MYOTONIA, MUSCLE STIFFNESS AND ELASTICITY IN NEUROMUSCULAR DISORDERS

Version: 1 [01-JUL-2019]

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This protocol was designed and will be conducted, recorded, and reported in accordance with the principles of Good Clinical Practice (GCP) as stated in the International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use and any applicable national and regional laws.

I have read and agree to abide by the requirements of this protocol.

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1 SYNOPSIS/SUMMARY

TITLE: **Myotonia, muscle stiffness and elasticity in neuromuscular disorders**
PROTOCOL NO.: Myotonia and Stiffness in NMD V1 [01. July 2019]

INVESTIGATOR STUDY SITE: Friedrich-Baur-Institute, Department of Neurology, Klinikum der Universität München. Ziemssenstr. 1, 80336 Munich, Germany

OBJECTIVES: The primary objective of this study is to assess stiffness, muscle tone, relaxation periods and elasticity of various muscles in patients with dystrophic or non-dystrophic myotonia. The secondary objectives are (1) to provide reference values for stiffness, muscle tone, relaxation periods and elasticity of various muscles in patients with dystrophic or non-dystrophic myotonia; to provide reference values for stiffness, muscle tone, relaxation periods and elasticity of various muscles in patients with non-myotonic neuromuscular disorders, (3) assess correlations between to compare result values for stiffness, muscle tone, relaxation periods and elasticity with clinical muscle function tests, measured by clinical evaluation (MRC-scale) and the 6-minute walk test; (4) assess correlations between subcutaneous fat and muscle thickness and echogenicity, measured by muscle ultrasound and result values for stiffness, muscle tone, relaxation periods and elasticity.

METHODOLOGY: This is a monocentric, blinded, cross-sectional explorative study in patients with neuromuscular diseases.

NUMBER OF PATIENTS: Approximately 70 patients will be enrolled, about 5 each in the following disease groups: Limb-Girdle-Muscular Dystrophy (LGMD), Myotonic Dystrophies Type 1 and 2 (DM1, DM2), Late-Onset-Pompe Disease (LOPD), Distal Myopathies, Congenital Myotonia, Spinal muscular atrophy Type 3 (SMA3), Amyotrophic lateral sclerosis (ALS) and peripheral Polyneuropathies (CIDP, HMSN). Approximately 20 healthy age- and gender-matched patients will be enrolled as a control group.

INCLUSION/EXCLUSION CRITERIA:

Inclusion Criteria: A patient must meet all of the following criteria to be eligible for this study. For patients, (1) the participant is ≥18 years of age and has a confirmed neuromuscular disease, (2) provides written consent and (3) is able and willing to perform study procedures. For healthy volunteers, age ≥18 and written informed consent is obligatory and there is no clinical sign for any neuromuscular disorder.

Exclusion Criteria: If one of the following criteria is met, a patient will not be able to participate in this study: (1) The patient has severe comorbidities; (2) the patient is participating in another clinical study using investigational treatment; (3) the patient cannot perform required muscle function tests; (4) the patient, in the opinion of the Investigator, is unable to adhere to the requirements of the study.

REGIMEN/STUDY PROCEDURES:

After obtained informed consent, the following procedures will be performed:

- at screening, patient's eligibility will be defined.

- at visit 1 (same day as Screening), the following procedures will be performed: (1) Collection of demographic and disease-related data (age, gender, diagnosis, age at onset of symptoms, age at diagnosis, severity and location of myotonia and severity and location of muscle stiffness, for both using numeric rating scale (NRS)). (2) Performing a 6-Minute-Walk-Test (meters). (3) Clinical evaluation of muscle strength of the following muscles, using the MRC-Scale (0-5): on both sides thenar and hypothenar, m. biceps brachii, m. triceps brachii, m. deltoideus, m. quadriceps femoris, m. tibialis anterior and gastrocnemius muscles. (4) Semiquantitative measurement of cutis and subcutis, subcutaneous fat and muscle thickness by muscle ultrasound of the following muscles: on both sides thenar and hypothenar, m. biceps brachii, m. triceps brachii, m. deltoideus, m. quadriceps femoris, m. tibialis anterior and gastrocnemius muscles. Measurements will contain thickness of cutis in millimeters, the thickness of subcutaneous fat in millimeters, muscle thickness in millimeters at the point of measurement of the MyotonPro® and semiquantitative measurement of muscle echogenicity, using Heckmatt scale I-IV. (5) Measurement of relaxation time, stiffness and elasticity of the following muscles, using the MyotonPro® device: on both sides thenar and hypothenar, m. biceps brachii, m. triceps brachii, m. deltoideus, m. quadriceps femoris, m. tibialis anterior and gastrocnemius muscles. For the documentation of the results, please see section "MyotonPro® device".
- at Visit 2 (+1-7 days after day 1), a measurement of relaxation time, stiffness and elasticity of the following muscles, using the MyotonPro® device: on both sides thenar and hypothenar, m. biceps brachii, m. triceps brachii, m. deltoideus, m. quadriceps femoris, m. tibialis anterior and gastrocnemius muscles will be performed for reliability purposes. For the documentation of the results, please see section "MyotonPro® device".

STATISTICAL METHODS:

All patients who participate in this study will be included in the analysis. Data collected in this study will be reported using summary tables, figures and patient data listings. Summary statistics (n, mean, median, standard deviation, minimum and maximum) will be calculated for the continuous variables and shift tables and/or frequencies, and percentages will be produced for the categorical variables. Due to the exploratory nature of this study, no formal inferential statistical tests will be performed.

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3 RESPONSIBILITIES

The scientific experts responsible for this study are Dr. S. Wenninger (PI) and Prof. B. Schoser (Backup PI). This is a non-funded study. All costs are covered by internal funds of the Friedrich-Baur-Institute.

4 BACKGROUND

In neuromuscular diseases, muscular involvement is extremely heterogeneous. The high variability is not only present in various neuromuscular diseases, but also within one type of disease itself. Since phenotypes vary among affected patients, a long delay between the onset of symptoms and diagnosis is common in neuromuscular disorders [1]. In myotonic dystrophies, specific muscles are affected by myotonia and stiffness, whereas other muscles or muscle groups may not or only be slightly affected or may show signs of weakness and atrophy [2]. In non-dystrophic myotonia like congenital myotonia Becker and Thomson, a generalized muscles stiffness is present in almost all patients at younger ages. For dystrophic and non-dystrophic myotonia, hallmarks are a delayed relaxation and stiffness of affected muscles after voluntary contraction [3, 4], but phenotypes show high variability. Non-invasively, myotonia and muscle stiffness is typically only described clinically, but not objectively measured. Semi-invasively, the severity of myotonia can be assessed by electromyography, requiring a high logistic and technical effort including sophisticated computerized measurements, which makes implementation in clinical practice almost impossible. In several Limb-girdle-muscular dystrophies (LGMD), muscles are selectively affected by fatty infiltration and atrophy. This becomes most evident in autosomal recessive LGMD2L, where the medial gastrocnemius muscles are selectively affected by fatty infiltration and atrophy at early stages of the disease, while proximal muscles are more likely to cause myalgia and reduced muscular endurance, but do not show signs of fatty infiltration [5]. In Late-Onset Pompe disease (LOPD), abdominal and paraspinal muscles are early and primarily affected [6-8]. In contrast, affection of the second motoneuron (Amyotrophic lateral sclerosis (ALS); spinal muscular atrophy (SMA)) as well as diseases with affection of the peripheral nervous system (chronic inflammatory demyelinating polyneuropathy (CIDP) or different forms of hereditary sensory and motor neuropathies (HMSN)) cause muscular atrophy without signs fatty infiltration, but are associated with muscular cramps in the majority of cases [9-12]. For assessing fatty infiltration of muscles or muscular atrophy, magnetic resonance imaging (MRI) is increasingly used as a complement to clinical and electrophysiological examination in neuromuscular disease, but costs and time are limitations [13]. Muscle ultrasound has proven to have a high sensitivity and specificity for characterizing and detecting neuromuscular disorders but is always complementary to clinical and electrophysiological findings [14]. It may be useful to detect and describe muscular movements like fasciculations and myokymia, but a correlation of myotonia or muscle stiffness with clinical disease severity is poor [15, 16].

To assess myotonia and muscle stiffness in neuromuscular disorders, we use a handheld device that allows non-invasive measurement of relaxation time, stiffness and elasticity. For this, the MyotonPro®, a commercially available device, is used to evaluate the stiffness, myotonia and elasticity of muscle fibers by applying short pulses to the underlying tissue [17]. Its validity and reliability were proven in a large cohort

of healthy volunteers as well as patients with different types of muscle diseases [17-22]. A brief pulse of a few seconds causes the underlying tissue to oscillate and send back a signal that is picked up and processed by the device. This allows to evaluate relaxation time, stiffness and elasticity of the tissue and may help to assess the severity of myotonia and stiffness in neuromuscular patients. The following measurements will be performed automatically, analyzing the oscillation curves and calculating the tissue tone (frequency (Hz)), the tissue stiffness (N/m), the decrement as parameter for the elastic stiffness of the tissue and viscoelastic parameters like relaxation time (in milliseconds) and creep as non-elastic tissue strain. The device has recently been used in patients with altered muscle elasticity and stiffness like Parkinson's disease, stroke and Duchenne muscular dystrophy [19-21, 23-25] as well as assessing muscular atrophy and viscoelasticity due to immobility in healthy subjects [22].

So far, the knowledge of muscle elasticity, myotonia and stiffness in various neuromuscular diseases is scarce. The study aims to test elasticity, myotonia and stiffness in various neuromuscular diseases and to provide reference values for different neuromuscular disease groups.

5 Study Objectives

5.1 Primary Objective

The primary objective of this study is to assess stiffness, muscle tone, relaxation periods and elasticity of various muscles in patients with dystrophic or non-dystrophic myotonia.

5.2 Secondary Objectives

The secondary objectives of this study are

- to provide reference values for stiffness, muscle tone, relaxation periods and elasticity of various muscles in patients with dystrophic or non-dystrophic myotonia,
- to provide reference values for stiffness, muscle tone, relaxation periods and elasticity of various muscles in patients with non-myotonic neuromuscular disorders,
- assess correlations between to compare result values for stiffness, muscle tone, relaxation periods and elasticity with clinical muscle function tests, measured by clinical evaluation (MRC-scale) and the 6-minute walk test,
- assess correlations between subcutaneous fat and muscle thickness and echogenicity, measured by muscle ultrasound and result values for stiffness, muscle tone, relaxation periods and elasticity

6. Study Population

6.1 Inclusion Criteria

A patient must meet all the following criteria to be eligible for this study. For patients, (1) the participant is ≥18 years of age and has a confirmed neuromuscular disease, (2) provides written consent and (3) is able and willing to perform study procedures. For healthy volunteers, age ≥18 and written informed consent is obligatory and

there is no clinical sign for any neuromuscular disorder.

6.2 Exclusion Criteria

If one of the following criteria is met, a patient will not be able to participate in this study: (1) The patient has severe comorbidities; (2) the patient is participating in another clinical study using investigational treatment; (3) the patient cannot perform required muscle function tests; (4) the patient, in the opinion of the investigator, is unable to adhere to the requirements of the study.

6.3 Number of patients

Approximately 70 patients will be enrolled, about 3-5 each in the following disease groups: Limb-Girdle-Muscular Dystrophy (LGMD), Myotonic Dystrophies Type 1 and 2 (DM1, DM2), Late-Onset-Pompe Disease (LOPD), Distal Myopathies, Congenital Myotonia, Spinal muscular atrophy Type 3 (SMA3), Amyotrophic lateral sclerosis (ALS) and peripheral Polyneuropathies (CIDP, HMSN). 20 healthy age- and gender-matched patients will be enrolled as a control group.

6.4 Recruitment

Patients will be asked to participate during their routine treatment at the in-patient clinic of the Friedrich-Baur-Institute or will be asked during appointments at the out-patient-clinic of the Friedrich-Baur-Institute. Healthy volunteers (e.g. relatives from patients) will be asked to participate during routine appointments of their relatives.

7 Investigational Plan

7.1 Study Design

This is a cross-sectional, blinded, monocentric, explorative study to evaluate the stiffness, muscle tone, relaxation periods and elasticity of various muscles in patients with neuromuscular diseases. Local ethic´s approval is obligatory before the recruitment of the first patient. The patient must provide informed consent prior to performing any protocol-related procedure at the screening visit. The estimated duration of this study is 12 months or as soon as a total of 50 patients with neuromuscular diseases and 20 healthy volunteers are evaluated. The person assessing the muscle stiffness and myotonia, using the MyotonPro® device, will be blinded to the diagnosis, to medical history as well as results from the clinical tests (clinical muscle strength, 6-minute-walk-test) and results of muscle ultrasound assessments. The study duration for one patient or one healthy volunteer is two to a maximum of seven days. All relevant data will be assessed within visit 1. At the second visit (visit 2), only measurements of the MyotonPro® will be repeated to evaluate the reliability of the measurements.

Both patients and healthy volunteers will perform the following assessments:

7.1.1 Screening

At screening, the patient´s eligibility will be defined.

7.1.2 Visit 1 (same day as Screening)

- Collection of demographic and disease-related data (age, gender, diagnosis, age at onset of symptoms, age at diagnosis, severity and location of myotonia and severity and location of muscle stiffness, for both using numeric rating scale (NRS)).
- Performing a 6-Minute-Walk-Test (meters).
- Clinical evaluation of muscle strength of the following muscles, using the MRC-Scale (0-5): on both sides thenar and hypothenar, m. biceps brachii, m. triceps brachii, m. deltoideus, m. quadriceps femoris, m. tibialis anterior and gastrocnemius muscles.
- Semiquantitative measurement of cutis and subcutis, subcutaneous fat and muscle thickness by muscle ultrasound of the following muscles: on both sides thenar and hypothenar, m. biceps brachii, m. triceps brachii, m. deltoideus, m. quadriceps femoris, m. tibialis anterior and gastrocnemius muscles. Measurements will contain the thickness of cutis in millimeters, the thickness of subcutaneous fat in millimeters, the muscle thickness in millimeters at the point of measurement of the MyotonPro® and a semiquantitative measurement of muscle echogenicity, using the Heckmatt scale I-IV.
- Measurement of relaxation time, stiffness and elasticity of the following muscles, using the MyotonPro® device: on both sides thenar and hypothenar, m. biceps brachii, m. triceps brachii, m. deltoideus, m. quadriceps femoris, m. tibialis anterior and gastrocnemius muscles. For the documentation of the results, please see section "MyotonPro® device".

7.1.3 Visit 2 (+1-7 days after day 1)

- Measurement of relaxation time, stiffness and elasticity of the following muscles, using the MyotonPro® device: on both sides thenar and hypothenar, m. biceps brachii, m. triceps brachii, m. deltoideus, m. quadriceps femoris, m. tibialis anterior and gastrocnemius muscles. For the documentation of the results, please see section "MyotonPro® device".

The total duration of assessments performed on screening and visit 1 will be approximately 2 hours. The total duration of visit 2 will be about 30 minutes.

The data collected will be stored in a local database, that is password protected and only read- and editable for persons directly involved in the study.

7.1.4 MyotonPro® Device

The MyotonPro® Device is a commercially available electronic device for non-invasive measurement of muscle stiffness, muscle tone, relaxation periods and viscoelasticity. The method of measurement consists of recording damped natural oscillation of soft biological tissue in the form of an acceleration signal and the subsequent

simultaneous computation of the parameters of State of Tension, Biomechanical and Viscoelastic properties. A damped natural oscillation is induced by an exterior, low force quick-release mechanical impulse under constant pre-load. The tip of the myometer will be placed on the tissue perpendicular to the underlying muscle. The device gets activated by a slight pressure on the tip which exerts a local impact on the tissue utilizing a brief mechanical impulse. This causes a minor deformation of the underlying muscle tissue. After the impact, the tip is quickly released, and the damped oscillatory behaviour of the tissue will be recorded by an accelerometer in the device. The viscoelastic stiffness of the underlying tissue will automatically be calculated using the following equations (S=stiffness; D=elasticity; R=relaxation):

$$S = \frac{a_{max} \cdot m_{probe}}{\Delta l} \qquad D = ln\left(\frac{a_1}{a_3}\right) \qquad R = t_R - t_1$$

S stands for stiffness, measured in N/m, and is the biomechanical property of a muscle that characterizes the resistance to a contraction or to an external force that deforms its initial shape. Elasticity **D** is the logarithmic decrement of a tissue's natural oscillation. Elasticity is the biomechanical property of a muscle that characterizes the ability to recover its initial shape after a contraction or removal of an external force of deformation. The Relaxation **R** is the time for a muscle to recover its shape from deformation. The functionality of the MyotonPro®, its way of measurement and the calculations have been tested and validated in a high number of clinical studies. Therefore, this study is not intended to validate the measurements of the MyotonPro® device itself nor its safety and accuracy of measurements, but to evaluate measurement results in patients with neuromuscular diseases and elaborate reference values.

7.1.5. Six-Minute walk test

The six-minute walk test (6MWT) measures the distance an individual is able to walk over a total of six minutes on a hard, flat surface. The goal is for the individual to walk as far as possible in six minutes. The individual is allowed to self-pace and rest as needed as they traverse back and forth along a marked walkway. The 6MWT has been used with a variety of other conditions than the chronic obstructive pulmonary disease (COPD) such as heart failure and stroke and is widely used in neuromuscular diseases [26]. The six-minute-walk-test will be conducted as recommended by the American Thoracic Society [27]. In this study, the six-minute-walk test will be performed once on visit 1 to detect the impact of muscle weakness, muscle stiffness and myotonia on muscular endurance.

7.1.6. Semiquantitative muscle ultrasound

Muscle ultrasound is an ideal imaging modality that allows for atraumatic, noninvasive, radiation-free point-of-care neuromuscular imaging [28]. Muscular dystrophies are typically associated with an increase in the echogenicity from the muscle substance, distal attenuation of muscle echo and a corresponding loss of bone echo. Spinal muscular atrophies and neuropathies also showed an increase in muscle echo along with atrophy of the muscle and increase in depth of subcutaneous tissue, but a persisting bone echo. In several other myopathies, similar changes are seen [29]. In semi-quantitative muscle ultrasound, muscle intensity will be documented using the 4-point Heckmatt score [28].

7.1.7. Hand-held Dynamometry of muscle strength

Muscle strength will be assessed by handheld dynamometry using the MicroFET2 myometer, produced by Hoggan Health Industries. This test is widely used in patients with neuromuscular diseases [30, 31]. To perform a test, the examiner holds the dynamometer stationary while the patient exerts a maximal force against the dynamometer. The patient makes a gradual increase in force and then completes an isometric hold for 4-5 seconds. The following muscle groups will be tested: Arm abduction, elbow flexion, elbow extension, knee extension, knee flexion, foot extension, foot flexion.

7.2 Completion of a Patient's Participation in the Study

7.2.1 Completion of a Patient's Participation in the Study

The length of a patient's participation will be from the time the informed consent form is signed until the last planned assessment/visit. A patient will be considered "completed" if all study procedures of the second visit have been carried out.

7.2.2 Premature Patient Discontinuation from the Study

Patients are free to withdraw consent and/or discontinue participation in the study at any time, without prejudice to further treatment. For patients who prematurely discontinue from the study, the Investigator or designee will contact the patient 4 to 7 days after withdrawal to assess any AEs. Post-study serious adverse events (SAEs) will be reported accordingly. A patient's participation in the study may also be discontinued at any time at the discretion of the Investigator. Patients who are prematurely withdrawn from the study will be asked to complete all discontinuation assessments prior to withdrawal, if possible. A patient will be considered early terminated if the patient does not complete the study after enrollment. Patients who are withdrawn from the study will not be replaced (i.e., a patient's study number will not be reused); however, additional patients may be enrolled to offset patient dropouts.

7.3 Discussion of Study Design, Including Choice of Control Group

This is a monocentric, blinded, cross-sectional explorative study designed to evaluate myotonia, muscle stiffness and elasticity in 70 patients with neuromuscular disorders. For statistical comparison, we plan to recruit 20 healthy subjects as a control group.

8 Risks and Benefits

8.1 Summary of Potential Risks

Study-specific procedures and associated risks are bulleted below:

- Functional testing (six-minute-walk-test): falls, shortness of breath, muscle soreness, myalgia and fatigue

8.2 Summary of Potential Benefits

Analyzing muscle stiffness and myotonia in an easy and non-invasive way could help to detect dystrophic

and non-dystrophic myotonia earlier on the course of the disease and support early diagnosis. Besides, treatment effects could be objectively measured and observed.

9 ADVERSE EVENTS REPORTING

Detailed listings of patients who experience AEs or SAEs will be presented. The incidence of treatment-emergent AEs and SAEs will be tabulated (frequencies and percentages) by severity, and by relationship to treatment. Adverse event severity will be categorized as mild, moderate, or severe. In tabulating severity of AEs on a per-patient basis, the greatest severity will be assigned to a patient should there be more than one occurrence of the same AE with different reported severities. Relationships of the AE to treatment will be categorized as not related, unlikely related, possibly related, or related. The highest level of the association will be reported in patients with differing relationships for the same AE. Listings of AEs and SAEs for all patients will be provided, which will include severity and relationship to the study procedures, the actions taken regarding the study procedures as well as the patient's outcome. A separate listing for patients who withdraw from the study due to AEs will be provided. The incidence of AEs leading to study discontinuations will also be summarized.

10 STATISTICAL METHODS AND PLANNED ANALYSES

10.1 General Considerations

All patients who participate in this study will be included in the analysis. Data collected in this study will be reported using summary tables, figures and patient data listings. Summary statistics (n, mean, median, standard deviation, minimum and maximum) will be calculated for the continuous variables and shift tables and/or frequencies, and percentages will be produced for the categorical variables. Due to the exploratory nature of this study, no formal inferential statistical tests will be performed.

10.2 Determination of Sample Size

50 patients and 20 healthy subjects will be enrolled in this study. No formal sample size calculations have been performed. This is an exploratory pilot-study and is not powered to make any statistical inferences.

10.3 Demographics and Baseline Characteristics

Demographic and baseline data on medical history and neuromuscular disease history as well as patient-related data will be summarized using exploratory and descriptive statistics for continuous variables and frequency distribution for categorical variables. To compare the mean values of two groups, the t-test will be used, to compare frequencies between two groups, the chi-square test. Correlations are calculated by using the Spearman and Pearson coefficient.

10.4 Patient Accountability

Data from all patients who are enrolled in the study will be included in the summary of patient accountability. The frequency and percentage of patients who are enrolled in the study, discontinued from the study, and

completed the study, along with reasons for discontinuation, will be summarized.

10.5 MyotonPro® measurement reliability

To ensure high reliability in measurements performed with the MyotonPro® device, a second measurement will be performed within 1-7 days after visit 1 (Test-Retest). The degree of similarity between the two measurements is determined by computing a correlation coefficient. In addition, 95% confidence intervals will be used to estimate the change from baseline to the second study visit. All data will be presented by study visit in by-patient listings.

10.6 Missing or Invalid Data

All data will be analyzed as they were collected in the database. It is not planned to impute missing data using statistical methods.

11 DATA MANAGEMENT AND DATA PROTECTION

The medical and study-related data (questionnaires, examination data) and the declaration of consent remain with the investigator or at the Friedrich-Baur-Institute of the LMU Munich and are kept strictly confidential. All data collected is stored and evaluated electronically in a database in a double pseudonymized form. Pseudonymized means that the patient's name will be deleted, and a number assigned to the data. Double pseudonymization means that the secret number is internally re-encoded to increase security. Only the age of patients is recorded from the personal data. No personal identifying numbers (e.g. identity card number) or addresses are used. To be on the safe side, the data is stored separately in different databases. The assignment between the name and the number is stored by the investigational doctor in a secure place, a corresponding decryption list remains in the Friedrich-Baur-Institute and is only accessible to authorized persons from the Friedrich-Baur-Institute. The decryption list remains at the Friedrich-Baur-Institute of the LMU Munich. The database is stored on a server of the University Hospital of Munich, to which only study staff of the Friedrich Baur Institute have access. Confidential and private aspects of the database are protected by special regulations. This includes double pseudonymization and the use of bug-proof connections.

All confidential documents are kept for up to 10 years after the end of the study and then destroyed. The obligation of confidentiality, data storage and storage are in accordance with the Data Protection Act Directive 95/46/EC.

12 SPECIAL REQUIREMENTS AND PROCEDURES

This protocol was designed and will be conducted, recorded, and reported in compliance with the International Conference on Harmonization (ICH)/Good Clinical Practice (GCP) guideline. These requirements are stated in the ICH Guideline Topic E6 entitled "Guideline for Good Clinical Practice".

12.1 Institutional and Ethics Review

This protocol and a patient informed consent form (ICF) must be reviewed and approved by an IRB/IEC before

enrollment of patients. Documentation of IRB/IEC and the approved consent form must be received by the Investigator or its designee prior to obtaining the patient's informed consent.

12.2 Changes to the Conduct of the Study or Protocol

Any changes in the study protocol, such as changes in the study design, objectives or endpoints, inclusion and exclusion criteria, and/or procedures (except to eliminate an immediate hazard) will be implemented only after the mutual agreement of the Investigator or designee. All protocol changes must be documented in the protocol amendment(s). Protocol amendment(s) must be signed by the Investigator and approved by the IRB/IEC prior to implementation. Any changes in study conduct that result from a pending amendment will be considered protocol deviations until IRB/IEC approval is granted. Documentation of IRB/IEC approval must be returned to the Investigator or designee.

12.3 Patient Informed Consent

Investigators must adhere to Good Clinical Practice, which includes ethical principles that have their origin in the Declaration of Helsinki, when developing the patient informed consent form and when obtaining consent from the patient. Written informed consent is required prior to enrollment in the study. It is the responsibility of the Investigator to document the consent process within the source documents and obtain consent using an IRB/IEC approved consent form.

12.4 Case Report Forms

Data will be entered by the site onto the paper CRFs (pCRF) and locally stored databases (Excel). Any erroneous entries made on the pCRFs should be corrected. Changes made to the data after initial entry into the pCRF will be unambiguously corrected and signed.

12.5 Record Retention

The Investigator is responsible for oversight and maintenance of the study records and patient source documents. The Investigator must retain study records for at least 10 years after the last marketing approval has been granted, or at least 10 years have elapsed since the formal discontinuation of the clinical program. However, these documents should be retained for a longer period, if required by other applicable requirements (e.g., applicable local regulatory requirement). Patient records or other source data must be kept for the maximum time mandated by the hospital, but not less than 10 years.

12.6 Clinical Study Report

A final clinical study report will be produced after study completion.

13 ABBREVIATIONS

Abbreviation	Term
6MWT	6-minute walk test
AE	adverse event

CRF	Case report form
GCP	Good Clinical Practice

14 LITERATURE

[1] Mary P, Servais L, Vialle R. Neuromuscular diseases: Diagnosis and management. Orthop Traumatol Surg Res 2018;104:S89-S95.

[2] Wenninger S, Montagnese F, Schoser B. Core Clinical Phenotypes in Myotonic Dystrophies. Front Neurol 2018;9.

[3] Montagnese F, Schoser B. [Dystrophic and non-dystrophic myotonias]. Fortschr Neurol Psychiatr 2018;86:575-583.

[4] Sansone VA. The Dystrophic and Nondystrophic Myotonias. Continuum (Minneap Minn) 2016;22:1889-1915.

[5] Liewluck T, Winder TL, Dimberg EL, et al. ANO5-muscular dystrophy: clinical, pathological and molecular findings. Eur J Neurol 2013;20:1383-9.

[6] Barba-Romero MA, Barrot E, Bautista-Lorite J, et al. Clinical guidelines for late-onset Pompe disease. Rev Neurol 2012;54:497-507.

[7] Carlier RY, Laforet P, Wary C, et al. Whole-body muscle MRI in 20 patients suffering from late onset Pompe disease: Involvement patterns. Neuromuscul Disord 2011;21:791-9.

[8] Schuller A, Wenninger S, Strigl-Pill N, Schoser B. Toward deconstructing the phenotype of late-onset Pompe disease. American journal of medical genetics. Part C, Seminars in medical genetics 2012;160C:80-8.

[9] Walter MC, Stauber AJ. [Spinal muscular atrophy - clinical spectrum and therapy]. Fortschr Neurol Psychiatr 2018;86:543-550.

[10] Verschueren A. Motor neuropathies and lower motor neuron syndromes. Rev Neurol (Paris) 2017;173:320-325.

[11] Ohyama K, Koike H, Katsuno M, et al. Muscle atrophy in chronic inflammatory demyelinating polyneuropathy: a computed tomography assessment. Eur J Neurol 2014;21:1002-10.

[12] Vasilescu C. Hereditary motor and sensory neuropathy. Clinical, genetic and electrodiagnostic studies. Rom J Neurol Psychiatry 1993;31:207-19.

[13] Simon NG, Noto YI, Zaidman CM. Skeletal muscle imaging in neuromuscular disease. J Clin Neurosci 2016;33:1-10.

[14] van Alfen N, Gijsbertse K, de Korte CL. How useful is muscle ultrasound in the diagnostic workup of neuromuscular diseases? Curr Opin Neurol 2018;31:568-574.

[15] Johansson MT, Ellegaard HR, Tankisi H, Fuglsang-Frederiksen A, Qerama E. Fasciculations in nerve and muscle disorders - A prospective study of muscle ultrasound compared to electromyography. Clin Neurophysiol 2017;128:2250-2257.

[16] Abraham A, Breiner A, Barnett C, Bril V, Katzberg HD. Quantitative sonographic assessment of myotonia. Muscle Nerve 2018;57:146-149.

[17] Zinder SM, Padua DA. Reliability, validity, and precision of a handheld myometer for assessing in vivo muscle stiffness. Journal of sport rehabilitation 2011;20.

[18] Bizzini M, Mannion AF. Reliability of a new, hand-held device for assessing skeletal muscle stiffness. Clin Biomech (Bristol, Avon) 2003;18:459-61.

[19] Chuang L-l, Wu C-y, Lin K-c. Reliability, validity, and responsiveness of myotonometric measurement of muscle tone, elasticity, and stiffness in patients with stroke. Arch Phys Med Rehabil 2012;93:532-540.

[20] Glemser PA, Jaeger H, Nagel AM, et al. 23Na MRI and myometry to compare eplerenone vs. glucocorticoid treatment in Duchenne dystrophy. Acta Myologica 2017;36:2.

[21] Marusiak J, Kisiel-Sajewicz K, Jaskólska A, Jaskólski A. Higher muscle passive stiffness in Parkinson's

disease patients than in controls measured by myotonometry. Arch Phys Med Rehabil 2010;91:800-802.

[22] Schoenrock B, Zander V, Dern S, et al. Bed Rest, Exercise Countermeasure and Reconditioning Effects on the Human Resting Muscle Tone System. Front Physiol 2018;9:810.

[23] Marusiak J, Żeligowska E, Mencel J, et al. Interval training-induced alleviation of rigidity and hypertonia in patients with Parkinson's disease is accompanied by increased basal serum brain-derived neurotrophic factor. Journal of rehabilitation medicine 2015;47:372-375.

[24] Lo WLA, Zhao JL, Chen L, Lei D, Huang DF, Tong KF. Between-days intra-rater reliability with a hand held myotonometer to quantify muscle tone in the acute stroke population. Sci Rep 2017;7:14173.

[25] Fröhlich-Zwahlen A, Casartelli N, Item-Glatthorn J, Maffiuletti N. Validity of resting myotonometric assessment of lower extremity muscles in chronic stroke patients with limited hypertonia: a preliminary study. Journal of Electromyography and Kinesiology 2014;24:762-769.

[26] Knak KL, Andersen LK, Witting N, Vissing J. Reliability of the 2- and 6-minute walk tests in neuromuscular diseases. J Rehabil Med 2017;49:362-366.

[27] ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med 2002;166:111-7.

[28] Heckmatt JZ, Leeman S, Dubowitz V. Ultrasound imaging in the diagnosis of muscle disease. J Pediatr 1982;101:656-60.

[29] Pillen S, Boon A, Van Alfen N. Muscle ultrasound. Handb Clin Neurol 2016;136:843-53.

[30] Angelini C, Semplicini C, Ravaglia S, et al. New motor outcome function measures in evaluation of late-onset Pompe disease before and after enzyme replacement therapy. Muscle Nerve 2012;45:831-4.

[31] de Boer A, Boukes RJ, Sterk JC. Reliability of dynamometry in patients with a neuromuscular disorder. Eng Med 1982;11:169-74.