Protocol Version 1.1

Clinical Trial Protocol

"E-monitoring of pulmonary function in patients with Duchenne Muscular Dystrophy undergoing respiratory rehabilitation at home". The controlled and observational 12-months study with prospective cohort design.

Sponsor: prive

Version and Date of Protocol: protocol version 1.1; dated: 01.Mar.2021

"**E**-monitoring of **PULMo**nary function in patients with **D**uchenne

Muscular **D**ystrophy undergoing respiratory rehabilitation at home".

acronym : **E-PULMoDMD**

ID: **E-PULMoDMD 001/2021**

SIGNATURES

I agree to conduct the study in accordance with the current protocol.

Principal Investigator's Name (print) _____

Principal Investigator's Signature_____

Date _____

STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP E6 (R2)).
The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Ethic Committee (EC) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IEC before the changes are implemented to the study. In addition, all changes to the consent form will be IEC-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.
The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from sponsor, and documented approval from the IEC, except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed ICH GCP Training.

KEY CONTACTS: NAME, TITLE, ADDRESS, TELEPHONE NUMBER INCLUDING PRINCIPAL INVESTIGATOR

Principal Investigator:
Sub-investigator:

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1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title:

"E-monitoring of pulmonary function in patients with Duchenne Muscular Dystrophy undergoing respiratory rehabilitation at home". The controlled and observational 12-months study with prospective cohort design.

Short title: E-monitoring of pulmonary function in Patients with Duchenne Muscular Dystrophy (DMD).

***Acronym: E-PULMoDMD**

Abbreviations:

DMD, Duchenne muscular dystrophy

BMI, body mass index

BS, Brooke scale

VS, Vignos scale

FVC, forced vital capacity

FEV1, forced expiratory volume in 1 second

PEF, peak expiratory flow

MEP, maximal expiratory pressure

MIP, Maximal inspiratory pressure

PUL, Performance of Upper Limb

Study Description:

The controlled and observational 12-months study with prospective cohort design and longitudinal data collection performs in paediatric patients with established diagnosis of DMD, at the Department of Allergy and Pulmonology Medical University of Gdansk, Poland.

The study includes 200 patients with DMD diagnosis confirmed by genetic testing, 7-17 years old (7 ≥and <18) at the study entry with the routine standardized a follow-up of up to 4 weeks, 12 weeks, 24 weeks and 12 months.

The study size is estimated at 200, with a dropout rate after the 12-month follow-up period being ca. 10% (N=180). The study consists of 4 arms: 3 actives and 1 control (each arm of n=50 participants; with dropout rate after 12 months ca.10%, n=45).

Duration of the project March 2021 - March 2025.

2 INTRODUCTION

Duchenne muscular dystrophy (DMD) is the most common and most severe muscular dystrophy recognized in childhood. DMD is a genetically determined, progressive, irreversible disease in which dystrophin dysfunction in skeletal and multiple organ muscles is fatal before the age of 20 years. Nowadays, multi-specialist care has resulted in an increase in patient survival by up to 10-25 years. However, the respiratory muscle failure is the most common cause of death [1,2], so assessment and treatment of respiratory system is one of the most important problems for DMD patients.

In the first years of life, the respiratory system of children with DMD is efficient and does not differ from that of healthy peers. From the age of 7 years, the parameters of lung function no longer increase, and between the ages of 10 to 12 years, when the child loses the ability to walk independently, the lung function rapidly deteriorates [3].

The basic tasks include the initiation of early monitoring of respiratory system functions. It is recommended that measurement of lung function is started from the age of 5 years [1,2,4]. Taking the measurement at such an early age is aimed at familiarizing and teaching the child about this type of systematic examination and determining the individual maximum parameters of lung function in each child. Thanks to systematic measurements, it is possible to detect any sharp deterioration as well as the moment when the decreasing lung function requires respiratory support, so-called non-invasive ventilation (NIV).

According to the standards, lung function is assessed by spirometry, which should be performed at least once a year, at least every 6 months after losing independent walking, and every 3 months after starting non-invasive ventilation [1,2].

The spirometry test assesses forced vital capacity (FVC), which is considered a marker of disease progression. An FVC value below 2.1 L is a rationale to start supporting the cough reflex, and below 1 L is an indication to start respiratory support, i.e. NIV [5,6,7,8,9,10].

Implementation of the above-mentioned tests often encounters difficulties which increase when the child loses independent walking. Additionally, during the COVID pandemic, which runs from March 2020 in Poland, spirometry was included in the procedures generating aerosols, i.e. high risk of SARS-CoV-2 virus transmission. Therefore, it has become necessary to look for other methods of measuring and monitoring lung function in children with DMD.

Because a major component of respiratory dysfunction seems to be decline of inspiratory muscle weakness [3,6] therefore also respiratory training and rehabilitation is recommended as one of the key elements of DMD patient care [1,3].

The time of the SARS-CoV-2 pandemic also makes it necessary to look for rehabilitation methods at home. The assumption of the exercises is the possibility of performing them independently, at home, without the assistance of a physiotherapist, and without the use of additional equipment.

Summary: Pulmonary rehabilitation is one of the key issues in DMD patient management. The combination of systematic home lung function measurement with home respiratory rehabilitation is an innovative project. It is a non-invasive test, and the measurement is intended to improve the quality of life of DMD patients.

3. AIMS, OBJECTIVE

Objectives:

The primary objective of this study is to evaluate whether using home electronical device to monitoring pulmonary function and/or home telerehabilitation can slow the rate of progression pulmonary function in males with DMD compared to control group without using home e-device and telerehabilitation.

The second aim of this study is:

to investigate whether it is possible to monitor pulmonary function at home using an electronical spirometry system called AioCare in boys with DMD.

to assess the acceptance of telerehabilitation of respiratory system in this group of patients.

to assess the acceptance of electronical home monitoring of respiratory system in this group of patients.

to assessment of the quality of life of patients subjected to electronic monitoring and pulmonary rehabilitation vs. not subjected (validated questionnaires)

4. STUDY DESIGN

4.1 Overall design

The study consists of 4 arms: 3 actives and 1 control (figure 1).

ARM 1: Patients in arm 1 use electronical home device to measurements pulmonary function,

ARM 2: Patients in arm 2 use electronical home device to measurements pulmonary function, and perform telerehabilitation,

ARM 3: Patients in arm 3 perform telerehabilitation

CONTROL GROUP: Patients without home e-monitoring pulmonary function and telerehabilitation.

Enrolment of a patient to one of four groups (active or control) takes place after enrolment visit in random order.

As a part of the clinical trial, diagnostic examinations evaluating progression of the disease, pulmonary function tests will be performed periodically. The compliance, efficacy, accomplishment in all patients will be monitored by questionnaire during follow -up visits. The problems with investigate procedures (e-spirometry, telerehabilitation) and patients' personal well-being will be controlled using telemedicine technologies. Additional visits in the research center will be provided if any adverse events occur. This model will be continued for 12 months from the enrolment. After 4 weeks the first home e-monitoring pulmonary function and respiratory telerehabilitation efficiency analysis will be performed.

The study may be continued for 12 months or in case of negative impact of the intervention on patients' health and well-being, terminated with further patients monitoring

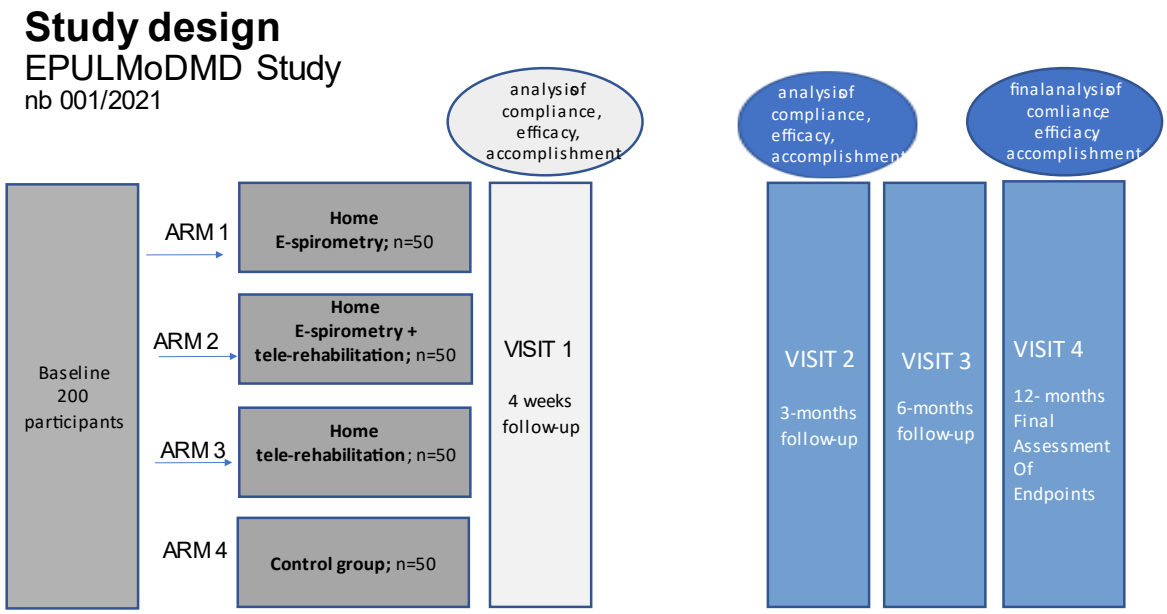

Figure 1. Study design

4.2 Investigate procedures

The presented project evaluates the measurement of lung function at home using an individual electronic spirometer called an AioCare [11] and home respiratory telerehabilitation.

4.2.1 Home e-spirometry

Home e-spirometry will be performed by AioCare system ((AioCare® spirometers, Healthup, Poland) [11]. The AioCare spirometer is a small, convenient device that can be used anywhere. The device enables systematic non-invasive monitoring of lung parameters (including FVC measurement) at home in children over 5 years of age. The child inhales and exhales forcefully through a mouthpiece with antibacterial and antiviral filters. The test results are sent from the AioCare spirometer via the AioCare application for iOS and Android (as used by all current smartphones). Communication between the AioCare spirometer and the application takes place via a Bluetooth 4.0 (BT LE) connection. The spirometry results are available to the practitioner in real time in the AIOCARE Doctor panel.

4.2.2 Home respiratory telerehabilitation

A module with exercises for training respiratory muscles, will be included in an additional smartphone application.

It will be a series of 4 exercises aimed at strengthening respiratory muscles, to be performed after the spirometry test; twice every day.

Participants will perform forced exhalation exercises with a relaxed epiglottis, exercises to improve the mechanics of the chest and the mobility of the shoulder girdle. The duration of the exercises is approximately 7 minutes. The exercise program will be recorded in the form of an instructional video.

4. STUDY POPULATION

4.1 Inclusion criteria

The study includes patients with signed informed consent, i.e. those who agree to participate in a clinical study and meet the inclusion criteria. The children will be enrolled in the study after their legal guardians read, agree to, and sign the informed consent form. All children older than 12 as well as adolescents will be enrolled in the study after signing the inform consent, as well. The subjects will be assesses for inclusion and exclusion criteria on first study visit after signing the informed consent.

Inclusion criteria are as follows: (1) male with DMD diagnosis based on current guidelines, the presence of typical clinical symptoms, genetic testing, and/or muscle biopsy results [1]. (2) male, ≥7 years and <18 years of age at time of enrollment in the study; (3) ability to perform spirometry; (4) do not use breathing support; and (5) stated willingness to comply with all study procedures and availability for the duration of the study.

4.2 Exclusion criteria

Autism

6. PARTICIPANT RECRUITMENT PROCESS

6.1 Recruitment Setting

In order to support the recruitment of patients the following actions will be undertaken:
I. Public study announcement and participant invitation

- preparation and distribution of the informational advertisements, fliers, and brochures
- preparation of the study website containing information for patients and their families
- establishment of contact and cooperation with patient's organizations throughout the country (such as "Parent Project DMD")
- other actions as outlined in the Recruitment strategies

II. Contacting potential participants

- answer any preliminary questions or doubts over a phone or internet communicator as suitable for the patient or guardian, point to the study website

6.2 Screening

Screening will take place at the study Site. Medical workup will include a visit to the nurse (measurements), interview and medical examination. Education on the study protocol. Signing the study consent form.

Every subject after giving the consent will be given one, unique screening number. The number will be used for identification of the subject for all procedures. In case a subject is screened more than once he will always have the same screening number.
The investigator agrees to complete a participant identification and enrolment log to permit easy identification of each participant during and after the study.

6.3 Informed Consent, Trial Records

The study will be conducted in accordance with the protocol, with established ethical principles in the Helsinki Declaration, in line with the harmonized ICH Guidelines for Good Clinical Practice (GCP), Relevant BI Standard Operating Procedures (SOP), and other relevant recipes. Investigators and plant employees must follow these rules. Deviation from protocol, rules of ICH GCP or applicable law that will be considered "protocol deviation".
Standard medical care (prophylactic, diagnostic and treatment procedures) still remains responsibility of the patient's doctor and participants are required to attend neurologist, rehabilitation specialist and other specialist if indicated for supervision of musculoskeletal function in accordance with current DMD treatment guidelines.
The Investigator will obtain the written informed consent from the subject's parent(s) or guardian(s) prior to any study-specific procedures.
Each subject's parent(s) or guardian(s) will receive an explanation of the nature and purposes of the study from the Investigator or designee.
Time will be given to the parent(s)/guardian(s) to ask questions and make their decision on whether they would like for their child to participate.
The Investigator or designee will ensure the study is appropriate for the subject.
Subjects are considered to be enrolled in the study at the time written informed consent is obtained.

6.3.1 Consent Process

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be IEC-approved, and the subject's parent(s) or guardian(s) and participant will be asked to read and review the document. The investigator will explain the research study and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. The participants should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.
The subject's parent(s) or guardian(s) will be asked if s/he understands that the study is for research purposes only and that it may not provide any benefit to the subject and that the subject is free to withdraw from the study at any time without prejudice. Each subject's parent(s) or guardian(s) will be

required to sign a study ICF before any procedures are performed for the study; both parents or guardians will sign the ICF in jurisdictions where this is required.
If applicable, the assent of the child himself will also be obtained, if possible, in writing per individual where a child is intellectually capable of assenting (and in accordance with local regulations: according to Art. 25 (in connection with Article 37h (1) (1) of the PF), if a minor is 16 or under 16 and is able to expressly express his opinion on his participation in the experiment, his consent is also required. At any time, the researcher is obliged to take into account the wishes of the minor (able to express an opinion) regarding the refusal to participate in the study or withdrawal from the study.), and with the permission of the parent(s)/guardian(s).
The Investigator or designee will obtain ICF in accordance with the principles outlined in the current version of the Declaration of Helsinki. Informed Consent Forms must be dated and signed by the Investigator or designee and the subject's legal representative(s) and the original signed consent form must be kept by the Investigator in the study subject's file.
"Legal representative" means an individual whom a judicial or other body authorized under applicable law to consent on behalf of a prospective study subject to the subject's participation in the procedure(s) involved in the research. The Study Monitor will ensure that the ICF has been signed by the subject's legal representative(s). The study subject's legal representative(s) will receive a copy of the signed consent form. Consent will be a continuing process; investigator will foster a continuous dialogue with participants and inform them of anything new related to the trial.

7. STUDY INTERVENTION

7.1 Description of all Trial Periods

7.1.1.Enrollment visit

During the enrollment visit, medical history data and clinical symptoms of the respiratory system will be collected from an interview. A physical examination, Vignos scale (VS) assessment, Brooke scale (BS) assessment, and spirometry (Jaeger, Germany), Respiratory muscle assessment (Maximal inspiratory pressure -MIP, and Maximal expiratory pressure- MEP), Assessment of the muscles of the upper limb (Performance of Upper Limb -PUL), Maximal grip strength (dynamometry) will be performed in all participants.

Participants on arm 1 and 2 will receive an electronic individual spirometer (AioCare) with a 2-h training session how to use it. Each participant was then asked to perform on their own three correct spirometry measurements twice daily (morning and evening) at home.

Participants from arm 2 and 3 will receive a 1-hour training with respiratory rehabilitation and link to video presentation with respiratory rehabilitation exercises to home daily using.

- Functional status

The functional status of the participants was assessed by the Vignos scale (VS). The scores on the VS range from 1 to 10 (1 - the subject can walk and climb stairs without assistance, 10 - the subject is

confined to a bed). The VS allows staging of the disease and focuses on functional ambulatory activities. This is the main scale used for characterizing the progression of disease [12]. Upper limb functional status was assessed with the 6-point Brooke scale (BS) (1 - the subject can abduct their arms in a full circle until they touch above their head, 6 - the subject has no useful function of the hands) [13].

- Respiratory muscle assessment

MicroRPM- Maximal respiratory pressure and reflecting respiratory muscle strength was measured using a calibrated portable hand-held mouth pressure meter (MicroRPM; Micro Medical Ltd., Rochester, England) in a sitting position. To measure maximal expiratory pressure (MEP), subjects performed maximal expiratory effort after maximal inspiration. Maximal inspiratory pressure (MIP) was measured by exerting maximal inspiratory effort after maximal expiration. To measure these pressures, effort was maintained for at least 1 second. The highest positive MEP value and the lowest negative MIP value in three or more attempts were chosen. We calculated percent predicted MEP (MEP%) and MIP (MIP%) values according to the formulas: **MEP%** MEP*100 / (7·619 + (7·806 * age) + (0·004 * height * weight)); **MIP%**-MIP*100 / (–27·020 – (4·132 * age) – (0·003 * height * weight))

- Pulmonary function tests

Lung function by spirometry using the calibrated, computerized spirometer Pneumo Screen (Jaeger, Germany) will be performed according to the European Respiratory Society and American Thoracic Society recommendations, by a certificated, experienced pediatric pulmonologist [6,14]. The highest value of forced vital capacity (FVC), forced expiratory volume in 1 second (FEV1), peak expiratory flow (PEF) expressed as liters (L) and liters per minute (L/min) accordingly, and percent predicted value (%pv) from correct acceptable attempts will be evaluated. The results will be compared with results from home electronical spirometry.

- Assessment of the muscles of the upper limb

Performance of Upper Limb (PUL)- a functional scale specifically designed for assessing upper limb function in Duchenne muscular dystrophy (DMD). It includes three domains (shoulder, mid- and distal), each including items exploring activities easily related to activities of daily living that both patients and clinicians identified as relevant. Each dimension (shoulder, mid, distal) can be scored separately. In the PUL 2.0 there is a maximum score of 12 for the shoulder level, 17 for the mid-level, and 13 for the distal level. A total score of 42 can be achieved by adding the three level scores [15].

Dynamometry – Maximal grip strength was measured using dynamometer FT-5988 – N1 (Spais, Poland) – very sensitive electronic device developed to measure grip in very weak patients, the device can be also applied in assessment of healthy subjects. The dynamometer measures forces from 0 to 1000N with a resolution of 0,02N. All measurements were conducted by the same experienced and trained evaluators. Grip strength was measured on the dominant side with a high precision dynamometer. All tests were performed with patients seated on a chair or in their wheelchair facing a table with the forearm placed on the table on the waist level. Trials were carried out with strong verbal encouragement asking the patients to provide maximal voluntary isometric contractions for 3 s. A rest period of about 30 s was respected between the trials. Patients were asked to perform three trials. The mean value was calculated from three valid trials.

- Telerehabilitation – respiratory exercises

Participants from arm 2,and 3 will received a 1-hour training with respiratory rehabilitation and link to video presentation with respiratory rehabilitation exercises to home daily using.

The video with the instruction of respiratory exercises: positive inspiratory pressure, glossopharyngeal breathing and positive expiratory pressure was showed to parents and patients at the Conference during the Physical Therapy panel. Participants were asked to perform the exercises themselves at home without physiotherapists' assistance. Patients were encouraged to train every day, three times a day. On the video patients received following instructions:

1. Breath stacking (positive inspiratory pressure): patients were asked to take maximal inhale, then without exhaling, to take another 1-3 inhales, and hold for 5 seconds. Boys were instructed to repeat the exercise 5 times. The aim of the exercise was to increase lung volume. The same exercise was presented with the use of balloon – inflated balloon – patients were instructed to inhale the air from balloon, without exhaling and try to hold as much air in the lungs as possible – hold for 5 seconds and repeat for 5 times.

2. Glossopharyngeal breathing – patients were instructed to push series of small volumes of air by tongue and pharynx into the lungs by saying 'cat' in Polish - patients were encouraged to repeat the exercise for 5 times.

3. Positive expiratory pressure – inflating the balloon – patients were instructed to inflate the balloon by inhaling by nose and exhaling by mouth – the exercise should be repeated for 3 times. Caregivers were encouraged to try different types of balloons to find optimal elasticity of material (optimal resistance). The video is available in the internet https://www.youtube.com/watch?v=AEaxOsuJimU&feature=youtu.be.

7.1.2 Follow-up visit

Visit 1

- Feasibility home e- monitoring pulmonary function survey

Participants from arm 1 and 2 during visit 1 (after 4 weeks training home using e-device), will be completed a survey that included questions about home spirometry (compliance, satisfaction, and problems with the use of the electronical spirometer at home). Patients were asked to express their general satisfaction and the intelligibility of the instructions from the AioCare home spirometry on a 5-point scale, where "1" meant the worst and "5" meant the best score. Participants who were not able to perform spirometric measurements regularly were also asked the reason why and what would help them to take measurements more regularly. The last question concerned the benefits of home spirometry (table 1).

Table 1. Survey of satisfaction and possibility of home e-monitoring pulmonary function in DMD patients.

Home AioCare e-spirometry				
1. Mark on the scale how you rate the monitoring of lung function with the home spirometer.				
1	2	3	4	5

the worst				the best
2. Mark on the scale how you assess the comprehensibility of the instructions received for self-measurements of spirometry.				
1	2	3	4	5
the worst				the best
3. If you have not been able to perform spirometric measurements regularly, what was the most common reason for this?				
- Correct blowing into the spirometer is too difficult for me				
- I don't have time to blow into the spirometer				
- I have no motivation for this assessment				
- I feel unwell				
- I forget to take the measurements				
- other reasons:				
4. What would be helpful for you to take measurements every day?				
- reminder sent via SMS				
- weekly online appointment with your doctor				
- weekly report of my results with interpretation				
- I am not able to take measurements everyday				
- other:				
5. Do you think that you benefit from home spirometry measurements?				
YES NO				
6. If 'YES', the benefits are:				
- I can breathe easier				
- I can clear my lungs easier				
- I feel more confident				
- I like to use new electronic spirometer				
- I will not be afraid of performing spirometry in the hospital				
- other:				

- Feasibility telerehabilitation

The structured questionnaire consisted of questions that covered several areas: (1) respiratory exercises; (2) additional information required with respect to exercises and home-based program; (3) satisfaction from program.
Respiratory exercises: The participants were asked: (a) whether the exercises were performed (yes/no, alone/with assistance), (b) possibility to implement this treatment to daily routine and about how often the exercises could be performed from patients perspective (3 times daily/ 1-2daily/ few times a week/not possible), (c) difficulty of the exercises (all exercises possible to perform/ possible to perform after practicing/some exercises too difficult, all exercises too difficult).
Additional information: The caregivers were invited to leave an e-mail contact in case they needed expanded individual consultation or training.
Satisfaction from program: Respondents were also asked to assess general satisfaction, appropriateness of the exercises and intelligibility in six points scale, where 0 meant the worse and 5 meant the best score.

Visit 2 and Visit 3

All patients will be followed up after 3, and 6 months from visit 1. Lung function testing, will be recorded and assessed at this visit.

Lung function by spirometry using the calibrated, computerized spirometer Pneumo Screen (Jaeger, Germany) will be performed according to the European Respiratory Society and American Thoracic Society recommendations, by a certificated, experienced pediatric pulmonologist [6,14]. The highest value of forced vital capacity (FVC), forced expiratory volume in 1 second (FEV1), peak expiratory flow (PEF) expressed as liters (L) and liters per minute (L/min) accordingly, and percent predicted value (%pv) from correct acceptable attempts will be evaluated. The results will be compared with results from home electronical spirometry.

End -point visit

- During the end-point visit (after 12 months), medical history data, clinical symptoms of the respiratory system will be collected from an interview. A physical examination, Vignos scale (VS) assessment, Brooke scale (BS) assessment, and spirometry (Jaeger, Germany), Respiratory muscle assessment (Maximal inspiratory pressure -MIP, and Maximal expiratory pressure-MEP), Assessment of the muscles of the upper limb (Performance of Upper Limb -PUL), Maximal grip strength (dynamometry) will be performed in all participants.

8. STATISTICAL CONSIDERATIONS

Sample size estimation

Estimation of the sample size was based on the expected differences of the FVC between the groups with and without intervention. In the reference studies annual decline of FVC is varied between 6-11% (mean 8.5%). Assuming that telerehabilitation with using mobile spirometry can decrease this decline to 6% per year with standard deviation of 3% we estimated sample size for the study arms. Taking into account 80% power of the test, 5% significant level, the number of the subjects in the arm 1 (AioCare

device spirometry + telerehabilitation of the respiratory system) and control group equals 46 per group; finally 50 subjects per group were established. The same number of subjects were determined in the rest arms of the study (arm 2 – only mobile spirometry and arm 3 – only telerehabilitation).

Statistical Analysis

The results of the statistical analysis will be expressed as mean ± standard deviation (SD) or median and interquartile range (IQR). The comparison analysis of the results will obtaine from ambulatory spirometry and results from home spirometry using the AioCare spirometer will be performed using paired, parametric Student t-test, or non-parametric Wilcoxon signed-rank test (depending on whether compared values were normally distributed, which was evaluated using the Shapiro–Wilk test) with a significance level of $p = 0.05$.

The relationship between the compliance of spirometry parameters, such as number of days with performed spirometry tests and clinical parameters (age, BMI, AS, VS, BS) and spirometry results (FEV1%pv or FVC%pv), will be evaluated using linear regression analysis. The goodness of fit of the model, based on Akaike's information criterion (AIC), will compare between the four models. The number of independent variables will reduce using backward, stepwise regression models. This method allows for a decrease in the variables from the model that do not contribute to the explanation of the variability of dependent variables, which reduces the complexity of the model (which was necessary due to the limited sample size). The correlation between continuous variables will be evaluated using Pearson correlation coefficients.

9. SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

9.1 Ethics

The study will be conducted in accordance with the law, local regulations, principles of GCP and Declaration of Helsinki.

Before the start of the study, the protocol, ICF, and any other appropriate documents will be submitted to the IEC for approval. The Investigator will keep the IEC informed regarding the status of the study. No changes will be made without IEC approval, except when required to eliminate immediate hazards to the subjects.

The Investigator will submit to the IEC the following:

1. Information on serious or unexpected AEs, as soon as possible

2. Periodic reports on the progress of the study

3. Final Study Summary upon study completion or closure.

Notification of the end of the trial will be sent to the IEC within 30 days after completion of the study close-out visit. The IEC will be notified within 15 days in case the study is ended prematurely.

9.2 Publication

The results will be disseminated through manuscript publications in high impact scientific journals, international workshops and presentations at scientific meetings and parents' forums.

9.3 Conflict of Interest Policy

There is no conflict of interest.

10. COVID-19 (SARS-COV-2 INFECTION) SAFETY MEASURES

COVID-19 PREPARADNESS AND PREVENTION

Based on the current data and understanding of both DMD and COVID-19 (Coronavirus Disease 2019) patients suffering DMD are potentially at higher risk of severe course of COVID-19. The following measures are to minimalize SARS-CoV-2 (Severe acute respiratory syndrome coronavirus 2 – the virus responsible for COVID-19) infection risk to other patients and their guardians, as well as personnel. One should be aware that total risk elimination in real life setting is nearly impossible. Two-fold measures will be implemented – patient specific and infrastructural.

As of now three main routes of transmission of SARS-CoV-2 are confirmed: respiratory droplets, aerosols and contact transmission. PFTs are non-invasive tests that are commonly performed in routine assessment and follow-up of patients with DMD. Most of the procedures planned by the study protocol (patient history and examination etc.) pose low to moderate risk of SARS-CoV-2 transfer and allow for both personnel and patient to were personal protective equipment (PPE). However, PFTs (pulmonary function tests, e.c. spirometry) may generate aerosols and require sharing common room and surfaces during forced expiration with the patient unable to wear a face mask. Thus, PFTs is considered high risk procedure. To address this risk the following measures will be implemented.

Patient specific recommendations

1. Every patient and guardian 1–2 day before each visit will be required to complete (by telephone or email or PRO-eCRF software)

a. epidemiological history

b. COVID-19 specific symptoms history

c. Local (regional) epidemiological status as reported by Polish Ministry of Health

If a. positive or b. positive or c. “Red” – COVID-19 PCR test at place of residence.

If PCR test doubtful or positive – visit postponed by 2 weeks, repeat a. to c. + PCR test.

2. Required from every patient and guardian accompanying during visit

a. Mask covering mouth and nose at all times

b. Hand disinfection before and after each procedure (on entering and leaving the room)

Study center, equipment and personnel (infrastructural preparedness)

I. Standard risk procedures (all except PFTs)

1. Personnel

a. Mask covering mouth and nose at all times

b. Hand disinfection after each patient

2. Room and equipment

a. Equipment and surfaces disinfection before each patient

II. High risk procedures (PFTs)

3. Personnel

a. Full set PPE: Gown, respirator (FPP2 or N95), full-face shield and disposable gloves

b. Hand disinfection after each patient

4. Room and equipment – Spirometry lab

a. Forced high efficiency room ventilation / air purifier

b. Post-test cleaning and disinfection procedures of the equipment and the testing room

c. Individual Antiviral/Antibacterial Filters for the equipment i.e. spirometer (high viral and bacterial filtration efficiency (99.999%), low resistance to airflow, minimal dead space)

The choice of specific types and brands of PPE, filters, disinfectants and air purification methods at the discretion and in accordance with recommendations, standards and practices of the specific study center.

This measures were prepared in compliance with Recommendation from ERS Group 9.1 (Respiratory function technologists/Scientists) Lung function testing during COVID-19 pandemic and beyond (https://ers.app.box.com/s/ zs1uu88wy51monr0ewd990itoz4tsn2h) and ERS COVID-19 resource centre (https://www.ersnet.org/the-society/ news/novel-coronavirus-outbreak--update-and-information-for- healthcare-professionals); Novel Coronavirus (COVID-19): The ATS Response (https://www.thoracic.org/professionals/clinical-resources/ disease-related-resources/novel-coronavirus.php)

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