively).

Repeated baseline TOFR measurements could be obtained in 47 of the 48 patients. In these patients, between 2 and 5 baseline TOF stimulations were recorded after induction of anesthesia but before neuromuscular blocking agent administration. The mean baseline TOFR measurements was higher with AMG (mean ± SD: 108.8% ± 7.2%, median: 108%, range: 93-141%) than with EMG (100.7% ± 1.5%, 101%, 96-108%; paired $t = 7.95$, $df = 46$, $p<0.0001$). The variance of baseline TOFR measurements with the AMG device was higher than the variance of measurements with the EMG device (51.85 vs. 2.22, respectively, $F = 23.35$, $p <0.0001$). As a result, the mean coefficient of variation was more than four times higher in baseline TOFR measurements by AMG (6.6) than by EMG (1.4). The calculation of repeatability by a random-effects one-way ANOVA showed higher repeatability for EMG (repeatability coefficient: 0.48 ± SE 0.09, 95% CI: 0.29 to 0.64) than for AMG (0.73 ± 0.06, 95% CI: 0.61 to 0.82).

The mean ± SD of baseline CMAP amplitudes recorded by the EMG device was 11.47 ± 4.36 mV (range: 3.2 – 20.9 mV).

Additional endpoints (based on AMG measurements)

PTC measurements in deep block

During deep block, 87 PTC measurements were recorded in 34 patients. The mean ± SD number of measurements per patient was 2.6 ± 1.6 (range: 1 to 9). The AMG monitor recorded twice as many signals (mean adjusted for repeated measurements: 8.6 ± SE 0.7, 95% CI: 7.3 to 9.9) as the EMG monitor (4.3 ± SE 0.7, CI: 3.0 to 5.6) ($F_{1,139} = 29.32$, $p <0.0001$). The Bland-Altman analysis showed a bias of 4.3 and suggested wide limits of agreement, heteroscedasticity of variances and higher between-subject than within-subject variance.

TOFC measurement in deep and moderate block

During deep and moderate neuromuscular block, 4186 data-pairs were recorded. In the Bland-Altman analysis, the TOFC measurements were biased towards AMG by 0.7 ± 0.1 responses (95% CI 0.4 – 0.9 responses), with limits of agreement of -1.5 to +2.8 responses. Clinically, this suggests that in general, the AMG monitor indicated a higher number of TOFCs than the EMG monitor by one response.

Transition from moderate to shallow block

At the time of recovery from moderate to shallow block, the median of the first TOFRs displayed by the AMG monitor was 12% (interquartile range 9.5-14%). This was significantly lower than the median of the first displayed TOFRs by the EMG monitor (19%, 17-24%, Wilcoxon signed rank test, $p<0.001$). The first recovery TOF ratios displayed by EMG were

influenced by the baseline CMAP amplitudes, so that higher baseline CMAP amplitudes yielded earlier detection of recovery TOFRs (linear regression, $R_2 = 0.55$, b = -1.30 ± SE 0.17, $F_{1,46} = 56.92$, $p < 0.0001$).

5. DISCUSSION

5.1. Postoperative residual neuromuscular blockade in our region

In recent years, several trials have demonstrated that PORNB is present in anesthesia practice and tried to give solutions. The incidence of PORNB varies widely in the literature (5-70%) due to methodological differences of the studies and clinical practice. Sasaki, Estevez and Kotake found incidences around 20% while the Canadian RECITE, the American RECITE-US and a large-scale Chinese trial found incidences higher than 60%. Nevertheless, none of the previous studies have examined the incidence of PORNB in such a complex was as we did in our randomized, controlled, double blind study in 2016.

This study has also shown that none of the reversal strategies are able to prevent PORNB in the absence of objective neuromuscular monitoring.

In our two-nation survey 84% of the respondents thought that the incidence of clinically significant PORNB is 1-5%. In Naguib's international survey the same proportion was 77%. Opposing these opinions, the incidence of PORNB in our trial was 22.4% at the time of PACU admission. 12.8% of the patients had nTOFR<0.7 at PACU admission and 11 patients (8.8%) needed rescue medication, which probably fulfills the criteria of "clinically significant PORNB". Nevertheless, the 22.4% PORNB incidence in our study is among the better results compared to other international trials, especially if we take it into consideration that our anesthesiologist could only rely on clinical signs. Interestingly the application of subjective monitoring has resulted in higher incidences in several studies, probably because it gives a false feeling of safety, yet it does not ameliorate the perception of events. In Hungary, and generally in Europe, subjective monitoring has no real tradition like in the US. The availability of subjective monitors is much lower than the availability of objective ones. Unfortunately, it is very likely that the favorable PORNB incidence rate in our study is partly a result of more attention induced by study environment and in reality, the PORNB rate is higher in our institution.

Similarly, to previous Australian (64%) and Portuguese (66.6%) trials, our anesthesiologists decided to give reversal agent in 60% of the cases. This was a little bit higher than what we had previously anticipated, which can also be explained by study environment. Anesthesiologists decided to omit reversal in those cases, when the operation was shorter, less NMBA was administered, and fewer top-up doses were used, and more time elapsed from the last administration of NMBA until the end of operation. It is interesting to see that although more than 90 minutes have passed since the last administration of rocuronium until extubation, TOFR has only recovered to 0.7 ± 0.29, although the clinical duration of rocuronium is supposed to be 30-70 min.

Allowing spontaneous recovery turned out to be less favorable than administering reversal agents (9.4% vs. 26%, OR: 0.3, 95% CI: 0.097 – 0.906, p=0.03). PORNB occurred less frequently after neostigmine (15.4%) than after spontaneous recovery (26%) but statistically this was significant difference. Nevertheless, sugammadex could not preclude PORNB either.

The routine use of neostigmine in previous trials proved to be unpredictable. In several studies higher PORNB rates were noted with neostigmine use than with spontaneous recovery. However, the articles often miss to describe the circumstances of neostigmine administration, like what kind of patients received it, in what dose, what level of block was reversed, how much time was allowed for neostigmine to exert its effect. The other thing is that most of these trials were not randomized, and objective neuromuscular monitoring was not used.

In our investigation to reason for neostigmine's failure was probably the short time it was allowed to exert its effect. Usually less than 10 min was allowed from administration till extubation. Knowing that the anesthesiologists had 33% chance to receive neostigmine, and another 33% to receive placebo, this short time might appear quite imprudent. This can be a result of deficiencies in general knowledge, as 83.2% of our survey respondents thought that neostigmine would do well in less than 10 min. This is similar to Naguib's results who found that only 5.5% of the European anesthesiologists knew that neostigmine needed more than 15 min. 87% thought that less than 10 min was enough.

The neostigmine dose used in the study was 0.05 mg/kg, which is the most often administered dose according to the literature. Considering the level of neuromuscular block at the time of reversal agent administration and the reversal times, this dose must have been sufficient to maximize the number of acetyl-choline molecules in the neuromuscular junction without risking relative overdose and paradox muscle weakness, described by Eikermann et al. Especially because the nTOFR values progressively increased in those 20 minutes which passed from extubation until PACU admission, then they stagnated.

Sugammadex could not preclude PORNB either. In our study one patient out of 27 (3.9%) had PORNB after sugammadex reversal. The level of neuromuscular block at PACU admission was nTOFR 0.87, which might be considered as clinically acceptable. Nevertheless, we were not the first to report PORNB after sugammadex administration. Kotake et al had a 4.3% (5/117) PORNB (TOFR<0.9) rate at extubation. Besides in the literature there are several reports on the ineffectiveness of sugammadex or slow onset of action. Knowing that the function of upper airway muscles is not normalized even at TOFR 1.0, we think that this PORNB rate is high.

From an ethical point of view, our study may be criticized for having included a placebo group. One could argue that the spontaneous recovery group would have sufficed for comparison with the reversal groups. This criticism can be rejected, as intended spontaneous recovery was not a random event but a selected strategy, and is not appropriate as a control group for comparison with the pharmacological reversal groups. In addition, the inclusion of the placebo group provides some evidence that the clinical identification of patients deemed not to need reversal of their neuromuscular blockade is inadequate. The design of the study may also raise an ethical issue about patient safety as the attending anesthetists were not informed of the results of acceleromyography. However, this study design provided a unique opportunity to evaluate the potential risks inherent in routine clinical practice using neuromuscular blocking drugs without guidance from neuromuscular monitoring, and we included safety measures to minimize these potential risks: permanent monitoring of patients by experienced anesthetists not involved in the routine clinical management, rescue medication with 2.0mg/kg of sugammadex, supplementary oxygen administration, bag–mask ventilation or re-intubation of the trachea as necessary.
In Naguib's 2010 international survey 85% of the respondents stated that they had never seen a serious PORNB case in their practice. Knowing the incidence of PORNB this number perfectly shows how difficult it is to recognize PORNB, and simple clinical signs will not help us. In our study, rescue medication was needed in 11 out of 28 PORNB cases. In 6 cases

the problem was evident right after extubation in the OR, in 5 cases the anesthesiologist (wrongly) decided that the patient could be transported to the PACU. The rescue medication promptly reversed the residual muscle relaxant effect, which was supported by the prompt normalization of TOFRs. Re-intubation was not necessary in any cases.
Another piece of evidence that shows how difficult is it to diagnose PORNB simply based on clinical signs is that clinical muscle strength points did not show any difference in any time points of the postoperative 1hour follow-up. The points at 0 min (PACU admission) were similar to points 20-40 and 60 min later, although the TOFRs were lower then. The possible explanation is that residual anesthetic, fentanyl effect and postoperative pain can strongly decrease patients' initiative to perform physical tests, which become absolutely unreliable. In previous studies the level residual neuromuscular blockade did not show direct relationship with patients' and volunteers' ability to pass clinical tests.
In conclusion we can say that none of the reversal strategies were able to prelude PORNB in the absence of neuromuscular monitoring. Although many anesthesiologists think that simple clinical test are reliable, and only high-risk patients need monitoring, our study refutes this. Yet, against all pieces of evidence and recommendations anesthesiologist do not take the trouble of routine monitoring. Usually those find neuromuscular monitoring unnecessary who do not use it. Those who start using it and learn the tricks, realize how much safer it is to use it than not.
Experts identified several factors that explain low willingness to monitor, one o them is the paucity of easy-to-use, reliable monitors. In our Hungarian-Romanian survey 74.1% of the respondents had access to objective monitors, yet only 7.7% used them routinely, 35.4% hardly ever and 27.4% never does. This data is really sad. In 2013 Australians showed larger willingness to monitor although 42% of the institutions had no monitors at all.

In our survey 26.3% of the clinicians said that monitors are complicated to use, 13.6% found hem unreliable and 44.7% would use them if their use had been easier and 9.3% if they had the proper knowledge. These numbers are not surprising if consider that even Dutch anesthesiologist face problems with neuromuscular monitoring although they have very long traditions of monitoring. But the problems do not keep them from using the monitors. The most common problems with acceleromyograph-based monitors are the fluctuation of displayed TOF values and frequent error signs. Both problems can be prevented with proper calibration and the use of preload.

5.2. A possible solution to prevent PORNB: the development of a new objective neuromuscular monitor

We joined the development of TetraGraph, a new-electromyography-based neuromuscular monitor in 2013. The constructor aimed to develop and easy-to-us, portable, reliable neuromuscular monitor which can be connected to electronic medical record system. At that time no monitors fulfilled all these criteria.

The reasons for choosing EMG technology were the followings:

1) At that time there was no portable EMG-based device on market.
2) EMG devices are faster to set up and easier to use as they are no subject to reverse fade and drift phenomena. Therefore, there's no need for preload, normalization and calibration is shorter.
3) EMG can be used when the arms are tucked as the free movement of the thumb is not necessary.

Since 2013 we have conducted several volunteer and clinical trials on different generations of the device in cooperation with the constructors and Mayo Clinic. These investigations served major data to software and hardware developments.

In the next chapter I would like to give a short summary of the development process.

5.2.1. Experiences with the first prototype of TetraGraph

We conducted two studies with the first prototype of TetraGraph (NEAT device) which served invaluable information to future developments. One of them was a volunteer lab test, the other was a clinical testing.

The aim of the first clinical trial was to test see the device working in clinical environment and compare the devices recordings to clinical notes. This trial proved the device able to elicit and record muscle action potentials and help to estimate the level of muscle relaxation. The devices recordings mirrored clinical events in 23/50 cases. Several factors were identified which explained the low parity. These observations later helped the designing of other studies:

1) The 30 mA stimulating current prescribed by the constructor proved to be too low because it could not ensure (supra)maximal stimulating conditions, therefore the amplitudes of evoked CMAPS were too low and often fell under the sensation threshold.
2) The 2 mV threshold (noise filter) proved to be too high and often made low amplitude CMAPs invisible.
3) It turned out to be difficult to define what a muscle twitch is. We did not find data in the literature telling us how big (1-5-10 mm) should be the movement of the thumb to take it as a “twitch”.

No unexpected adverse events were noted during the study, the stimulations did not cause erythema. The device was electrically safe.

The experiences of the first clinical investigation were supported by our observations made during our first volunteer trial:

1) The two examined muscle showed clinically different characteristics, which was previously described. The adductor pollicis muscle needed 20 mA higher stimulating current intensity to evoke muscle contractions, and the stimulating current intensity requirements for supramaximal stimulation was also higher (50 mA).
2) The mean of recordable CMAPs was around the expected value of 1,0 but their variability was higher then in the previous study. 83.17% of the TOFRs fell in the accepted range of 0.9-1.1. The larger variability was explained by the withdrawal movements of awake volunteers provoked by stimulation discomfort.
3) VAS point increased in line with increasing stimulating current intensity. The sex of volunteers did not affect the VAS scores. In this study the VAS scores were lower than in our next volunteer trial. Maximal stimulating conditions required a discomfort level of 3 (2-5).

5.2.2. The second volunteer trial with the last prototype

Our first volunteer trial was followed by another 3-center trial in 2018. The aim of the study was the testing of the preproduction prototype of TetraGraph and the TetraSens electrodes. The comparator device was the AMG-based TOF-Watch. We aimed to compare the discomfort evoked by the two devices and the repeatability of the measurements.

Although TOF stimulation poses less discomfort than tetanic or double burst stimulation, this discomfort is still considerable, as this was also supported by our first volunteer trial. In this investigation, 8 volunteers reported VNRS score ≥6 at 20 mA stimulating current intensity with either device. Although these volunteers agreed to continue the measurements, 4 volunteers could not tolerate the stimulations at higher intensities, and they were ultimately excluded from final analysis. These data imply that there is a need to optimize nerve stimulation conditions to decrease patient discomfort, as neuromuscular monitoring is performed not exclusively in anesthetized patients. One way could be the development of specially designed stimulating electrodes.

Most currently available neuromuscular monitors utilize $Ag/AgCl_2$ ECG electrodes for neurostimulation and for signal recording by EMG devices. However, the optimal electrode size for diagnostic nerve stimulation has not been extensively investigated; rather the polarity and optimal positioning of electrodes have been studied. In the field of electrotherapy, numerous investigations have been conducted to explore the effect of electrode size on pain intensity induced by muscle stimulation. Though several studies indicated that larger stimulating surfaces are more comfortable, as they decrease the current density, the results are not consistent. At the same time, the temporal and spatial characteristics of repetitive stimuli used for muscle stimulation are not directly corresponding to currents used for diagnostic nerve stimulation.

The TetraGraph neuromuscular monitor utilizes a silver/silver chloride surface electrode strip for both neurostimulation and CMAP recording. The surface area of the stimulating electrodes is roughly twice as large as that of commercially available adult ECG electrodes, which was designed to disperse the potentially painful electrical stimulus through the skin and the subcutaneous tissues. These electrodes also have an elongated shape and are applied perpendicularly to the course of the ulnar nerve, which could reduce the chance for suboptimal electrode placement. The authors had hypothesized that stimulation with TetraSens electrodes would be less painful compared to the standard ECG electrodes. However, results obtained from this large cohort of 135 volunteers did not support this hypothesis. The TetraSens electrodes and the standard ECG electrodes induced identical discomfort to the volunteers at each current intensity. The VNRS scores increased as a function of stimulating current intensities, which is in agreement with previous reports. The age or sex did not influence the level of pain perception. Higher anchor VNRS scores at 30 mA single twitch stimulation yielded higher VNRS scores for all current intensities of TOF stimulation.

Currently, there are no guidelines describing the ideal stimulating parameters for postoperative patients. To reduce patient discomfort, several investigations have advocated the use of lower stimulating intensities (20–30 mA) in the postoperative setting, finding that decreasing the stimulating current did not influence the reliability of measurements. But these previous investigations that reported that TOFRs can be obtained reliably at lower stimulating intensities used MMG and AMG where the fingers are fixed. In the current investigation,
the EMG-based device had a significantly lower success rate in obtaining TOFRs at 20 and 30 mA stimulating current intensities (18% and 8.9% vs 5.2%), likely due to the fact that lower current intensities were not able to reliably activate a sufficient number of nerve fibers. The noise filter was also set at 2 mV in the investigation. In the first volunteer trial 30 mA was usually required to evoke palpable muscle contractions and recordable CMAPs.

In addition, the short duration of individual investigations (approximately 10 minutes) could have been insufficient for optimal curing of the silver–silver chloride gel of the electrodes. This could adversely affect both stimulating and sensing conditions, especially at low current intensities. Yet, once the stimulating intensity was sufficient to evoke detectable CMAPs, the

detection of peak-to-peak amplitudes was equally effective at all current intensities, as the stimulating current intensity did not influence the distribution of the measurements. Of note, the mean of the 1469 TOFRs obtained with TetraGraph was 100.43% ± 7.74% (standard error = 0.2%), which is nearly identical to the 100% expected in unmedicated volunteers. The mean ± SD of the TOFRs was more ideal in this investigation than in the first trial, (102 ± 13%) which was probably due to the software developments.
The secondary aim of the study, to compare the repeatability of the AMG- and EMG-derived TOFRs, could not be achieved, as 1 center was unable to consistently obtain TOFRs using the TOF-Watch SX. This resulted in the authors being unable to perform a detailed comparison and draw conclusions on the repeatability in obtaining TOFR with the TOF-Watch and TetraGraph. Faulty or damaged piezoelectric transducer has been implicated in the past as a source of error with AMG devices and could have contributed to the failures experienced by one of the sites.

5.2.3. Further steps of development and their funding clinical investigations

The first clinical trial with the first prototype was followed by several others in the next years in different arrangements. The later prototypes of TetraGraph were compared to different AMG-based devices. According to the 2007 Good Clinical Practice guidelines new neuromuscular monitors should be validated against mechanomyography. However, mechanomyography is no longer commercially available. Although several investigations showed that AMG-derived TOF stimulation results are not interchangeable with mechanomyography-derived measurements, the AMG-based TOF-Watch monitor has been used in multiple investigations as the reference device.
These studies were crucial for the development of the device. Not just the electronical system and analyzing algorithm were developed but calibration protocol, trend display were also implemented and the appearance and interface were also optimized and modernized.
Another major question of the development process was to determine the optimal lower sensing threshold. Subthreshold signals (CMAPs of low amplitude) are considered as noise and do not get analyzed which can impair PTC and TOFC measurements. After the first tests it has to be admitted that the 2 mV threshold was too high, therefore it was lowered to 1 mV.
The first clinical trial with 1 mV threshold was performed at Mayo Clinic. The circumstances to conduct this investigation were less than ideal, therefore the results were not published but the experiences were later used to design an ideal investigation.
In 2018 we conducted our most important AMG-EMG comparative study at the University of Debrecen. Home environment helped to idealize patient enrollment and the performance of measurements. We tried to obtain slow recovery curves to gain as much data as possible.
The most important achievement of this investigation was the synchronization of the two neuromuscular monitors via fier optic link. This made it possible to analyze both the electrical and mechanical function of the very same muscle. Previous comparisons of AMG and EMG technologies have been hampered by the inability to simultaneously record AMG and EMG responses from the same muscle, introducing another potential source of variability. The synchronization of the two neuromuscular monitors in this investigation via fiber optic link provided a unique opportunity to exclude the arm-to-arm variability factors, such as hand dominance, electrode positioning, blood perfusion, temperature, drug administration site and lack of stimulation synchronization. Because of simultaneous stimulation and recording in the same muscle afforded by the two monitors, any differences observed in the study can only be attributable to the two monitoring technologies (and the fact that they are recording different physiologic phenomena), and not to external factors or differences in individual muscle response.

Despite its unique design, our study has several limitations. First, the type and dose of neuromuscular blocking agent were not standardized. This was a conscious decision during study design; it allowed us to obtain spontaneous recovery curves from several neuromuscular blocking agents, since there is no reason to suspect that AMG and EMG recovery curves would be differentially influenced by the type of neuromuscular blocking agent.

Second, the calibration process was showed some differences to recommendations. We did not use tetanization to avoid T1 drift as it is not part of routine clinical practice and we did not intend to use T1 data. Instead of performing CAL2 calibration, which would have identified the supramaximal current intensity as well as set the gain. we used a pre-determined stimulating current intensity (60 mA) The reason for this was that the two devices could not be calibrated separately; the same AMG device was used to provide nerve stimulation, while both monitors recorded the responses simultaneously. In a previous volunteer study using the TetraGraph and in which no neuromuscular blocking agent was administered, 20, 30, and 40 mA stimulating current intensities proved insufficient to evoke detectable muscle twitches in 18%, 9% and 5% of the volunteers, respectively. Therefore, using low stimulating current intensity in the clinical setting could have artificially decreased the performance of the EMG-based monitor when monitoring deep and moderate neuromuscular block by decreasing the baseline (and subsequent) CMAP amplitudes.

The primary aim of our study was to see if TetraGraph is reliably able to detect safe extubation conditions. The EMG-derived TOFRs showed good agreement with AMG-derived measurements in shallow and minimal neuromuscular block. The bias in the primary endpoint was 1.3 or 0.5, depending on whether AMG ≥80% or EMG ≥80% was used to determine the range boundary, and this bias was less than the acceptable difference set *a priori* (10%). However, the bias based on the non-normalized (raw) data was considerably larger (9.3 or 7.8), and this bias was close to the acceptable limit (10%). In contrast, the limits of agreement (-14.0 to 13.6 or -7 to 23.7 at the minimum) were wider than the acceptable difference (-5 to 5).

Nevertheless, our results of raw TOFRs showed a lower bias between AMG- and EMG-derived TOFRs than previous reports did; the overall bias of raw TOFRs was 7.0 ± 1.1 with limits of agreement of -12.0 to +25.9. Some investigators reported a bias of 14.9, while others reported an overall bias of 17.6, with similarly wide limits of agreement (-4.5 to 39.6). These differences might be explained by the different study designs and statistical approaches, and by the recording of evoked responses from different devices and muscles. A likely explanation for the lower bias between EMG and AMG devices reported in our study is its unique design.

In line with previous investigations, this study has also shown that the normalization of AMG-derived TOFRs is crucial to correctly identify the threshold of recovery. In this study the normalization of AMG TOFRs not only significantly decreased the bias between the two techniques, but also improved the precision of AMG measurements. In the clinical setting, however, normalization of AMG-derived TOFRs prior to tracheal extubation is rarely, if ever, performed. It is therefore likely that the patients' degree of recovery immediately prior to extubation is overestimated; this may explain the persistently high incidence of residual neuromuscular block when tested in the postanesthesia care unit. Second, in light of existing data that the patients' hypoxic ventilatory response may well be blunted at a TOFR of 90%, while the vital capacity is depressed by 16% at this level of recovery,[37] a difference of 10% between non-normalized and normalized TOFRs may well be clinically significant for patient safety. This difference also lends support to the contention that the minimum threshold of neuromuscular recovery with AMG should be a TOFR ≥100%, rather than the current threshold of TOFR ≥90%. From the side of device manufacturers it would be worth

considering to implement automatic normalization into AMG devices to ease the work of clinicians.

Similar to previous observations,[28] EMG had slightly higher repeatability (lower coefficients) in the primary endpoint (except in the AMG ≥80% range due to larger sample size), and lower variance and higher repeatability in the secondary endpoint in our investigation. The difference was even more evident when EMG data were compared to raw AMG measurements. Based on all these observations, our results suggest that the EMG-based device is a better indicator of adequate recovery from neuromuscular block and readiness for safe tracheal extubation than the AMG monitor.

As previously reported, baseline TOFRs obtained with EMG were more consistent and showed less deviation from the baseline TOFR of 100% than with AMG. As recommended for AMG-based investigations, we performed our measurements using the TOF-Watch$_R$ Hand Adapter to improve the stability of baseline responses. This application of preload to the thumb and fixation of the monitored extremity also improves the precision of the EMG-derived baseline TOFRs. Nevertheless, the low variation coefficient of the baseline measurements with EMG suggests that the use of preload and normalization of responses to baseline value is unnecessary with this device (as opposed to AMG-based monitors), improving its clinical acceptance and routine use.

The primary aim of our study was to describe the EMG-based monitor's ability to accurately indicate recovery from neuromuscular block. Precise measurement of neuromuscular block is essential throughout surgery to maintain adequate muscle relaxation, and to facilitate making correct decisions about additional dosing of neuromuscular blocking agents, as well as the timing, type, and dose of antagonist. Therefore, we aimed to also examine the performance and reliability of the EMG monitor during deep, moderate, shallow and minimal phases of neuromuscular block.

Unfortunately, when analyzing the data of deep and moderate neuromuscular block, our analyses could not include cases when the simultaneous measurements recorded distinct types of data, e.g., TOFR, a continuous variable on one device, and TOFC, a discrete variable on the other device. Because measurements could not be compared quantitatively in such cases, it is possible that our results may be biased in the direction of minimizing differences between the two devices, which needs to be considered in the interpretation of our results.

The agreement between AMG and EMG responses during deep and moderate block was not as narrow as during shallower degrees of block. The EMG showed a delay (i.e., indicated slower recovery from neuromuscular block) in PTCs and TOFCs compared to the AMG monitor; this difference was not attributable exclusively to the differences between the two techniques, but rather to a relatively high sensing threshold (noise filter) of the EMG- based monitor. During recovery from neuromuscular block, the difference in the time to first reappearance of the fourth twitch of TOF (T4) between the EMG and AMG monitors was variable. While some patients showed good synchrony between the start of TOF recovery (first return of T4) with both monitors, others exhibited significant delay in the return of T4 by EMG. We investigated the factors that might explain this difference and found a correlation between the amplitude of baseline CMAP and the recovery of T4: the greater the baseline CMAP signal (the better the signal quality), the earlier the EMG monitor displayed TOFRs, and the closer it correlated with the AMG-obtained TOFR values.

Further studies are needed to determine the optimal lower threshold. At the moment, we cannot tell if a fix threshold (eg. 0.5 mV) or a proportional threshold (eg. 5-10% of baseline amplitude) would be more ideal. What makes the question tricky i the definition of the smallest muscle twitch and corresponding electrical signal that should be analyzed. If very low twitches are considered, we might overestimate recovery, which would result in unnecessary, superfluous administration of NMBAs. In addition, it is difficult to define a

“twitch” as the sensation threshold during clinical examination, and such tactile evaluations of response (contractions) do not necessarily correlate with quantitative measurements. According to prior investigations, clinicians are likely to overestimate the number of subjectively determined twitches compared to objective measurements by AMG. In a recent study, the order of sensitivity in detecting twitch count was mechanomyography (most sensitive); EMG was most similar to palpation; and AMG was least sensitive.
Although in recent years TetraGraph has undergone major developments, it is still not ready. Finding the optimal lower sensing threshold will be a big challenge. Hopefully the data we have gathered so far will help this work. We feel that it would be important to test the device in special patient populations (eg. in old patients and in patients with neuromuscular disease). The greatest challenge will be the monitoring of the pediatric population which will necessitate further hardware and software developments and extensive testing.

6.0. SUMMARY

In the first half of the thesis the incidence and causative factors leading to postoperative residual neuromuscular block were examined. Therefore, we conducted a partially randomized, placebo controlled, double blind investigation at the University of Debrecen, Department of Anesthesiology and Intensive Care. Then an internet-based survey was spread among Hungarian and Romanian anesthesiologist to survey their reversal and monitoring habits. Based on our results the following conclusions can be drawn:

1) Postoperative residual neuromuscular blockade is a significant problem nowadays even though anesthesiologists still seem reluctant to admit this. The occurrence of PORNB at our center was 22.4%.
2) PORNB was apparent in all four study groups (spontaneous recovery, neostigmine reversal, sugammadex reversal, placebo group). In the absence of objective neuromuscular monitoring none of the reversal strategies were able to prevent it.
3) Opposing both our own and international results, a significant proportion of the Hungarian and Romanian anesthesiologists consider PORNB as a negligible problem in everyday anesthesia practice.
4) 26.3% of our respondents think that objective neuromuscular monitoring is unnecessary to exclude PORNB and simple clinical tests do well.
5) Only 7.7% of our respondents use neuromuscular monitors routinely and 27% of them never monitor neuromuscular function.

6) A cause of low willingness to monitor neuromuscular function might be the relatively low availability of objective neuromuscular monitors and the unease of application.

In the second half of my thesis, I described the development of a new electromyography-based neuromuscular monitor. The first clinical tests were conducted at the University of Debrecen, Department of Anesthesiology and Intensive Care. These tests allowed the device to appear on market. As a result of the development process the device has become an ideal indicator of adequate recovery from neuromuscular blockade and ideal tool to signal optimal extubating conditions. Its easy-to-use interface will hopefully make it popular among anesthesiologists and helps to enhance monitoring willingness. Further developmental process is needed to improve the device's capabilities to monitor deep and moderate neuromuscular block and certain special patient groups, like patients with neuromuscular diseases and pediatric patients. These developmental processes are currently ongoing.

7.0. ACKNOWLEDGEMENTS

First, I would like to say thank you to my supervisor, Béla Fülesdi, MD, PhD, DSci for his trust, support and all his help.

I also have to thank Edömér Tassonyi MD PhD DSci's support. His huge experience has helped designing the studies and writing the manuscripts.

I cannot express how grateful I am to Prof. Sorin J Brull, for all his fatherly support, guidance and encouragements and for inviting me to Mayo Clinic.

Thank you for the Hungarian Fulbright Commission for making it possible for me to spend six months in the US at Mayo Clinic. This turned out to be a cornerstone in the development process.

Thank you to Dave Hampton and Martyn gray for the work we had together.

Huge thank you to Szabolcs Lengyel PhD, DSci for his immense help in statistical analysis.

Thank you to my dear colleagues and friends Adrienn Pongrácz, László Asztalos, György Nagy and J Ross Renew.

Thank you to Erzsébet Igbonu-Nagy for her help with the authorities.

Thank you for all my anesthesiologist colleagues who helped the studies, and especially our anesthesia nurses.

Thank you to the colleagues from the Surgical Department for their patience.

Last but not least, thank you for my family and friends for all their patience and support over the years.

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

Registry number: DEENK/310/2021.PL
Subject: PhD Publication List

Candidate: Réka Nemes
Doctoral School: Doctoral School of Neurosciences

List of publications related to the dissertation

1. **Nemes, R.**, Nagy, G., Murphy, G. S., Logvinov, I. I., Fülesdi, B., Renew, J. R.: Awake Volunteer Pain Scores During Neuromuscular Monitoring.
   Anesth. Analg. 130 (4), 941-948, 2020.
   DOI: http://dx.doi.org/10.1213/ANE.0000000000004326
   IF: 4.305 (2019)

2. Pongrácz, A., **Nemes, R.**, Breazu, C., Asztalos, L., Mitre, I., Tassonyi, E., Fülesdi, B., Mitre, C.: International survey of neuromuscular monitoring in two European countries: a questionnaire study among Hungarian and Romanian anaesthesiologists.
   Rom. J. Anaesth. Intensive. Care. 26 (1), 45-51, 2019.
   DOI: http://dx.doi.org/10.2478/rjaic-2019-0007

3. **Nemes, R.**, Fülesdi, B., Pongrácz, A., Asztalos, L., Szabó-Maák, Z., Lengyel, S., Tassonyi, E.: Impact of reversal strategies on the incidence of postoperative residual paralysis after rocuronium relaxation without neuromuscular monitoring: a partially randomised placebo controlled trial.
   Eur. J. Anaesth. 34 (9), 609-616, 2017.
   DOI: http://dx.doi.org/10.1097/EJA.0000000000000585
   IF: 3.958

4. Fuchs-Buder, T., **Nemes, R.**, Schmartz, D.: Residual neuromuscular blockade.
   Curr Opin Anaesthesiol. 29 (6), 662-667, 2016.
   DOI: http://dx.doi.org/10.1097/ACO.0000000000000395
   IF: 2.369

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

List of other publications

5. Renew, J. R., Hernandez-Torres, V., Logvinov, I. I., **Nemes, R.**, Nagy, G., Li, Z., Watt, L., Murphy, G. S.: Comparison of the TetraGraph and TOFscan for monitoring recovery from neuromuscular blockade in the Post Anesthesia Care Unit.
J. Clin. Anesth. 71, 1-6, 2021.
DOI: http://dx.doi.org/10.1016/j.jclinane.2021.110234.
IF: 6.039 (2019)

6. **Nemes, R.**, Renew, J. R.: Clinical Practice Guideline for the Management of Neuromuscular Blockade: what Are the Recommendations in the USA and Other Countries?
Curr. Anesthesiol. Rep. 10, 90-98, 2020.
DOI: https://doi.org/10.1007/s40140-020-00389-3

7. **Nemes, R.**, Renew, J. R., Brull, S. J.: Physiology of neuromuscular transmission.
In: Faust's Anesthesiology Review, 5th Edition. Ed.: Terrence L. Trentman, Brantley D. Gaitan, Bhargavi Gali, Rebecca L. Johnson, Jeffrey T. Mueller, Steven H. Rose, Toby N. Weingarten, Elsevier, Philadelphia, 91-94, 2020.

8. **Nemes, R.**, Renew, J. R., Brull, S. J.: Reversal Agents of neuromuscular block.
In: Faust's Anesthesiology Review, 5th Edition. Ed.: Terrence L. Trentman, Brantley D. Gaitan, Bhargavi Gali, Rebecca L. Johnson, Jeffrey T. Mueller, Steven H. Rose, Toby N. Weingarten, Elsevier, Philadelphia, 179-183, 2020.

9. Renew, J. R., **Nemes, R.**, Cammu, G., Van de Velde, M., Brull, S. J., Naguib, M.: Belgian anesthesiologists' overconfidence in their perceived understanding of neuromuscular blockade management.
Acta Anaesthesiol Belg. 70, 161-165, 2019.

10. **Nemes, R.**, Diószeghy, B., Pongrácz, A., Tassonyi, E., Fülesdi, B.: Performance Assessment of a New Electromyography-based Neuromuscular Monitor and Subjective Discomfort in Unmedicated Volunteers.
J Anesth Clin Res. 9 (8), 1-8, 2018.
DOI: http://dx.doi.org/10.4172/2155-6148.1000848

11. Iwasaki, H., **Nemes, R.**, Brull, S. J., Renew, J. R.: Quantitative Neuromuscular Monitoring: current Devices, New Technological Advances, and Use in Clinical Practice.
Curr. Anesthesiol. Rep. 8 (1), 1-11, 2018.
DOI: https://doi.org/10.1007/s40140-018-0261-x

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

12. Tassonyi, E., Asztalos, L., Szabó-Maák, Z., **Nemes, R.**, Pongrácz, A., Lengyel, S., Fülesdi, B.: Reversal of Deep Pipecuronium-Induced Neuromuscular Block With Moderate Versus Standard Dose of Sugammadex: a Randomized, Double-Blind, Noninferiority Trial.
Anesth. Analg. 127 (6), 1344-1350, 2018.
DOI: http://dx.doi.org/10.1213/ANE.0000000000003719
IF: 3.489

13. Asztalos, L., Szabó-Maák, Z., Gajdos, A., **Nemes, R.**, Pongrácz, A., Lengyel, S., Fülesdi, B., Tassonyi, E.: Reversal of Vecuronium-induced Neuromuscular Blockade with Low-dose Sugammadex at Train-of-four Count of Four: a Randomized Controlled Trial.
Anesthesiology. 127 (3), 441-449, 2017.
DOI: http://dx.doi.org/10.1097/ALN.0000000000001744
IF: 6.523

14. **Nemes, R.**, Berhés, M., Sárkány, P.: Carotis-endarterektómia miatt kialakuló stroke.
In: Anesztéziai szövődmények megelőzése és kezelése. Szerk.: Bogár L, Medicina Könyvkiadó Zrt., Budapest, 373-379, 2016.

15. **Nemes, R.**, Fülep, Z., László, I., Sárkány, P., Fekete, K., Mechler, F., Fülesdi, B.: Assessment of severity and time course of critical illness neuropathy in septic patients: a prospective observational study.
Ideggyogy. Szle. 68 (1-2), 30-36, 2015.
IF: 0.376

16. Tassonyi, E., Pongrácz, A., **Nemes, R.**, Asztalos, L., Lengyel, S., Fülesdi, B.: Reversal of Pipecuronium-Induced Moderate Neuromuscular Block with Sugammadex in the Presence of a Sevoflurane Anesthetic.
Anesth. Analg. 121 (2), 373-380, 2015.
DOI: http://dx.doi.org/10.1213/ANE.0000000000000766
IF: 3.827

17. **Nemes, R.**, Molnár, L., Fülep, Z., Fekete, K., Berhés, M., Fülesdi, B.: Critical illness associated neuromuscular disorders: keep them in mind.
Ideggyogy. Szle. 67 (1-2), 1-12, 2014.
IF: 0.386

18. Sárkány, P., Lengyel, S., **Nemes, R.**, Orosz, L., Páll, D., Molnár, C., Fülesdi, B.: Laparoszkópos cholecystectomia során alkalmazott CO2-inszufflációkardiovaszkuláris hatásainak non-invazív monitorozása pulzushullám analízissel.
Anaesthesiol. Intenziv Ther. 44 (4), 162-168, 2014.

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

19. Sárkány, P., Lengyel, S., **Nemes, R.**, Orosz, L., Páll, D., Molnár, C., Fülesdi, B.: Non-invasive pulse wave analysis for monitoring the cardiovascular effects of CO2 pneumoperitoneum during laparoscopic cholecystectomy- a prospective case-series study.
BMC Anesthesiol. 14 (98), 1-7, 2014.
DOI: http://dx.doi.org/10.1186/1471-2253-14-98
IF: 1.375

20. Molnár, L., Simon, É., **Nemes, R.**, Fülesdi, B., Molnár, C.: Postcraniotomy headache.
J. Anesth. 28 (1), 102-111, 2014.
DOI: http://dx.doi.org/10.1007/s00540-013-1671-z
IF: 1.176

21. Pongrácz, A., Szatmári, S., **Nemes, R.**, Fülesdi, B., Tassonyi, E.: Reversal of neuromuscular blockade with sugammadex at the reappearance of four twitches to train-of-four stimulation.
Anesthesiology. 119 (1), 36-42, 2013.
DOI: http://dx.doi.org/10.1097/ALN.0b013e318297ce95
IF: 6.168

22. Sárkány, P., Tassonyi, E., **Nemes, R.**, Timkó, A., Pongrácz, A., Fülesdi, B.: Testing rocuronium-induced neuromuscular blockade at the stapedius muscle using stapedius reflex measurements.
Acta Physiol. Hung. 98 (4), 472-479, 2011.
DOI: http://dx.doi.org/10.1556/APhysiol.98.2011.4.11
IF: 0.821

23. **Nemes, R.**, Sárkány, P., Berhés, M., Varga, Z., Fülesdi, B.: Regionális és általános anesztézia carotis endarterectomia során: tapasztalatok és a magyar betegpopuláció sajátosságai a GALA tanulmány tükrében.
Anaesthesiol. Intenziv Ther. 40 (3), 137-145, 2010.

Total IF of journals (all publications): 40,812
Total IF of journals (publications related to the dissertation): 10,632

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

18 May, 2021

DEBRECENI EGYETEM Egyetemi és Nemzeti Könyvtár