C2. Onderzoeksprotocol

Title: Evaluation of the TOF CUFF for perioperative neuromuscular transmission monitoring during recovery of moderate and deep neuromuscular block compared to acceleromyography and electromyography – *TO CUFF study*

Date: June 2019

VERSION: 6.0

PROTOCOL nr: p17.050

CCMO nr: NL60865.058.17

Investigators:

M. Honing MD, PhD student

M. Boon MD PhD, Anesthesiologist, Resident (LUMC)

B. Torensma, PhD student (Nederlandse Obesitas Kliniek, The Hague)

C. Martini MD PhD, Anesthesiologist (LUMC)

L. Aarts MD PhD, Anesthesiologist (LUMC)

A. Dahan MD PhD, Anesthesiologist (LUMC, PI)

External Subjects Advisor:

Dr. E. Sarton, Department of Anesthesiology, Leiden University Medical Center

Address for correspondence:

Prof. dr. A. Dahan

Dept. of Anesthesiology. LUMC, H5-Q, 2300 RC Leiden, The Netherlands

P +31 71 52 **62301** *F* +31 71 526 6230 *E* **a.dahan@lumc.nl**

List of abbreviations and definitions

AE: Adverse event. Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the investigational product and or the experimental treatment.

CCMO: (Centrale commissie mensgebonden onderzoek) Central Human Ethics Committee

CRF: Case record form.

DNB: Deep neuromuscular block

MNB: moderate neuromuscular block

GCP: Good clinical practice

ICH: International conference on harmonisation

LOAA/S: Leiden Observer's Assessment of Alertness/Sedation

LUMC: Leiden University Medical Center

METC: (Medisch Ethische Commissie) Medical Ethics Committee

NMBA: non-depolarizing neuromuscular blocking agent

Train of four (TOF). Four supramaximal stimuli of 2 Hz are provided to the ulnar nerve via the skin at the wrist. The effect of the stimuli can be measured at the adductor pollicis muscle (in our study with the TOF watch) as muscle contractions. With increasing muscle blockade a fade (*i.e.,* the amplitude decreases) appears followed by the disappearance of muscle contractions. The reverse is true when the muscle blockade disappears. Under conditions of deep or profound neuromuscular blockade (with absence of any twitches) the TOF is of limited use.

Train of four ratio (TOF ratio). The ratio of the amplitude of the last twitch of the TOF

relative to the first twitch (*i.e,* T4/T1). The lower this ratio, the greater the extent (depth) of muscle relaxation.

Post Tetanic Count (PTC). In contrast to TOF stimulation, PTC can be used to determine the degree of neuromuscular blockade under conditions of a deep neuromuscular block. A 50 Hz stimulus, given for 5 seconds, is applied at the skin over the ulnar nerve. Three seconds after this stimulus, muscle contraction is counted in response to single 1 Hz stimulation. In an intense neuromuscular block, no PTC response may be observed.

Acceleromyography (AMG): method to measure neuromuscular transmission. A piezoelectric device measures thumb acceleration after stimulation of the ulnar nerve

Electromyography (EMG): Gold standard to measure neuromuscular transmission. Evoked action potentials after stimulation of a peripheral nerve are recorded in a muscle innervated by that nerve. Usually

Abstract

Introduction

Neuromuscular monitoring is mandatory during anesthesia. Acceleromyography (AMG) is the most wide spread used method because it is easy to apply and accurate enough for daily practice. However AMG is known to be inaccurate when compared to the gold standard in neuromuscular transmission monitoring, electromyography (EMG). Furthermore when the patients arms require to be positioned next to the body and beneath surgical drapes, AMG measurements are often hindered and inaccurate. The TOF cuff is a new device which measures NMB at the upper arm with a blood pressure cuff. It overcomes the previously mentioned disadvantages of AMG. However, it validity compared to EMG has not yet fully been investigated.

Aim

To compare the bias, limits of agreement and precision of the TOF cuff relative EMG on both the ipis- and contralateral arm during recovery of moderate and deep neuromuscular block in patients with normal body mass index and morbidly obese patients.

Methods

This is an observational, non-inferiority trial in which we will compare the TOF cuff device to electromyography (EMG). All patients will receive general total intravenous anesthesia (propofol/remifentanil/rocuronium). Patients will have the TOF cuff and EMG placed on the same extremity (hand for EMG, upper arm for TOF cuff). Measurements will be done during induction, maintenance and recovery from a moderate (GROUP 1) and deep (GROUP 2) neuromuscular block. Patients will enter the study in group 1 or 2 based on the required procedure. In other words, the procedure dictates the desired level of neuromuscular block (moderate or deep), not the study. We will perform a pilot study with 200 patients (150 patients with normal BMI (< 30 kg/m^2) and 50 patients with morbid obesity (>35 kg/m^2) to ensure that reliable estimates of repeatability coefficient, bias, and limits of agreement are obtained. After 100 patients (50 non-obese/50 obese) we will perform an interim analysis and calculate the remaining number of patients needed to complete the study. The protocol was amended (19-11-2018) to include 50 extra patients to investigate relative bias between ipsi- and contralateral comparisons between TOF Cuff and EMG in patients. Total number of included patients will be 250. The interim analysis at 100 included patients showed a significant difference between the TOF Cuff and the EMG regarding the time to complete spontaneous recovery of neuromuscular block. The time to complete recovery is clinically the most relevant outcome as this reflects zero chance of residual NMB by definition. To increase the emphasis on time until full spontaneous recovery, the protocol was amended (19-6-19) to include 50 patients at the LUMC that were originally scheduled for inclusion at the Nederlandse Obesitas Kliniek (NOK). The very reason for this, is the fact that at the NOK, sugammadex reversal is routinely applied to facilitate quick changeover of patients. However, this practice interferes with the objective of

evaluating time until complete spontaneous (unreversed) recovery of the NMB. Transferal of 50 intended study subjects from the NOK to the LUMC will allow us to gather more data on the relevant final stages of the spontaneous recovery of NMB

1. Introduction

Neuromuscular monitoring is mandatory during anesthesia when using muscle relaxants. Lack of monitoring has for years been associated with increased risk of postoperative residual curarisation and pulmonary complications.(1, 2) In addition, monitoring is of utmost importance during the application of a deep neuromuscular block. Monitoring is important for optimal steering of the depth of the neuromuscular block (NMB) during surgery and reversal at the end of surgery. As such, optimal neuromuscular monitoring is of importance for various reasons (1) optimizing surgical conditions; (2) to prevent complications during surgery (e.g., sudden patient movement) and (3) to prevent postoperative complications due to incomplete recovery of the NMB. (3, 4) There are several methods to monitor a NMB; each technique has its specific advantages and disadvantages. Gold standards are electromyography (EMG) and mechanomyography (MMG). However, these methods require specific equipment. Hence, for practical reasons, acceleromyography (AMG, such as the TOF Watch) of the thumb is the most widespread neuromuscular monitor in use. This method uses a piezoelectrical detector, which measures acceleration of the thumb after ulnar nerve stimulation. Major disadvantages of AMG are that it is not very accurate, and it requires a free and unrestricted moving thumb (which may not always be available due to specific patient positioning requirements) and a thumb preload (which is often unavailable, yielding inaccurate results). In conclusion, AMG is suitable in daily practice for *most* procedures due to its ease of use. However, during prolonged procedures, or when the thumb is covered by surgical drapes, AMG monitoring is often inadequate.(5)

The TOF cuff is a new device that overcomes most of the issues associated with the use of an AMG technique. The TOF cuff measures neuromuscular transmission on the upper arm with a blood pressure cuff. Two electrodes in the cuff elicit a response in the upper arm musculature. The device records the pressure changes in the blood pressure cuff following stimulation and calculates the depth of the NMB.(6) It has the advantage of not being influenced by surgical positioning, as it does not require a free moving thumb.

We have successfully used the TOF cuff in the BLISS 2 and Neuropa studies.(7, 8) We find it to be far superior in ease of use over AMG. However, several reviewers questioned the validity of the device during the review process of both studies. A small validation study the TOF cuff has been conducted several years ago and produced acceptable bias and limits of agreement when compared to mechanomyography during recovery of a moderate neuromuscular block. However, the TOF cuff was

not assessed during deep neuromuscular block and not compared to EMG and the authors concluded that additional validation of the device is necessary.(6)

In this study we intend to further validate the TOF cuff and compare it to EMG during recovery of both moderate and deep neuromuscular block. We will study patients of normal and high body mass index values.

2. Objectives

To compare the bias, limits of agreement and precision of the TOF cuff relative to EMG during recovery of moderate and deep neuromuscular block in patients with normal body mass index and morbidly obese patients.

Hypothesis

The TOF cuff behaves similarly to EMG in terms of bias, limits of agreement and precision when comparing the TOF cuff to EMG.

3. Study design

This is an observational, non-inferiority trial in which we will compare the TOF cuff device to electromyography (EMG). We will do this in 100 patients with BMI < 30 kg/m^2 and 50 patients with BMI > 35 kg/m^2. The protocol was amended (19-11-2018) to include 50 extra patients to investigate relative bias between ipsi- and contralateral comparisons between TOF Cuff and EMG in patients with normal BMI. Total number of included patients will be 250. The preliminary analysis after 100 patients showed that a significant difference occurs in the comparison between the TOF Cuff and the EMG regarding the time to full recovery of neuromuscular block. The time to complete recovery is clinically the most relevant outcome. To increase emphasis on time until full recovery, the protocol was amended (19-6-19) to include 50 patients in the LUMC which were originally scheduled for the NOK. In the NOK sugammadex is routinely administrated which disables evaluation of the time until complete recovery.

All patients will receive general total intravenous anesthesia (propofol/remifentanil/rocuronium). Patients will have the TOF cuff and EMG placed on one extremity (hand for EMG, upper arm for TOF cuff).

Measurements will be done during induction, maintenance and recovery from a moderate (GROUP 1) and deep (GROUP 2). neuromuscular block. Patients will enter the study in group 1 or 2 based on the required procedure. In other words, the procedure dictates the desired level of neuromuscular block (moderate or deep) not the study. Patients will therefore enter the study in a non-randomized fashion.

In group 1, the target level of neuromuscular block is TOF 1-3 twitches (moderate neuromuscular block). Time to 90% depression of the first twitch will be recorded. Measurements will be performed during spontaneous recovery to from T1 until full recovery to a TOF ratio of 1.0. After that, if time allows, a new rocuronium bolus will be given to reach a target TOF 1-3 twitches. Every 60 seconds, EMG measurements will be followed by TOF cuff.

In group 2 target depth of neuromuscular block after induction is 1 PTC (deep neuromuscular block). Time to 90% depression of the first twitch will be recorded. After that, spontaneous recovery will be allowed to a PTC of 10. After that, if time allows, a new bolus of rocuronium will be administered to again reach a PTC of 1 followed by spontaneous recovery. Again, EMG measurements will be followed by TOF cuff measurements on the other arm measurements at 5 min intervals.

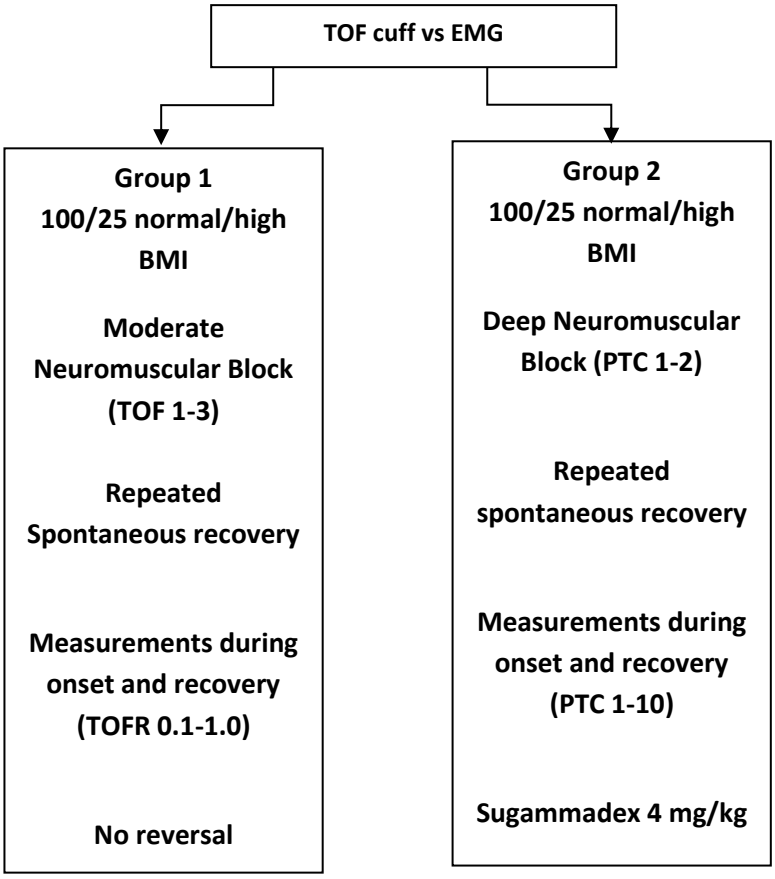

Fig 1. Study outline

4. Study Population

4.1 Patients and patient requirements/procedures

A total of 200 patients (150 BMI < 30 kg/m^2 and 50 BMI >35 kg/m^2) scheduled for general anesthesia with the use of muscle relaxants will be included in this study.

The protocol was amended (19-11-2018) to include 50 extra patients to investigate relative bias between ipsi- and contralateral comparisons between TOF Cuff and EMG in patients with normal BMI. Total number of included patients will be 250. The preliminary analysis after 100 patients showed that a significant difference occurs in the comparison between the TOF Cuff and the EMG regarding the time to full recovery of neuromuscular block. The time to complete recovery is clinically the most relevant outcome. To increase emphasis on time until full recovery, the protocol was amended (19-6-19) to include 50 patients in the LUMC which were originally scheduled for the NOK. In the NOK sugammadex is routinely administrated which disables evaluation of the time until complete recovery.

4.2 Patient inclusion criteria

(i) ASA class I-III

(ii) > 18 years of age;

(iii) Ability to give oral and written informed consent.

4.3 Patient exclusion criteria

(i) Known or suspected neuromuscular disorders impairing neuromuscular function;

(ii) Allergies to muscle relaxants, anesthetics or narcotics;

(iii) A (family) history of malignant hyperthermia;

(iv) Women who are or may be pregnant or are currently breast feeding;

(v) Renal insufficiency, as defined by a glomerular filtration rate < 30 ml/min

(vi) Scheduled for anesthesia without the use of muscle relaxants.

5. Study procedures

Pre operative procedures. Eligible patients will be informed about the study and asked to complete the informed consent on the preoperative screening visit. This will generally be one or two weeks prior to the surgery. General preoperative examination and testing will be done as usual at the preoperative outpatient department. At all time periods prior to the induction of anesthesia the patient may withdraw from the study.

Recruitment. Subjects will be recruited after approval of the study protocol by the medical ethics committee of the LUMC. Eligible subjects will be informed and recruited at the preoperative outpatient department by an anesthesiologist.

Informed consent. Patients will receive verbal and written information about the study. A written informed consent must be completed for a subject to enter the study.

Medical examination. Full medical examination and relevant blood laboratory tests will be completed at the preoperative outpatient department.

Pre-study requirements. Pre study requirements will be no different compared to patients undergoing surgery under general anesthesia. This includes refraining from eating six hours before surgery and refraining from drinking two hours before surgery.

5.1.1 Anesthesia

Standard anesthesia monitoring will be applied (blood pressure, electrocardiography, plethysmography) and will include bispectral index (BIS module, Philips, Eindhoven, The Netherlands) to assess the level of hypnosis. Intravenous access will be obtained in the antecubital fossa on the EMG arm such that it doesn't hinder EMG measurements. Central body temperature will be measured at the nasopharynx and maintained at 35.5-37.5 degrees Celsius. Peripheral (skin) temperature measured on the palms of the hands on which neuromuscular monitoring is applied will be maintained at >32 degrees Celsius. All patients will be covered by forced warm air blankets. For induction and maintenance of anesthesia, propofol combined with remifentanil and rocuronium will be used. Depth of anesthesia is aimed at a bispectral index of 50 +/- 5.

In case of a moderate neuromuscular block (GROUP 1), rocuronium 0.6 mg/kg ideal body weight (IBW) bolus will be given at induction. After that, spontaneous recovery is allowed to a TOF ratio of 1.0. After that, if time allows, a new bolus rocuronium is given to reach the target depth of NMB of TOF 1-3. No reversal agent will be given in this group unless deemed necessary by the attending anesthesiologist.

In case of a deep neuromuscular block (GROUP 2), rocuronium 1.0 mg/kg IBW will be given at induction, aimed at a PTC of 1-2. If necessary extra rocuronium is given to reach the target depth of NMB. When PTC is 1 or 2, spontaneous recovery is allowed to a PTC of 10. After that, if time allows, a new bolus rocuronium is given to reach a target depth of NMB of PTC 1-2. For reversal, 4 mg/kg IBW sugammadex will be given at a NMB depth of 1-2 PTC.

Extubation in both groups will be performed if EMG TOF ratio values are > 0.9

The attending anesthesiologist will be responsible for the anesthesia and safety of the patient at any time during the experiment. He or she may decide to discard the protocol if this is required for safety reasons. Measurements will be performed and recorded in the CRF by a separate researcher in the operating theater.
In case of suboptimal surgical conditions, extra muscle relaxants may be given at any time as needed.

5.1.2 Neuromuscular monitoring

During this study we will use two or three devices to monitor neuromuscular block. The TOF cuff and EMG will be applied on the same arm. See next paragraphs for detailed explanation.

Application of the TOF cuff

The TOF cuff (*RGB medical devices, Spain*) blood pressure cuff will be applied on a upper arm (50 dominant and 50 non dominant arm) according to the manufacturers guidelines.

The electrodes in the TOF cuff blood pressure cuff are placed on the medial aspect of the upper arm in the arm nerve pathway. See fig 3.

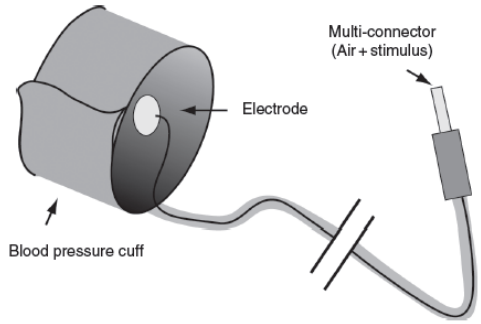

Fig 3. TOF cuff (from rodiera et al (6))

Application of electromyography

A Neuromuscular Transmission module (E-EMT-01) with the Carescape B450 monitor (both *GE Healthcare, Finland)* will be used for EMG measurements. Electrodes will be applied after degreasing the skin with alcohol and shaved if necessary to reduce impedance. Studies have demonstrated that EMG TOF ratio measured at the first dorsal interosseus muscle is equivalent to MMG during the late phase of recovery (TOF ratio > 0.70). Hence, EMG at the first dorsal interosseus is an alternative gold standard for detecting residual neuromuscular block in clinical settings. Additionally, EMG yields more consistent responses because it is not affected by restriction of movement of the muscle and is devoid of the staircase phenomenon.The stimulating negative electrode (brown) will be positioned 1 cm proximal to the wrist skin crease over the ulnar nerve and the positive electrode (white) 3 to 5 cm proximal. The black earth electrode will be placed at the proximal wrist crease. The green sensing electrode will over the FDI muscle and the red electrode at the insertion of the muscle (see Fig 3. (9)) .

In the 50 additional patients where the AMG is not applicated, the EMG will be applicated on both arms following the procedure stated above.

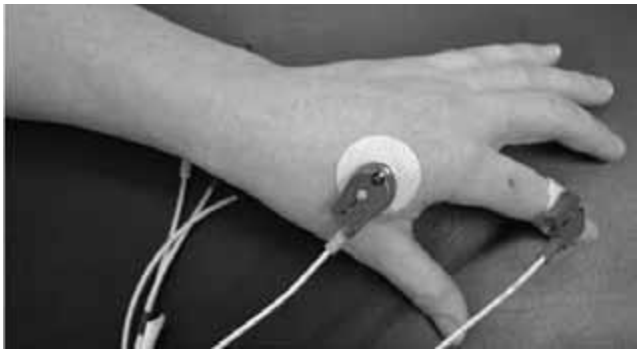

Fig 3. Application of the EMG on the first dorsal interosseus (from Phillips et al.(9))

Calibration

After induction, but before administration of the muscle relaxant rocuronium, all neuromuscular monitoring devices will be calibrated according to the manufacturers guidelines and good clinical research practice guidelines in pharmacodynamic studies of neuromuscular blocking agents to ensure supra maximal stimulation.(3) Supra maximal stimulation is the maximal stimulation current (*eg.* a greater stimulus does not result in a greater response) with an added 20%. All devices have an inbuilt calibration program to detect the maximal stimulation current. Pulse width in all devices is set at 200 microseconds (default). After calibration, 3 consecutive measurements will be performed to ensure precise measurements. In case of >5% difference between measurements, the device will be recalibrated.

5.2 Withdrawal of individual subjects

Subjects can decide to leave the study at any time, for any reason if they wish to do so, without any consequences. The responsible investigator can also decide to exclude a subject if by continuing participation the subjects' wellbeing is harmed in any way. Subjects can also be excluded in case of protocol violations and noncompliance.

In case of dropping out from the study at the subject's own request, the subject is asked permission for using the data already collected. The subject is allowed to decline this request, without giving any reason, and again without any consequences. When permission is not granted to use already available data, this specific data is deleted from the database and any paperwork will be disposed of.

In case of withdrawal the subject will be replaced by another patient.

6. STUDY MEASUREMENTS

General

- Patient characteristics including age, gender, weight, height, BMI, ASA class, underlying disease, medication, comorbidity.
- Duration of surgery and anesthesia;
- Drug dosages and administration times (propofol, opioid, muscle relaxant, reversal agent, other agents used during anesthesia);
- Temperature (central and periferal)
- Time of extubation

Neuromuscular data

Group 1

- Onset:
    - T1 90% depression time will be recorded after administration of rocuronium for all devices
- Recovery:
    - Time to reappearance of T1, T2, T3 and T4
    - Time to T1=25%
    - Time to TOF ratio 0.9
    - Continuous comparisons between the devices at 30 seconds intervals (as outlined before) until TOF ratio 1.0

Group 2

- Onset:
    - T1 90% depression time will be recorded after administration of rocuronium for all devices
- Recovery:
    - Continuous comparisons between the devices at 5 minutes intervals (as outlined before) between PTC 1-10
    - Continuous comparisons between the devices at 30 seconds intervals (as outlined before) until TOF ratio 1.0 after reversal.

7. Statistics

Recovery of neuromuscular block will be compared by the Bland–Altman analysis for repeated measurements for TOF cuff vs. EMG (See Olofsen et al.(10)). This analysis allows estimation of agreement between the devices by estimating bias (*D*) and the 95% limits of agreement (*L*). Small bias and narrow limits of agreement indicate strong agreement. Additionally, this analysis accounts for the effect of instrumental imprecision by evaluating the repeatability coefficient of each device. A small repeatability coefficient indicates high precision of the device. The limits of agreement capture 95% of differences between the compared devices. We defined clinically acceptable agreement as a bias <0.025 and limits of agreement within −0.050 to 0.050.

The protocol was amended (19-11-2018) to include 50 extra patients to investigate relative bias between ipsi- and contralateral comparisons between TOF Cuff and EMG in patients with normal BMI. Bias of ipsi- and contralateral comparisons of TOF Cuff vs. EMG will be compared. A relative bias of <5% is defined acceptable.

Regarding precision, a one-way analysis of variance will be performed on the sets of consecutive TOF ratios measured by the same device during steady-state NMB maintenance conditions. The 95% repeatability coefficient (r) is defined as 1.96 times the standard deviation of the differences between 2 consecutive measurements.

Additionally we will construct Receiver Operating Curves (ROC) to assess the specificity and sensitivity of the TOF cuff versus EMG (gold standard) at various levels of NMB depth.

7.1 Sample size

Previous results of Rodiera et al. yielded a bias (*D*) of -0.02 and limits of agreement (*L*) of -0.035 to -0.012 for the comparison of TOF cuff vs EMG at a TOF ratio of 0.7 and *D* 0.05 and *L* 0.04 – 0.06 at TOF 0.1.(6) We are not informed about *D* and *L* during deeper levels of neuromuscular block, and at TOF ratio 0.9 which is a clinically important level for safe extubation of a patient. Furthermore, precision was not assessed in this study and no comparisons were made with AMG and EMG.
We will therefore perform a pilot study with 200 patients (100 patients with normal BMI (< 30 kg/m^2) and 100 patients with morbid obesity (>30 kg/m^2)) to ensure that reliable estimates of repeatability coefficient (*r*), bias (*D*), and limits of agreement (*L*) are obtained. After 100 patients (50 non-obese/50 obese) we will perform an interim analysis and calculate the remaining number of patients needed to complete the study.
The protocol was amended (19-11-2018) to include 50 extra patients to investigate relative bias between ipsi- and contralateral comparisons between TOF Cuff and EMG in patients with normal BMI. The total number of included patients will be 250. A sub analysis of the first 20 patients shows that it takes 30 (SD 18.0) minutes until spontaneous recovery to a Tof Ratio of 0.9 is achieved. The EMG group required 47 minutes. To exclude possible outliers in our previous study we expect a mean time to a Ratio 0.9 of 42 minutes. With an α of 0.05 and β of 0.9 we require 24 patients. In the same sub analysis we saw that approximately half of the patients require additional muscle relaxants,

meaning that complete recovery is not achieved in half of the cases. In order to acquire a proper sample size to compare recovery to a Tof Ratio of 0.9 we will need a total of 50 patients to assess neuromuscular recovery on both ipsi- and contralateral extremities with the Tof Cuff.

7.2 Handling and storage of data and documents

All patients will be addressed to a random patient identification code. Patient identifying data will be omitted. The codebook will be stored digitally and will be safeguarded by the investigator. The digital form will be encrypted. Other involved parties (monitor, DSMB, ethical reviewing committee, Inspectie, Gezondheidszorg en Jeugd) could be granted access to patient data, also patient identifying data, to review if the research is being executed safely. These involved parties will handle the patient identifying data in a confidential manner. The sponsor, local researchers and project leader are responsible for data processing. When a subject withdraws consent, data collected until that moment will be used. All data will be stored for the length of the study and for 15 years afterwards, for further publication. All handling of personal data will comply with the Dutch Personal Data Protection Act.

The Functionaris Gegevensbescherming from the LUMC has been informed about the data handling in the ToCuff-trial. When subjects have questions or complaints about data handling they can contact the Functionaris Gegevensbescherming (contact information is mentioned in the patient information letter).

Only data needed to assess primary- and secondary objectives will be collected (see paragraph 8).

8. SAFETY

8.1 Adverse Events

An adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product, including placebo, and which does not necessarily have to have a causal relationship with treatment. An AE can be:

- Any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of study medication, whether or not considered related to the study medication.
- Any new disease or exacerbation of an existing disease.
- Any deterioration in protocol-required or non-protocol-required measurements of laboratory value or other clinical tests (e.g. ECG or X-ray) that results in symptoms, a change in treatment, or discontinuation from study medication.

- Recurrence of an intermittent medical condition (e.g. headache) not present at baseline.

Subjects with AEs that are ongoing at the subject's completion/discontinuation visit (last treatment visit) will be followed up for *7* days and the follow up information will be recorded in the CRF. New AEs that are reported in the *7* days following the subject's completion/ discontinuation visit will be recorded in the AE section of the CRF. Any AE that is still ongoing 7 days after the completion/discontinuation visit will have an end date of 'ongoing' in the CRF, however the investigator will continue to follow up ongoing AEs and record information in the source documents. SAEs will be followed until the event resolves or the event or sequels stabilize and this information will be reported to the Sponsor using the SAE Data Form.

8.2 Reporting of Adverse Events

For subjects who receive study medication, all AEs (learned through spontaneous reports, subject interview) starting from providing informed consent for study participation through the period beyond study completion will be collected on the AE pages of the CRF. In addition, a note should be made in the source documentation of the subject.

For each AE on the AE pages of CRF, the following information will be recorded: AE (e.g. headache), start date, start time, stop time, severity, study medication action taken, other action taken, relationship to study medication, outcome, seriousness. A cluster of signs and symptoms that results from a single cause should be reported as a single AE (e.g. fever, elevated WBC, cough, abnormal chest x-ray, etc. can all be reported as "pneumonia.").

8.3 Criteria for Assessing Severity

The Investigator will evaluate the comments of the subject and the response to treatment in order that he/she may judge the true nature and severity of the AE. Severity refers to the accumulated intensity of discomfort/impairment of health since the last recording of AEs and will be assessed according to the following criteria:

Mild: Awareness of sign, symptom, or event, but easily tolerated.

Moderate: Discomfort enough to cause interference with usual activity and may warrant intervention.

Severe: Incapacitating with inability to do usual activities or significantly affects clinical status and warrants intervention.

The criteria for assessing severity are different to those used for seriousness (see below for the

definition of an SAE).

8.4 Criteria for Assessing Causality

The question of the relationship of an AE to study medication should be determined by the Investigator after thorough consideration of all facts that are available. Assessment of causality is based on considering associative connections (time or place), pharmacological explanations, previous knowledge of the drug, presence of characteristic clinical or pathological phenomena, exclusion of other causes, and/or absence of alternative explanations. The causal relationship of an AE to study medication will be assessed according to the following criteria (based on World Health Organisation definitions):

Not related: Temporal relationship to study medication administration is missing or implausible, or there is an evident other cause.

Related

Unlikely to be related: Temporal relationship to study medication administration makes a causal relationship improbable; and other drugs, chemicals, or underlying disease provide plausible explanations.

Possibly related: Reasonable time sequence to administration of study drug, but event could also be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal may be lacking or unclear.

Probably related: Reasonable time sequence to administration of study drug, but unlikely to be attributed to concurrent disease or other drugs or chemicals, and which follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required.

Definitely related: Plausible time relationship to study medication administration; event cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (dechallenge) should be clinically plausible. The event must be definitive pharmacologically or phenomenologically, using a satisfactory rechallenge procedure if necessary.

8.5 Serious Adverse Events

A serious adverse event (SAE) is any untoward medical occurrence that at any dose:

- Results in death;
- Is life-threatening (i.e. the subject was at immediate risk of death from the AE as it occurred. This does not include an event that, had it occurred in a more severe form or

was allowed to continue, might have caused death);

- Requires inpatient hospitalisation or prolongation of existing hospitalisation;
- Results in persistent or significant disability/incapacity;
- Is a congenital anomaly/birth defect (in the child of a subject who was exposed to the study medication);
- Is a medically important event or reaction (see below).

Other important medical events that may not be immediately life-threatening or result in death or hospitalisation but may, based on appropriate medical judgment, jeopardize the subject or require intervention to prevent one of the outcomes in the definition of SAE listed above should also be considered SAEs. Examples of such events are intensive treatments in an emergency room or at home for allergic bronchospasm, blood dyscrasias or seizures that do not result in hospitalisation, or development of drug dependency or drug abuse. These events may be considered to need rapid reporting by the Sponsor to competent authorities.
All SAEs, including those occurring up to 7 days following the subject's completion/ discontinuation visit (subject's last treatment visit), will be recorded on the AE pages of the CRF. In addition, SAEs must be reported to the Sponsor using the SAE Data Form. Subjects with SAEs must be followed until the event resolves or the event or sequels stabilize.

8.6 Reporting of SAEs

All SAEs must be reported to the Sponsor within one business day of first knowledge of the SAE using the SAE Data Form. In the initial report, all of the information requested that is available should be provided. The SAE Data Form must be signed by the Investigator prior to submission to the Sponsor. Follow-up information, or new information available after the initial report, should be actively sought and reported to the Sponsor as it becomes available using the SAE Data Form. SAE Data Forms must contain the following information, at a minimum: the reportable event, the study medication (if known), the protocol number, the subject number, and the Investigator name.

8.7 Expedited Reporting

Adverse Reaction. Any untoward and unintended responses to an investigational medicinal product related to any dose administered. All AEs judged by either the reporting Investigator or the Sponsor as having a reasonable causal relationship to a medicinal product qualify as adverse reactions. The expression "reasonable causal relationship" means to convey in

general that there is evidence or argument to suggest a causal relationship (i.e. causality is at least “unlikely”).

Unexpected Adverse Reaction. An adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g. Investigator' Brochure for an unregistered investigational product or summary of product characteristics for a registered product). When the outcome of the adverse reaction is not consistent with the applicable product information this adverse reaction should be considered as unexpected.

Suspected Unexpected Serious Adverse Reaction (SUSAR). A SUSAR is an adverse drug reaction, which is both serious and unexpected. If an SAE was assessed to be a SUSAR by the Sponsor, the Competent Authorities, ECs and Investigators must be informed as soon as possible but no later than 15 calendar days after the sponsor has first knowledge of the minimum criteria for expedited reporting. If the SUSAR was immediately life-threatening or fatal, it must be reported as soon as possible but no later than 7 calendar days after the sponsor has first knowledge of the minimum criteria for expedited reporting. Relevant follow-up information should be communicated to the competent authority and the Ethics Committee within an additional 8 calendar days. The Sponsor is responsible for expedited reporting of SUSARs and other reportable events and safety issues to the Competent Authorities, according to local legislations. Investigators will be informed by the Sponsor. The Investigator or Sponsor, depending on local regulations, must inform the EC/IRB about SUSARs and other reportable events and safety issues in accordance with ICH guidelines and the practices of the governing ECs.

8.8 Data Safety Committee (DSC)

A data safety committee will assess the data collected in the CRF at regular time intervals and in case of occurrence of serious adverse events (SAE). In case a SAE occurs, the DSC will advise the research team on the measures that are required to enable a successful completion of the study. The independent physician is part of the DSC.

The primary concern of the DMC is patient safety. If AEs or SAEs do occur they will advise the Investigator on the continuation of the study. Reporting to the DMC is independent of the reporting to sponsor, METC and CCMO.

9. ETHICS

Ethics Committees. The protocol, possible protocol amendments, the patient information form, informed consent form and any other study related information or documents will be reviewed and approved by the LUMC ethics committee (METC) before subjects are screened for study entry. The Investigator will submit periodic reports and inform the METC of any reportable adverse events (AEs) per ICH guidelines and local EC standards of practice.

Ethical Conduct of the Study. This study will be conducted in accordance with LUMC standard operating practices, which are designed to ensure adherence to Good Clinical Practice (GCP) guidelines as required by the following:

- Declaration of Helsinki, 1964 ("Recommendations Guiding Physicians in Biomedical Research Involving Human Patients"), and all its accepted amendments to date concerning medical research in humans.
- ICH Guideline for GCP (CPMP/ICH/135/95) of the European Agency for the Evaluation of Medicinal Products, Committee for Proprietary Medicinal Products, International Conference on Harmonization of Pharmaceuticals for Human Use. (Note for Guidance on Good Clinical Practice, 2002).
- European Union (EU) Clinical Trials Directive 2001/20/EC on the regulation of clinical trials in the EU and the implementation of GCP.
- GCP Directive 2005/28/EC.

This study will be conducted in accordance with national and local laws.

Subject Information and Consent. All subjects will be provided with oral and written information describing the nature and duration of the study and the procedures to be performed. Each subject will be given a copy of the informed consent and written information. The subject will be asked to sign an informed consent form prior to any study specific procedures being performed.

Benefits and risks assessment.

Patients will not have any benefit nor have a higher risk for adverse events when participating in this study. All devices used are noninvasive and pose no harm for the patient. We therefore content that there is no additional risk or burden for the patient.

Investigators and study personnel. Qualified Investigators under the sponsorship of the Leiden University Medical Center will conduct this study. The names of the investigators, independent physician and contact person are given on page 1 of this protocol. The Principle Investigator is GCP/GRP certified by the LUMC Boerhaave Committee.

11. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

11.1 Approval and registration

This study will be done in patients with normal and extremes of BMI. Patients with normal BMI (<30kg/m2) will be enrolled in the LUMC. Patients of high BMI (>30 kg/m2) will be enrolled in the Nederlandse Obesitas Kliniek (NOK), the Hague, which is situated at the Haaglanden MC (HMC). All study procedures will be the same in both hospitals. The protocol will have to be approved by local authorities as required before initiation.

The study will be registered at clinicaltrials.gov

11.2 Handling and storage of data and documents

Data will be recorded in the case report form (CRF). Patient hospital ID, birth day and gender information will be recorded as well as coexisting disease and medication use. All data will be handled confidentially.

11.3 Amendments

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favourable opinion.

11.4 End of study report

Estimated study duration is 20 weeks (planned inclusion 5 patients/week in the LUMC and 5 in the Nederlandse Obesitas kliniek)

The investigator will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient's surgery.

In case the study is ended prematurely, the investigator will notify the accredited METC within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

11.5 Public disclosure and publication policy

We plan to publish the study in either an anesthesia-related journal (Anesthesiology)

12. References

1. Brull SJ, Murphy GS. Residual neuromuscular block: lessons unlearned. Part II: methods to reduce the risk of residual weakness. Anesth Analg. 2010;111(1):129-40.
2. Murphy GS, Brull SJ. Residual neuromuscular block: lessons unlearned. Part I: definitions, incidence, and adverse physiologic effects of residual neuromuscular block. Anesth Analg. 2010;111(1):120-8.
3. Fuchs-Buder T, Claudius C, Skovgaard LT, Eriksson LI, Mirakhur RK, Viby-Mogensen J, et al. Good clinical research practice in pharmacodynamic studies of neuromuscular blocking agents II: the Stockholm revision. Acta Anaesthesiol Scand. 2007;51(7):789-808.
4. Claudius C, Viby-Mogensen J. Acceleromyography for use in scientific and clinical practice: a systematic review of the evidence. Anesthesiology. 2008;108(6):1117-40.
5. Larsen PB, Gatke MR, Fredensborg BB, Berg H, Engbaek J, Viby-Mogensen J. Acceleromyography of the orbicularis oculi muscle II: comparing the orbicularis oculi and adductor pollicis muscles. Acta anaesthesiologica Scandinavica. 2002;46(9):1131-6.
6. Rodiera J, Serradell A, Alvarez-Gomez JA, Aliaga L. The cuff method: a pilot study of a new method of monitoring neuromuscular function. Acta Anaesthesiol Scand. 2005;49(10):1552-8.
7. Boon M, Martini C, Broens S, van Rijnsoever E, van der Zwan T, Aarts L, et al. Improved postoperative oxygenation after antagonism of moderate neuromuscular block with sugammadex versus neostigmine after extubation in 'blinded' conditions. Br J Anaesth. 2016;117(3):410-1.
8. Boon M, Martini C, Hellinga M, Bevers R, Aarts L, Dahan A. Influence of variations in arterial PCO2 on surgical conditions during laparoscopic retroperitoneal surgery. Br J Anaesth. 2016;117(1):59-65.
9. Phillips S, Stewart PA, Freelander N, Heller G. Comparison of evoked electromyography in three muscles of the hand during recovery from non-depolarising neuromuscular blockade. Anaesth Intensive Care. 2012;40(4):690-6.
10. Olofsen E, Dahan A, Borsboom G, Drummond G. Improvements in the application and reporting of advanced Bland-Altman methods of comparison. J Clin Monit Comput. 2015;29(1):127-39.