- **RESEARCH PROTOCOL**

Validation of the 1-minute sit to stand test (1MSTS) for assessment of breathlessness at exertion in individuals with chronic disease in out-patient care.

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Sponsor:	Blekinge Institute of Technology, Lund University
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1. Contact information

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2. List of used acronyms and abbreviations

Abbreviation	**Term/Explanation**
1MSTS	1-minute sit-to-stand test
AT	Anaerobic threshold
CAT	COPD Assessment Test
COPD	Chronic obstructive pulmonary disease
CI	Confidence interval
CPET	Cardio Pulmonary Exercise Testing
CRF	Case Report Form
DLCO	Diffusing capacity of the lungs for carbon monooxide
EPM	Etikprövningsmyndigheten (English: Swedish Ethical Review Authority)
FEV	Forced Expiratory Volume
FVC	Forced Volume Capacity
GCP	Good Clinical Practice
GOLD	Global Initiative for chronic obstructive Lung Disease.
HF	Heart Failure
IC	Inspiratory capacity
ICH	International Council for Harmonization
ILD	Interstitial Lung Disease
IQR	Interquartile range
VE	Minute ventilation
MDP	Multidimensional Dyspnoea Profile
NRS	Numerical rating scale
PI	Principal Investigator
RER	Respiratory exchange ratio
SD	Standard Deviation
SF-12	12-item Short Form Survey
SpO2	Oxygen saturation
SV	Study Visit

tLIM	Time to the limit of tolerance
V'O2	aerobic exercise capacity
VT	Tidal volume
W	Workload
WHO	World Health Organization

3. Synopsis

Title:	Validation of the one-minute sit to stand test (1MSTS) for assessment of exertional breathlessness in individuals with chronic cardiorespiratory conditions.
Study code:	1MSTS-PC
Short background/ Rationale/Aim:	Individuals suffering from Chronic Obstructive Pulmonary Disease (COPD), asthma, interstitial lung disease (ILD) and/or hearth failure (HF) are primarily managed within outpatient care such as primary care of pulmonary clinics. Breathlessness is largely unrecognised and underassessed. Current clinical guidelines mainly rely on patients' self-assessment of symptoms to determine treatment. The most prevalent scales are mMRC and CAT, but these fail to identify the majority (88%) of people with significant breathlessness on standardized cardiopulmonary exercise testing (CPET). Many individuals with breathlessness are therefore at risk of being incorrectly examined, referred and categorized, with their symptom levels and disease severity underestimated, and thus insufficiently treated. There is currently no validated and available exercise test for use in outpatient care, such as primary care. We now aim to investigate 1MSTS as a possible tool for enhanced identification and categorization of breathlessness within outpatient care such as primary care. 1MSTS is a short and easily performed test validated for measuring physical capacity, but not breathlessness, in COPD, asthma, ILD and HF and has been correlated with increased mortality [1-3]. We now aim to investigate whether this test can be used to identify and measure exertion-induced breathlessness and thereby improve recognition of breathlessness and its categorization among patients in outpatient care.
Study objectives:	1. To validate the 1MSTS test for standardized measurement of breathlessness severity in people with cardiorespiratory illness using calculations for validity, reliability and responsiveness. 2. To establish whether 1MSTS have better sensitivity and specificity in identifying abnormal breathlessness than mMRC and CAT when compared to CPET and the 6-minute walk test (6MWT), and its potential for use in a clinical setting. 3. To evaluate the physiological demands and predictive capacity of the 1MSTS by comparing it with CPET and 6MWT, and to identify clinically relevant threshold values for pathological breathlessness. 4. To explore the potential of wearable sensors to enhance and extend the assessment of sit-to-stand performance. 5. To assess feasibility and prognostic potential in a clinical outpatient setting.
Study design:	Clinical validation study of 1 minute sit-to-stand test
Study population:	Adult patients with a diagnosis of COPD confirmed by spirometry
Number of participants:	At least 30-40 per disease group
Inclusion criteria:	● Have a physician diagnosis of COPD/ILD/asthma/HF. ● Age 18 years or older

	● Able to cycle ● Able to use the legs and stand up for sitting without risk of falling. ● Able to understand and talk Swedish to participate in the study procedures, as judged by the Investigator. ● Provides written informed consent
Exclusion criteria:	● Resting peripheral oxygen saturation (SpO2) < 92% ● Hospitalization or clinical instability during the last four weeks ● Treatment with supplementary oxygen at rest or during exercise ● Contraindication to exercise testing in accordance with clinical practice guidelines ● Expected survival shorter than six months as judged by the Investigator
Study endpoints:	**Primary endpoints** • To determine the test–retest reliability of the 1MSTS test. • To assess the concordant and discordant validity of the breathlessness score after the 1MSTS test by comparing it to validated numerical rating scales (VNRS) at peak exercise during CPET and 6MWT. • To evaluate the responsiveness of the breathlessness score to change over time. • To establish the correlation and agreement between the breathlessness score after 1MSTS and VNRS at peak CPET and peak 6MWT. • To assess if 1MSTS is better than CAT and mMRC in identifying pathological breathlessness using CPET as reference and golden standard. • Feasibility of 1MSTS for identification of abnormal breathlessness in a clinical setting **Secondary endpoints** • To compare physiological responses and estimated mechanical work between 1MSTS and CPET, and to determine threshold values of 1MSTS performance that best predict pathological breathlessness as defined by CPET and breathlessness questionnaires. • To explore the use of wearable sensors as a digital tool for recording and assessing the 1MSTS test. • To derive and analyse additional performance metrics from wearable sensor data during the 1MSTS, including time to complete the first five repetitions, 30-second performance, and temporal changes in sit-to-stand velocity across the test, and to explore their association with physiological responses and breathlessness outcomes.
Study period:	*August 2026 – June 2028*

4. Background and rationale

Chronic breathlessness (dyspnoea) is a dominant symptom in heart and lung diseases,[4] and as many as 10% in general populations if affected by exertional breathlessness in their daily life activities [5]. Breathlessness contributes to reduced physical activity, decreased social interactions, significantly lowered quality of life, more frequent exacerbations, and premature death [6] Disabling breathlessness often persists despite optimal treatment of the underlying cause(s), defined as *chronic breathlessness syndrome* [7]. The symptom often leads to a vicious circle of reduced physical activity, deconditioning and further worsening breathlessness [8].

Relieving chronic breathlessness is a major management target across cardiorespiratory disease [9-11] and advanced illnesses. However, chronic breathlessness might often be overlooked and insufficiently treated in clinical practice [12]. Primary care plays a crucial role in the early identification, assessment, and long-term management of chronic breathlessness. As the first point of contact for most patients, primary care providers are uniquely positioned to recognize persistent symptoms, initiate appropriate diagnostic investigations, and coordinate multidisciplinary care.

Measurement of breathlessness is insufficient in COPD patients

In chronic obstructive pulmonary disease (COPD), breathlessness is commonly assessed through self-reporting using the mMRC and CAT scales. However, these scales have very poor sensitivity [13, 14], missing up to 88% of patients with significant symptoms [13]. This can lead to delays in correct GOLD (Global Initiative for chronic obstructive Lung Disease) categorization and a worsening of the condition before appropriate measures are implemented, such as intensified medication, smoking cessation, physiotherapy, and COPD education programs [11]. Evaluation of exertional breathlessness needs to be performed using standardized exercise testing [13].

The exertion tests currently used, such as CPET and the 6-minute walk test (6MWT), are not validated for measuring breathlessness, and have low accessibility [15] especially in a primary care setting. Often, a confirmed diagnosis and initiated treatment are required before a patient can gain access to these tests and CPET is only available at specialized centres [16]. Since most individuals presenting with breathlessness remain in primary care, there is a need for easy accessible, reliable and validated test to guide clinicians in further assessment and categorization.

The "1-minute sit-to-stand" test (1MSTS)

1MSTS is a short and easily performed test validated for measuring physical capacity, but not breathlessness, in COPD and heart failure[17] and has been correlated with increased mortality [1-3]. 1MSTS test has not been previously tested for use in a primary care setting and is not validated for measuring breathlessness. We now aim to investigate whether this test can be used to identify and

measure exertion-induced breathlessness and thereby improve the categorization patients in primary care.

The overall objective of the project is to determine whether the 1MSTS test is superior to current methods in uncovering symptoms and categorizing COPD patients who seek help in primary care.

5. Study objectives

1. To validate the 1MSTS test for standardized measurement of breathlessness severity in people with cardiorespiratory illness using calculations for validity, reliability and responsiveness.
2. To establish whether 1MSTS have better sensitivity and specificity in identifying abnormal breathlessness than mMRC and disease specific questionnaires such as CAT when compared to CPET and the 6MWT, and its potential for use in a clinical setting.
3. To evaluate the physiological demands and predictive capacity of the 1MSTS by comparing it with CPET and 6MWT, and to identify clinically relevant threshold values for pathological breathlessness.
4. To explore the potential of wearable sensors to enhance and extend the assessment of sit-to-stand performance.
5. To assess feasibility and prognostic potential in a clinical outpatient setting.

6. Study design and procedures

a. Overall study design

This is a prospective multicentre clinical validation study which comprises three visits to a research laboratory. The first two visits will be separated by at least 24 hours and maximum of 1 week. Visit 1 include evaluation of inclusion- and exclusion criteria, medical history, chronic activity-related breathlessness questionnaires, pulmonary function testing, and a symptom-limited incremental CPET with breathlessness assessment as the golden standard for identification of pathological breathlessness. Visit 2 will be performed within 1 week and will include questionnaires, and the 1MSTS performed twice as well as the 6MWT with at least 30 minutes for recovery between the tests. The focus in the first two visits will be on calculating validity and test-retest reliability for the 1MSTS test as well as the potential for identification and severity assessment compared to the mMRC and disease specific questionnaires such as CAT. Day 3 will be performed approximately 3months later and will include questionnaires, 1MSTS and another symptom-limited incremental CPET. This visit will be used for calculating responsiveness. 1MSTS is easier to perform and recovery times will be shorter, and 1MSTS will thereby be performed before the CPET.

b. Primary endpoint

- To determine the test–retest reliability of the 1MSTS test.
- To assess the concordant and discordant validity of the Borg CR10 (or equivalent 0-10NRS) breathlessness score after the 1MSTS test by comparing it to the validated numerical rating scale (VNRS 0-10) at peak exercise during CPET and 6MWT.
- To evaluate the responsiveness of the 0-10 NRS score to change over time.
- To establish the correlation and agreement between 0-10 NRS after 1MSTS and at peak CPET and peak 6MWT.
- To assess if 1MSTS is better than disease specific questionnaires (such as CAT) and mMRC in identifying pathological breathlessness using CPET as reference and golden standard.
- Feasibility of 1MSTS for identification of abnormal breathlessness in a clinical setting

c. Secondary endpoints

- To compare physiological responses and estimated mechanical work between 1MSTS and CPET, and to determine threshold values of 1MSTS performance that best predict pathological breathlessness as defined by CPET and breathlessness questionnaires.
- To explore the use of wearable sensors as a digital tool for recording and assessing the 1MSTS test.
- To derive and analyse additional performance metrics from wearable sensor data during the 1MSTS, including time to complete the first five repetitions, 30-second performance, and temporal changes in sit-to-stand velocity across the test, and to explore their association with physiological responses and breathlessness outcomes.

7. Study population

a. Inclusion criteria

To be included in the study, participants must meet the following criteria:

- Have a physician diagnosis of COPD/ILD/Asthma/HF.
- The participant has given written consent to participate in the study
- Age 18 years or older
- Able to cycle
- Able to use the legs and stand up for sitting without risk of falling

- Able to understand and talk Swedish to participate in the study procedures, as judged by the Investigator.

b. Exclusion criteria

Participants must not be included if any of the following criteria is met:

- Resting peripheral oxygen saturation (SpO2) < 92%
- hospitalization or clinical instability during the last four weeks
- treatment with supplementary oxygen at rest or during exercise
- contraindication to exercise testing in accordance with clinical practice guidelines
- expected survival shorter than six months as judged by the Investigator
- medical conditions including acute coronary artery disease, neuromuscular disease or severe psychiatric illness.

c. Recruitment

Participants will be recruited at the participating clinical and research units including internal medicine and primary care outpatient clinics, cardiopulmonary rehabilitation programs or evaluation by physiotherapist at the participating centres. In addition, participants will be recruited through advertisements in newspapers and patient organizations, and through postal invitations to patients at the participating centres. In the case of a postal invitation, the potential participants will be identified through electronic health records using a relevant diagnostic code after approved by the responsible department head, in accordance with the established procedures for data access within the regional healthcare organization. The extraction and handling of identifiable information (diagnostic code and postal address) is performed solely within the healthcare organisation, and the research team does not access or store identifiable data during this process. The extraction will be approved by the responsible department head in accordance with regional procedures for data access. Individuals meeting the criterion receive a postal invitation containing brief study information and instructions for how to contact the research team if they wish to learn more, and no follow-up occurs unless they initiate contact.

The participating research centres also have knowledge of, and will invite, individuals who have attended the clinic previously or participated in previous studies and have given permission to be contacted in upcoming studies.

d. Withdrawal criteria

Participants can discontinue their participation in the study at any time without any consequence to his/her continued treatment. The investigator/sponsor can at any time terminate the study for a participant due to, e.g., because the participant does not follow procedures in the study protocol.

e. Sample size calculations

The primary objective of this study is to assess the validity of the breathlessness score (Borg CR10 or other validated 0-10NRS) after the 1MSTS by comparing it to the equivalent 0-10 NRS at peak exercise during CPET. The primary endpoints are the correlation (validity), reliability and responsiveness between these two measures. Based on previous data [1], we expect a moderate-to-strong correlation ($r \approx 0.5–0.6$). Using Fisher's Z transformation for correlation analysis with $\alpha = 0.05$ and power = 0.80, the required sample size is approximately 20 participants for $r = 0.6$ and 29 participants for $r = 0.5$. To account for potential dropouts and ensure robust analysis, we plan to recruit at least 30 participants.

Reliability will be assessed using the Intraclass Correlation Coefficient (ICC) between two repeated measurements. For an expected ICC of 0.8, with $\alpha = 0.05$ and power = 0.80, previous methodological recommendations [18] suggest a sample size of approximately 22 participants. Including 30-40 participants per disease group will provide sufficient precision for reliability estimates.

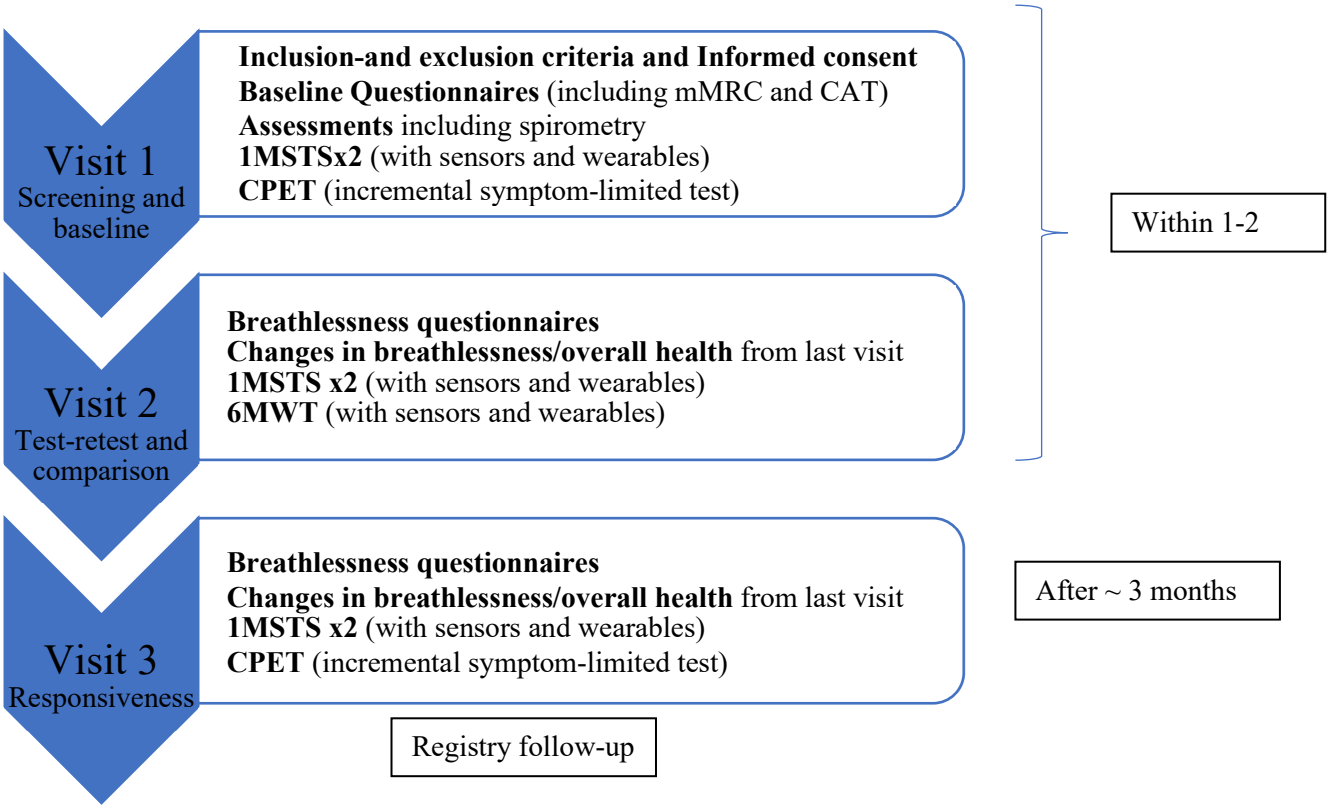
Responsiveness will be analysed using paired comparisons and effect size calculations. The same sample size ($n > 30$) is considered adequate for detecting clinically meaningful changes, given the repeated-measures design.

In summary, a target sample size of 30 participants is justified to meet all three endpoints: validity, reliability, and responsiveness.

8. Study procedures

a. Timeline of Assessments - overview

Flowchart 1:



b. Data collection methods

Electric records review

Data will be extracted from the patient's journal, after getting the informed written consent, regarding:

- Diagnoses
- Most recent values from cardiac and lung function tests
- Medications and treatments
- Any contraindications for CPET according to clinical guidelines.

Demographic and background questionnaires

BASELINE QUESTIONNAIRE

The baseline questionnaire will be administered on the baseline visit 1, after screening and informed consent, and will include:

- Swedish social security number
- Date of visit
- Age, sex
- Smoking (year of starting, cessation and mean number of cigarettes per day)
- Work/occupational status
- Conditions diagnosed by a physician
- Medications and treatments
- Self-reported physical activity level
- Self-reported change in health status and health conditions since last visit (for visit 2 or 3)

ASSESSMENT OF QUALITY OF LIFE (EQ5D)

The EQ-5D is a standardized instrument for measuring health-related quality of life [19, 20]. It consists of five dimensions: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. Each dimension is rated on a scale reflecting severity (e.g., no problems, some problems, extreme problems). In addition, the EQ-5D includes a visual analogue scale (VAS) where participants rate their overall health on a scale from 0 (worst imaginable health) to 100 (best imaginable health). The instrument is widely used in clinical research and health economics to assess patient-reported outcomes.

Questionnaires on breathlessness

STANDARDIZED VALIDATED 0-10 RATING SCALE

The Borg CR10 scale is a validated tool for assessing perceived exertion or symptom intensity during physical activity. It uses a 0–10 numeric rating, where 0 represents “no exertion at all” and 10 represents “maximal exertion.” Intermediate descriptors (e.g., “moderate,” “strong,” “very strong”) help participants anchor their perception. Newer, not yet published research has shown that a simpler numerical rating scale (0-10), anchored at none and maximal, might be a better choice for rating breathlessness during exercise testing. In this study we will use the alternative that is the most suitable and validated for measuring breathlessness. These standardized scales are all commonly used to monitor breathlessness, fatigue, or overall effort during exercise tests such as the 6MWT or CPET.

MODIFIED MEDICAL RESEARCH COUNCIL (MMRC) DYSPNEA SCALE

The mMRC scale is a simple questionnaire used to assess the degree of breathlessness experienced by a patient during daily activities [4, 21]. It consists of five grades (0–4):

Grade 0: Breathless only with strenuous exercise.

Grade 1: Short of breath when hurrying or walking up a slight hill.

Grade 2: Walks slower than people of the same age because of breathlessness or has to stop for breath when walking at own pace.

Grade 3: Stops for breath after walking about 100 meters or after a few minutes.

Grade 4: Too breathless to leave the house or breathless when dressing.

DISEASE SPECIFIC QUESTIONAIRES

In studies related to COPD, pulmonary fibrosis, asthma, and heart failure, the assessment scales will be adapted to the specific condition. Either the COPD Assessment Test (CAT), the New York Heart Association (NYHA) scale (heart failure), the Asthma Control Test (ACT) (asthma), or the King's Brief Interstitial Lung Disease (K-BILD) questionnaire (pulmonary fibrosis) will be used instead of the CAT.

The CAT is an 8-item questionnaire designed to quantify the impact of COPD symptoms on health status. Each item is scored from 0 (no impact) to 5 (severe impact), giving a total score range of 0–40 [22]. Items cover: Cough, Phlegm, Chest tightness, Breathlessness, Activity limitation, Confidence leaving home, Sleep quality, Energy levels.

NYHA is a widely used scale to assess the severity of heart failure based on symptoms and limitations in physical activity, ranging from Class I (no limitation) to Class IV (severe limitation).

ACT: A validated 5-item questionnaire that evaluates asthma control over the past four weeks, including symptoms, activity limitation, and use of rescue medication. Scores indicate well-controlled, partially controlled, or uncontrolled asthma.

K-BILD Questionnaire: A patient-reported outcome measure assessing health-related quality of life in individuals with interstitial lung disease. It covers breathlessness, chest symptoms, psychological impact, and activities of daily living.

OTHER BREATHLESSNESS MEASURES:

- Change in breathlessness since previous visit on a 7-point Global Impression of Change (GIC)

Clinical and physiological measurements

- Height, weight
- SpO2 at rest
- Systolic and diastolic resting blood pressure
- Spirometry post-bronchodilator (FEV1, FVC, FEV1/FVC) according to the American Thoracic Society / European Respiratory Society (ATS/ERS) standards [23].
- Single breath diffusing capacity of the lungs for carbon monoxide (DLCO)

Exercise tests

1MINUTE SIT TO STAND TEST (1MSTS)

The 1MSTS test will be performed twice in the same visit but with sufficient recovery time (>30min) between. The test itself will be conducted using a chair of 45-48m height, without wheels, armrests and stuffing and with a straight back [24]. The chair will be placed in an area with sufficient space and with the back towards a wall for support. Standardized instructions will be given to all staff as well as a seminar with training and cases. Breathlessness will be recorded before the test, directly after the test and after 1 and 2 minutes. The study nurse will also record the total number of complete repetitions after counting manually. The test will be terminated at occurrence of any of the predefined termination criteria (adverse symptoms such as chest pain, nausea or vertigo), any worry for the safety of the participant (such as risk of falling) or if the participant needs to use their hands. In case of termination, the completed time and number of repetitions is recorded, and breathlessness, saturation and the reason for termination. Wearables such as sensors, portable VO_2 measurements and recording of heart rate, SpO_2 and other relevant physical aspects of exercise will be used during the test, as described below.

5 TIMES SIT TO STAND (5TSTS)

The 5TSTS measures lower limb strength and mobility [25]. Participants rise from a standard chair to a full standing position and sit back down five times as quickly as possible without using their arms. The total time to complete five repetitions is recorded. This test will be performed simultaneously as the longer 1 minute test. The time it takes for the individuals to rise the 5 first times will be recorded both manually and using the wearables described below.

WEARABLES DURING EXERCISE TESTS

To objectively quantify physical performance and investigate its relationship with breathlessness, we will use a wearable 9-degrees-of-freedom (9-DoF) inertial measurement unit (IMU) that includes a triaxial accelerometer, a triaxial gyroscope, and a triaxial magnetometer. The use of this type of device in research has grown in recent years, and have been used in multiple different areas, but not specifically for 1MSTS or 6MWT testing [26].

The device will be placed at belt level on the side of the body, close to the iliac crest. This placement provides a stable and unobtrusive position for capturing dynamic trunk movements during stand-up activity.

Collected movement data will be used to detect the upward and downward phases of each repetition and to derive detailed performance metrics. In addition to total repetition count over 60 seconds, sensor-derived parameters will include time to complete the first five repetitions, performance at 30 seconds,

repetition cycle duration, peak and mean movement velocity, and changes in velocity across the test to quantify fatigability. These digital metrics will allow exploration of alternative and extended sit-to-stand performance outcomes beyond total repetition count. Sensor-derived parameters will be analysed in relation to self-reported breathlessness (Borg CR10 or other validated 0-10NRS), physiological responses, and estimated mechanical work to determine whether movement dynamics provide additional clinically relevant information regarding functional limitation and exertional breathlessness. In addition, participants will be equipped with other suitable and commonly used wearables (such as pulse-band, ring, portable VO_2 equipment or watch) for assessment of heart rate, Sp02, estimated V'O2 and other aspects of exercise during both the 6MWT and the 1MSTS. These recordings will aid in further comparison the findings with the CPET examination (which also collects these data).

This approach aims to establish wearable sensor–based analysis as an efficient and scalable method for enhanced sit-to-stand assessment while investigating how breathlessness influences movement quality, endurance, and intra-test performance dynamics.

INCREMENTAL SYMPTOM-LIMITED CPET

The CPET is a standardized, progressive exercise test designed to evaluate cardiopulmonary function and exercise capacity under controlled conditions [27]. The test will be performed on a cycle ergometer with continuous monitoring of respiratory gases, heart rate, blood pressure, and oxygen saturation. The incremental CPET will consist of a 2-min resting period, followed by 3-min of unloaded pedalling (warm-up) and then using a suitable ramp protocol until the participant reaches their symptom-limited maximum effort or any of the predefined safety criteria (adverse symptoms such as chest-pain, abnormal ECG changes or dizziness). The ramp will be individually calculated based on expected work rate to achieve a test duration of approximately 7-11 minutes to symptom-limitation. The test is ended with a recovery phase on the bike of 2 min. Heart rate and ECG will be monitored throughout the test and blood pressure will be measured at regular intervals by study nurse. Breathlessness intensity and unpleasantness as well as leg-fatigue (Borg CR10 scales or other validated 0-10 NRS) will be assessed before the test, every 2 minutes during the test, at the end of the test and after 1 and 2 minutes.

THE SIX-MINUTE WALKING TEST (6MWT)

The 6MWT is a standardized submaximal exercise test used to assess functional exercise capacity and breathlessness [28]. Participants are instructed to walk back and forth along a flat, straight corridor of 20 or 30 meters for six minutes (both lengths are validated [29]), aiming to cover as much distance as possible at their own pace. They may slow down or stop if necessary but should resume walking as soon as they are able. The total distance walked in six minutes is recorded in meters.

The test will be conducted indoors on a marked 20 or 30-meter walkway. Participants will receive standardized instructions and encouragement according to international guidelines (ATS/ERS) [30, 31].

Heart rate, oxygen saturation, perceived exertion (Borg scale or other validated 0-10NRS) and perceived breathlessness (Borg Scale or other validated 0-10NRS) will be measured before and immediately after the test. Safety measures include monitoring for symptoms such as chest pain, severe breathlessness, or dizziness. The test will be stopped if adverse symptoms occur.

1. Registry Follow up

Participants may be followed for up to ten years using national or regional health registers to collect information on clinical outcomes, hospitalizations, medication use, and mortality. This allows long-term monitoring of health status without additional burden to participants. All data will be handled in accordance with applicable data protection regulations, and only de-identified or pseudonymized data will be accessed by the research team.

6. Data Management and Analysis

a. Data Handling and storage

All collected data will be handled in accordance with the General Data Protection Regulation (GDPR) and Swedish data protection laws. Personal identifiers will be replaced with a unique study code to ensure pseudonymization. The key linking codes to personal data will be stored separately in a secure, password-protected file accessible only to authorized study personnel. Research data will be stored on encrypted servers provided by the institution, with regular backups and restricted access. No data will be transferred outside the EU/EEA.

b. Case Report Form

An electronic Case Report Form (eCRF) is used for data collection (REDcap). The investigator must ensure that data is registered and any corrections in the eCRF are made as stated in the study protocol and in accordance with the instructions. The investigator must ensure that the registered data is correct, complete, and that reporting takes place according to the timelines that have been predefined and agreed. The investigator signs the completed eCRF. A copy of the completed CRF will be archived at the study site.

If an examination/test is not performed and data does not exist, ND (Not done) or NK (Not known) is marked. If the question is irrelevant NA (Not applicable) is written. Corrections in the paper CRF are done by striking out the incorrect information and adding the correct information next to the incorrect information, signing, and dating the correction.

Data from the CPET is recorded through the Vyntus software installed on a computer without internet connection and coupled with the CPET equipment. The same method is frequently used in clinical care and in other research projects. CPET-data is extracted from the software using imbedded functions and exported in .csv or excel format for further processing in separate statistical software.

c. Statistical analysis plan

A detailed statistical analysis plan will be established before each analysis in collaboration with a biostatistician. Patient characteristics and data will be summarized using mean with standard deviation (SD) and median with range or interquartile range (IQR) for continuous variables with normal and skewed distribution, respectively. Categorical variables will be expressed as frequencies and percentages.

Abnormally high exertional breathlessness will be defined as a validated 0-10 NRS scale breathlessness intensity rating at peak exercise of more than upper limit of normal in relationship to peak V′O2 and peak V′E, using published normative reference equations. (REF)

Validity/ correlation

The primary validity analysis will focus on the correlation. Both will be measured using the Borg CR10 scale or another validated 0-10 NRS. Pearsons's correlation coefficient will be used, if results seem normally distributed (expected). Correlation coefficients will be reported with 95% confidence intervals. Additional validity analyses will explore correlations between breathlessness scores from the 1MSTS and other established measures, including breathlessness scores after the 6MWT, health-related quality of life instruments and physiological markers from CPET such as VO_2-peak and VE/VCO_2 slope. All analyses will be adjusted for possible confounding effects from age and sex.

Test- retest reliability

To assess the consistency of the 1MSTS test, test–retest reliability will be evaluated. Participants will perform the test on two separate occasions during the same visit and under similar conditions. The intraclass correlation coefficient (ICC) with 95% confidence intervals, will be calculated

Responsiveness

To evaluate the ability of the 1MSTS test to detect clinically meaningful changes in breathlessness over time (MCID defined as >1 point on Borg rating [32] or on other validated 0-10NRS), regression analyses will be conducted. Changes in breathlessness scores between visit 2 and 3 will be analysed in relation to changes in other clinical and physiological outcomes. Standardised response means and effect sizes will be calculated in accordance with established guidelines for responsiveness assessment.

Estimation of mechanical work during 1MSTS

To further characterize the physiological demands of the 1MSTS, mechanical work will be estimated using a simplified biomechanical model. The calculation will be based on participant body weight, chair height, and the number of completed repetitions, using the formula:

$$Work\ (J) = Body\ weight\ (kg) \times 9.81 \times Chair\ height\ (m) \times Repetitions$$

Average power output (Watt) will be derived by dividing total work by 60 seconds. This estimated power will be used to compare the mechanical demands of 1MSTS with those obtained during CPET and may serve as an additional parameter for assessing functional capacity and responsiveness.

Threshold Identification

The values obtained in 1MSTS testing will be compared to physiological responses obtained during CPET, including VO_2-max, RER, and heart rate. ROC curve analyses will be used to identify threshold values of power output or repetitions that best discriminate between normal and pathological breathlessness, as defined by CPET parameters and breathlessness questionnaires (mMRC, CAT). Results will also be compared with established reference values for breathlessness on CPET [33]. This approach may allow the identification of clinically relevant cut-off values for 1MSTS performance indicative of impaired functional capacity or disproportionate breathlessness.

Sensor-based measurement and extended analysis

In addition to manual counting, the 1MSTS will be recorded using wearable sensors (9-degrees-of-freedom inertial measurement unit) connected to a data acquisition system. This approach enables precise segmentation of the test which will be used to explore additional performance metrics, including changes in movement velocity and frequency between different phases of the test (e.g., first versus last 10-20 seconds). These parameters may provide novel insights into muscular endurance and fatigue patterns and will also be related to the breathlessness measurements. Potentially improving the characterization of functional capacity beyond total repetitions.

Data collected from the wearable 9-degrees-of-freedom (9-DoF) inertial measurement unit (IMU) will be processed to extract objective performance metrics, including repetition count, movement amplitude, and cycle duration. These continuous variables will be summarized using descriptive statistics (mean, standard deviation, median, interquartile range) and compared across subgroups defined by breathlessness severity (e.g., mMRC categories) using appropriate parametric or non-parametric tests. Correlation analyses (Pearson or Spearman) will be performed to examine associations between IMU-derived features and self-reported breathlessness scores (e.g., Borg CR10, mMRC). Additionally, multivariable regression models may be used to explore whether movement

characteristics predict perceived exertion after adjusting for potential confounders such as age, sex, and baseline lung function. This integrated approach aims to provide a comprehensive analysis of objective physical performance and subjective symptom burden

7. Ethics

a. Benefit-risk evaluation

The 1MSTS provides a quick, non-invasive and practical measure of lower limb strength and endurance, which is valuable for assessing functional capacity and monitoring progress in patients with respiratory or mobility limitations. The test is simple, requires minimal equipment, and reflects everyday movements, making it clinically relevant. The risks associated with the 1MSTS are minimal. Participants may experience temporary fatigue or mild discomfort in the legs, and there is a small risk of dizziness or loss of balance. These risks are mitigated by close supervision during the test and immediate termination if any adverse symptoms occur.

The 6MWT offers an objective measure of functional exercise capacity that closely reflects daily activities. It is non-invasive, widely validated, and provides important information about endurance and overall health status in individuals with respiratory or cardiovascular conditions. The risks of the 6MWT are low. Participants may experience shortness of breath, fatigue, or mild musculoskeletal discomfort during or after the test. Rarely, adverse symptoms such as chest pain or severe dyspnea may occur. These risks are minimized through continuous monitoring, standardized safety procedures, and immediate termination of the test if necessary.

CPET is considered a safe test to evaluate many symptoms, one of those being breathlessness. Various studies report complication rates at 2-5 per 100.000 tests. Most patients will not experience any symptoms during the test and serious complications are extremely rare. Possible symptoms that may arise are chest pain or heart rhythm disturbances that almost always disappear by themselves. If not, there will always be a physician there to react. Through pretest preparation and adherence to safety recommendations will help to minimize the risk.

Written informed consent will be obtained from all participants before commencement of the trial procedures. The participants will be generally informed on the trial's research aims but not specifically on the primary and secondary endpoints (such as exercise duration or breathlessness during the test), to avoid bias, from participants' expectations and motivation, on the trial results. The project will be evaluated and needs to be approved by the Ethical Review Authority before recruitment of the first participant.

b. Compliance with the protocol, GCP and regulations

The study will be performed in compliance with the study protocol, the Declaration of Helsinki, ICH-GCP (Good Clinical Practice) guidelines and current national and international regulations governing this clinical trial. This is to ensure the safety and integrity of the study participants as well as the quality of the data collected.

c. Ethical review of the study

The final study protocol for clinical trials must be approved, as a part of the application for a permit for clinical trials, by the Swedish Ethical Review Authority (Etikprövningsmyndigheten, EPM) before the trial can be conducted. The final version of the informed consent form and other information provided for participants, must be approved or given a written positive opinion by EPM. EPM must be informed of any changes in the study protocol in accordance with current requirements.

d. Procedure for obtaining informed consent

The principal investigator shall ensure that the participant is given full and adequate oral and written information about the study, its purpose, any risks and benefits as well as inclusion and exclusion criteria. Participants must also be informed that they are free to discontinue their participation in the study at any time without having to provide a reason. Participants should be given the opportunity to ask questions and be allowed time to consider the provided information. If the person chooses to participate, both the participant and the investigator shall sign the informed consent form. A copy of the participant information as well as the informed consent form shall be provided to the participant. The participant's signed and dated informed consent must be obtained before performing any study-specific activity in the study. Each participant who participated in the study will be identified by a participant number on a participant identification list. If new information is added to the study, the participant has the right to reconsider whether he/she will continue their participation.

e. Insurances

The Swedish healthcare regions have signed a patient insurance (Patientskade- försäkring) with Landstingens Ömsesidiga Försäkringsbolag, Löf.

8. Substantial changes to the study

Substantial changes to the signed study protocol are only possible through approved protocol amendments and by agreement from all responsible persons. Information on non-substantial changes should be clearly noted in the amended protocol.

In the event that substantial changes to the protocol (e.g., changing of the main objective, primary or secondary variables, method to measure the primary variable) will be made during the course of the study, approval from the Swedish Ethical Review Authority (Etikprövningsmyndigheten, EPM) shall be obtained before any changes are implemented.

Non-substantial changes will be recorded and later entered in documentation that is submitted, for example in any subsequent notifications of a substantial change or in connection with End of Trial reporting.

9. Notification of study completion, reporting, and publication

Within two years after the study is completed, the main results shall be analysed and published in international, peer-reviewed scientific medical journals.

10. References

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11. Attachments

See attached questionnaires.