Supplementary material for “Effect of PEP flute-selfcare versus usual care in early COVID-19: a non-pharmacological, open-label, randomised trial”

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CLINICAL STUDY PROTOCOL
[PEP flute-selfcare to prevent respiratory deterioration and hospitalization among COVID-19 patients: a randomized trial]

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TRIAL IDENTIFIER

Full title
PEP flute-selfcare to prevent respiratory deterioration and hospitalization among COVID-19 patients: a randomized trial

Acronym
PEP-CoV

Short title
PEP flute-selfcare to prevent respiratory deterioration

Health Research Ethics Committee Number

Clinicaltrials.gov Trial registration identifier and date

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Internal protocol number

Version number & Date

Revision history

| Version # | Issue date | List of major changes |
|-----------|------------|-----------------------|
| 1.0       | 04/06/2020 | Version for first submission for authority approval |
| 2.0       | 19/06/2020 | Version for second submission for authority approval |
| 3.0       | 14/07/2020 | Version for third submission to comply with authority approval H-20035929, dated 09/07/2020 |
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BACKGROUND

Infection with severe acute respiratory syndrome Corona Virus 2 (SARS-CoV-2) may result in only non-specific symptoms like fever, fatigue and dyspnoea or it may progress to severe pulmonary disease Corona Virus Disease 2019 (COVID-19) with reports of a median time from symptom onset of approximately 5 days (1). COVID-19 seems to damage the respiratory system due to an overreaction of the immune system with individual risk profiles of age and comorbidity (2). This may lead to acute respiratory distress syndrome (ARDS), an inflammatory reaction in the lungs including extravascular fluid distribution and atelectasis. In these cases, the median time from symptom onset to severe hypoxemia and intensive care unit (ICU) admission has been reported to be approximately 7-12 days (1). When critically ill, the intensive care treatment involves mechanical ventilation with high oxygenation and positive end expiratory pressure.

At present, the trajectory of disease is not easy to predict (1), and little is known of any measures or medication to alter the course of disease. Positive expiratory pressure (PEP) is used in chronic inflammatory pulmonary diseases like chronic obstructive pulmonary disease (COPD) because of the possible beneficial effects on lung function i.e. airway clearance techniques (ACTs) (3). Furthermore, ACT appears to be safe and the PEP flute has been shown to be as effective as other ACTs (3).

Most research about SARS-CoV-2 and COVID-disease relate to screening measures, development of vaccines and optimising treatment of hospitalised patients. It is likely that we may have this pandemic for several years until we have reached a high level of immunity in the population or a vaccine has been developed. Thus, there is a need of measures to help the SARS-CoV-2 infected individual at home to overcome the course of disease with less symptoms and strain. A PEP flute is feasible for home use and it is possible that regular use of PEP flute may prevent the progression of respiratory symptoms in non-hospitalized individuals with COVID-19 disease.
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**Evidence Base of the Trial**

Preliminary evidence of the trajectory of COVID-19 points out that there may be a window of opportunity for treatment e.g. with the antiviral drug, remdesivir. To be most effective, this should be early in the course of disease when the patient is hospitalized and in need of oxygen (4). When a COVID patient is hospitalized, also the use of airway clearance therapy e.g. positive expiratory pressure has been highlighted as very important measures to avoid a critical course.

Little is known of the potential effects of PEP flute other than related to COPD. Among patients with acute myeloid leukaemia (AML), patient-performed daily spirometry alongside lung training with PEP flute at least twice daily was superior to daily spirometry solely in preventing pneumonia (5). In the trial, 25 incidences of X-ray-verified pneumonia were reported throughout the study period affecting six patients from the intervention group and 17 patients from the control group. The difference in first pneumonia incidence between intervention versus control group was significant with a rate ratio of 3.004 (95 % confidence interval: 1.184-7.619, p = 0.021) (5).

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**Study Aims, Hypothesis and Objectives**

**Aim**

The aim of the present study is to examine the effectiveness on respiratory symptoms by regular use of PEP flute among SARS-CoV-2 infected, non-hospitalized patients.

The PEP flute-selfcare intervention is feasible and easy to use. If it proves to be effective, it will be easy to implement as a public health intervention. This may result in less sick leave and less strain for the individual and the family. Moreover, potentially less severe courses of COVID-19 disease will reduce the overall burden of the health care system and the society.

**Hypotheses**

We hypothesize that the PEP flute has positive effects on SARS-CoV-2 infected individual’s self-reported respiratory symptoms such as dyspnoea, coughing and perceived mucus clearance through beneficial effects on lung function and airway clearance (3). Furthermore, we expect a lower rate of hospitalization and use of antibiotics in the intervention group as compared to the control group.
Objectives
The primary objective of the study is to examine the effect of PEP flute use among SARS-CoV-2 infected, non-hospitalized patients on self-reported change in COPD Assessment Test (CAT) score during 30 days of follow-up.

The secondary objectives are to compare the development in hospitalization rates and use of antibiotics in the intervention group and the control group during the follow-up period.

Finally, potential subgroup effects by gender, age, comorbidity and BMI at study entry will be explored for all outcomes.

STUDY DESIGN

Trial Design
This study is designed as a randomised, controlled, open-label trial with two parallel groups and a primary endpoint of COPD Assessment Test (CAT)-score after 30 days of intervention.

Duration of study
For each study participant, the active intervention period is 30 days with assessments of primary outcome i.e. CAT-score after 30 days. However, the active intervention period is shortened and ended in case of hospitalisation within this period. This study period of 30 days is equivalent to a previously reported study of community-acquired pneumonia which also reported long-term health impairment at 90 days follow-up (6). Thus, in the present study, longer-term effects will be evaluated 3 and 6 months after baseline.

Patient involvement
Personal communication with convalescents by COVID-19 has contributed to the designing process of this study. Otherwise, since this is a new disease, we have not been able to involve patient partners in the development phase of the trial. We expect to involve patients partners i.e. patient organisations when disseminating the results. This also includes the implementation phase in case of an effectful intervention.
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**SELECTION AND ALLOCATION OF PARTICIPANTS**

**Number of participants planned**
The number of study participants to be randomised for this study is 400 based on a sample size calculation (see section 0). Randomised participants who withdraw during study participation or who are prematurely terminated will not be replaced.

**Diagnosis under study**
In ICD-11, the code for the confirmed diagnosis of COVID-19 is RA01.0 ([https://www.who.int/classifications/icd/covid19/en/](https://www.who.int/classifications/icd/covid19/en/)).

**Inclusion Criteria**
An individual will be eligible for study participation if he/she meets the following criteria:

1. Positive SARS-CoV-2 (information of these data from medical files provided from the microbiological departments in the Capital Region and Region Zealand with permission to contact individuals eligible for study participation given by Head of Departments in compliance with the Danish Health Act (‘Sundhedsloven’) § 46, stk. 1 and 3)
2. Symptoms of SARS-CoV-2 infection e.g. fever, cough and shortness of breath. Recent reports indicate the most frequent self-reported respiratory symptoms among COVID-19 positive (n = 308) to be cough (71%) and shortness of breath (54 %) (7).
   A screening manual has been developed and symptoms are asked for according to surveillance by the Danish Health Authority, the COVIDmeter ([https://beredskab.digst.dk/form/covid-19](https://beredskab.digst.dk/form/covid-19))
3. Access to use a smartphone
4. Can reply to a questionnaire (sent on email, SMS or via telephone interview) in Danish
5. Given informed consent (via REDCap)

**Exclusion Criteria**
A participant will be excluded from the study if he/she meets any of the following criteria:

1. Age < 18 years.
2. Any condition or impairment that, in the opinion of the investigator, makes a potential participant unsuitable for participation or which obstruct participation, such as psychiatric disorders.
3. Hospitalised patients or citizens living in nursing homes
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Allocation of participants and sequence generation
Randomization lists will be computer-generated using an appropriate statistical software and based upon permuted random blocks. The allocation ratio will be 1:1 stratified for the following baseline conditions:
- Sex
- Age (≥60 and <60 years)

Blinding
As this is an “open-label” trial neither the health professionals delivering the interventions, nor the participants will be blinded to treatment allocation.
Outcome assessors will be blinded to treatment allocation where possible. This is of outmost importance, and participants are requested not to disclose their allocation when outcomes are assessed. To test the blinding efficacy, the outcome assessors are asked what treatment strategy they think a patient has received after assessments.

STUDY TREATMENTS

[Treatment] The rationale for the potential effect of a PEP flute is a mechanically supported inflation of the alveoli and loosening of secretions (5). This may prevent atelectasis and lower tract infection thus the SARS-CoV-2 infection may be hindered to progress to COVID-19 with more severe lung infiltrates.

[Regular use of PEP flute in combination with standard care] We advise the participants allocated to the intervention group to use the PEP flute at least three times daily. Ideally, each session consists of 15 breaths (for approximately 1 minute) repeated twice at an upright position. The participants will be handed three airway resistances equivalent to a resistance of 10-20 cm H2O alongside one PEP flute. Two videos (to be developed) will guide the participants in use of the PEP flute; one with instructions of the rationale and how to use the flute, including how to choose the suitable resistance and one video, which gives instructions of hygienic maintenance.
Participants in the intervention group will be advised to continue use of their PEP flute in the active intervention period of 30 days or at least if they still have respiratory symptoms. They will receive daily text-messages to prompt their reporting of CAT-scores (by links to a questionnaire in
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REDCap) and to use the PEP flute according to instructions. Also, they will be asked to report their present choice of airway resistance and the number of PEP flute sessions the previous day. To avoid attrition of the trial due to early recovery of symptoms, the project manager will call the participants by phone at day 15 to ask about their present condition (i.e. CAT-score) and address potential concerns of continued participation in the trial.

[Control condition]

[Standard of care only]
The participants in the control group will receive daily text-messages to prompt their reporting of CAT-scores by links to a questionnaire in REDCap. To avoid attrition of the trial due to early recovery of symptoms, the project manager will call the participants by phone at day 15 to ask them about their present condition (i.e. CAT-score) and address potential concerns of continued participation of the trial. Otherwise, they will receive usual care.

Warnings

[Treatment]
The PEP flute is widely used as an airway respiratory technique and according to guidelines, the only reason for not using the PEP flute is the presence of pneumo- and/or haemothorax without adequate drainage (5, 8). It is not likely that any participant in the intervention is at home with an acute condition of pneumothorax. The study poses minimal risks to the patients as the intervention is harmless. The patients are instructed in using the PEP flute with a pressure of approx. 10 cmH2O. If the patients blow with full power, they might reach a pressure of approx. 50 cmH2O, whereas coughing generates a pressure in the lungs of 80-120 cm H2O (9). The use of a PEP flute is therefore considered safe for even the weakest patient with lung disease (10). Thus, we consider this to be a safe self-treatment. The participants allocated to the intervention group will be advised to stop the PEP flute session in case of any discomfort. They may contact the project manager or a designated hotline in such circumstance.

Hygienic maintenance of a PEP flute is usually recommended on a weekly basis. However, due to the manifest SARS-CoV-2 infection, we advise the participants to daily hygienic maintenance (i.e. video instructions).
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Treatment adherence
During the follow-up period treatment adherence will be assessed through daily self-reported use of the PEP flute in an administered questionnaire, prompted by text-messages. The advised dose is presented in section 8.1.1.

Concomitant Therapy
Standard care for SARS-CoV-2 infection.

OUTCOME ASSESSMENTS/VARIABLES

Primary Outcome
- CAT score
The primary outcome is the self-reported CAT-score. CAT is an acronym for COPD Assessment Test, and it is widely used in treatment of COPD e.g. both as a telemonitoring tool and as a means to stratify the patients into groups based upon the severity of symptoms (11). As part of the TeleKOL in Region Zealand, the patients in the most unstable phase of disease are encouraged to report their CAT-score daily (11) and it is considered quick and easy for patients to use (12). The latter is important to ensure adequate data collection among the cohort of SARS-CoV-2 infected, non-hospitalized patients in the present trial.

Secondary Outcomes
The following outcomes are assessed as secondary outcomes
- Hospital admissions
- Use of antibiotics in case of superinfection

Safety outcomes
- Number of participants with Serious Adverse Events (SAEs) during the 30-day intervention period.

COVID-19 is related to a high mortality in case of severe and critical disease. Among 55,924 laboratory-confirmed cases in China, 6.1% were classified as critical having respiratory failure, shock, and multiple organ dysfunction or failure (1). Also, coagulopathies of diverse aetiology have been described in critical COVID-19 cases as the SARS-CoV-2 infection itself promotes an immunological response (13). In the critical case pathway, when the pneumonia leads to ARDS
with a fatal outcome, this may also involve coagulation dysfunction. Furthermore, in ICU-treatment of extracorporeal membrane oxygenation (ECMO), deep attention to the coagulation profile during the ECMO should be considered (13). Hence, coagulopathies may be inevitable during a critical course of COVID-19. It is not likely that the PEP-intervention per se causes such physiological responses. Even among patients acutely ill with leukaemia and having neutropenia, no adverse events were detected related to PEP-usage (5). However, the participants in the intervention group are encouraged to inform the project managers in case of any adverse events during the trial. These adverse events will be documented and reported. Moreover, we emphasize that participants from both the control and the intervention group consult with their general practitioner in case of deterioration as they would do without trial participation.

Other outcomes
- Compliance assessment

**DESCRIPTION OF THE OUTCOMES**

[Self-reported CAT score]
The CAT-score is based on a validated questionnaire designed to evaluate symptoms in COPD patients (14). The CAT-scale is free of use by courtesy of GlaxoSmithKline. The eight items in the scale covers symptoms of cough, sputum, chest pain, dyspnoea, activities of daily living at home, feeling safe at home despite symptoms (modified for the present study from feeling safe at leaving home despite symptoms), sleep quality and vigour (12). The eight items sum up to a range of 0-40 with higher scores indicating more respiratory impairment. Although validated for COPD-use, the CAT-scale is considered useful in the present study because several of the items (dyspnoea, cough, fatigue, sputum and pleuritic chest pain) previously have been used as outcome variables in pneumonia studies (6). Based upon anecdotal evidence, a single course of COVID-19 disease revealed changes in CAT-score from CAT=5 prior to onset of disease to a peak of CAT=31 and a CAT=14 after a total of 40 days (ref: personal communication). Other COVID-19 convalescents report long term breathlessness, chest pain and fatigue (15, 16).

CAT-scores will be measured at baseline and after 30 days of intervention using a telephone administered questionnaire. Also, the participants in both the control and the intervention group are encouraged to self-report CAT-scores daily. A text-reminder function will prompt a direct link to the REDCap-database, where the CAT-scores may be reported. Participants in the intervention group are also asked about their use of the PEP-flute i.e. frequency and actual airway resistance. Long term follow-up of both groups at three months and six months will be conducted by use of a
similar follow-up questionnaire administered by telephone interviews. This will also address the CAT-score and issues related to self-reported sick leave and present health status.

[Hospital admissions]
Will be obtained from the Danish National Patient Registry, from which also data of comorbidity will be retrieved.

[Use of antibiotics]
Will be obtained from the Danish National Prescription Registry.

[Serious Adverse Events]
Assessed by Common Terminology Criteria for Adverse Event (CTCAE)

**PARTICIPANT SAFETY**

The investigator and research assistants will monitor each participant for evidence of adverse events (AEs) throughout the study. During all telephone outcome assessments, we will ask participants whether they have experienced any AEs. Moreover, we will encourage participants report AEs at any point during the trial period. The investigator will assess and record any AE in detail including the date of onset, description, severity, duration and outcome, relationship of the AE to study treatment, and any action(s) taken. AEs, whether in response to a query, observed by site personnel, or reported spontaneously by the participant will be recorded.

All AEs will be followed to a satisfactory conclusion.

**Adverse Event**

An AE is defined as any untoward medical occurrence in a study participant which does not necessarily have a causal relationship with the allocated treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the investigational treatment, whether or not the event is considered causally related to the treatment.

**Serious Adverse Events**

If an AE meets any of the following criteria, it is to be reported to the trial sponsor (The Parker Institute) as a serious adverse event (SAE) within 24 hours of the site being made aware of the SAE.

- Results in death
The intervention to prevent one of the other outcomes listed in the definition above.

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above. These should also in general be considered serious.

**Relationship to Study Treatment**

The investigator will use the following definitions to assess the relationship of the AE to the study intervention:

**Probably Related**

An AE has a strong temporal relationship to study treatments or recurs on re-challenge and an alternate aetiology is unlikely or significantly less likely.

**Probably Not Related**

An AE has little or no temporal relationship to the study treatments and/or a more likely alternative aetiology exists.

**Not Related**

An AE is due to an underlying or concurrent illness or effect of another exposure and is not related to the study treatments (e.g., has no temporal relationship to study treatments or has a much more likely alternative aetiology).

If an investigator's opinion of probably not, or not related to study drug is given, an alternate aetiology must be provided by the investigator for the AE.

**Adverse Event Collection Period**

All AEs reported from the time of randomisation until 30 days after randomisation will be collected, whether elicited or spontaneously reported by the participant.
Suspected unexpected serious adverse reaction (SUSAR)
A serious adverse reaction (SAR) can be a suspected unexpected serious adverse reaction (SUSAR), which means that it may or may not be related, but is unexpected, as it is not consistent with current information. If the Sponsor and Principal Investigator judge that a SAE is SUSAR they are responsible for reporting it to the Danish Health Authority and the local health research ethics committee. A SUSAR that is life-threatening must be reported to the national competence authority within 7 days (15 days in case of not life-threatening SUSAR).

DISCONTINUATION

Participant withdrawal
A participant may withdraw from the study at any time without this impacting on any future investigations and/or treatments at the site, by the Investigators in this study or by other staff associated with the study.
If a participant withdraws from the study, the procedures outlined for the closest assessment telephone call is sought to be completed within 1 week, and preferably prior to the initiation of another therapy. However, these procedures should not interfere with the initiation of any new treatments or therapeutic modalities that the investigator feels are necessary to treat the participant's condition.
It is important to avoid any lost to follow-up participants for the efficacy assessment and meaningful analysis of the study.

Individual participant discontinuation
The investigator may discontinue any participant’s participation for any reason, including an AE, safety concerns or failure to comply with the protocol.
Participants will be discontinued from the study immediately if any of the following occur:
- Clinically significant abnormal results or AEs, which rule out continuation of the study treatment, as determined by the investigator.
- Death
- Other illness
- Failure to adhere to the protocol.

If at any point in time from randomization to the end of trial the investigator feels that the patient’s clinical course is not acceptable within the normally applied paradigms, the patient should be taken out of the study. The clinician’s judgment will be required to decide on a case-by-case basis whether to implement this step or not.
It is important to avoid any loss to follow-up participants for the efficacy assessment and meaningful analysis of the study.

**Discontinuation of Entire Study**

The Sponsor has the right to terminate this study at any time. Reasons may include the following, but are not restricted to:

- The incidence of events in this or other studies that indicate a potential health hazard to participants.
- Unsatisfactory participant enrolment.

**STUDY PROCEDURES**

**Study schedule**

Study procedures will be performed as summarized in the visit and assessment schedule below.

All potential participants (see section 13.3.1) will receive oral information on the study, undergo screening (assessment of eligibility) and medical history assessment through telephone up to two days before randomisation.

CAT-scores will be measured at baseline and after 30 days of intervention using a telephone administered questionnaire (primary outcome), and daily tracking of CAT-scores will be collected via a text-message prompted and REDCap administered questionnaire during the active intervention period. Similarly, information on hospital admissions and use of antibiotics will be followed during the 30-day active intervention by use of registers. Moreover, potential longer-term effects on all outcomes will be evaluated 3 and 6 months after baseline through a telephone administered questionnaire and data from registers.

Additionally, for the intervention group only, compliance will be assessed through the daily text-messages prompted administered questionnaire and at the primary outcome assessment.
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| Study phases                  | Screening | Randomization (baseline) | Intervention | End of active intervention (primary outcome assessment) | 3-month Follow-up | 6-month Follow-up |
|-------------------------------|-----------|--------------------------|--------------|----------------------------------------------------------|------------------|--------------------|
| Day no.                       | -2-0      | 0                        | 0-30         | 30                                                       | 90               | 180                |
| Information                   |           |                          |              |                                                          |                  |                    |
| Eligibility screening         |           |                          |              |                                                          |                  |                    |
| Medical history               |           |                          |              |                                                          |                  |                    |
| Informed consent              |           |                          |              |                                                          |                  |                    |
| Demographic information       |           |                          |              |                                                          |                  |                    |
| CAT-score                     |           |                          |              |                                                          |                  |                    |
| Habits, sick leave            |           |                          |              |                                                          |                  |                    |
| Hospital admissions           |           |                          |              |                                                          |                  |                    |
| Use of antibiotics            |           |                          |              |                                                          |                  |                    |
| Compliance assessment         |           |                          |              |                                                          |                  |                    |

**Assessment visit windows**

The visit windows are as follows:

- Screening will be done no more than 2 days before randomisation
- Baseline measurements will be taken on the day of randomisation
- The 30-day outcome assessments can be taken within +/- 1 week for the scheduled visit
- The 3-month outcome assessments can be taken within +/- 1 week for the scheduled visit
- The 6-month outcome assessments can be taken within +/- 1 week for the scheduled visit

**Pre-screening procedures and recruitment**

Potential trial participants will be identified by the microbiological departments in the Capital Region and Region Zealand using patient records. No other information will be extracted from the patient records. Complying to the Danish Health Act (‘Sundhedsloven’) §46, stk. 1, information about identified eligible participants will be given to the investigator through daily reports from the microbiological departments of the Capital Region and Region Zealand.

Current expectations are that about 20-25 individuals will be tested positive/day in the two regions. Screenings suggest that about 70% have symptoms at diagnosis. A conservative estimate is that about 50% will accept the invitation to participate in the RCT. According to these numbers, 25 x
0.70 x 0.5 = 8.75 patients can be included per day. Hence, 1.5 months should be enough to recruit 400 patients at the current rate. We will allow for 4 months of recruitment, should these estimates not be conservative, but will terminate enrolment at n = 400.

Complying to ‘Sundhedsloven’ §46, stk. 3, when a potential trial participant i.e. an individual with a SARS-CoV-2 positive test, is identified by an investigator, written information material (Appendix) will be sent by use of the person’s e-Boks (Nordic provider of secure platforms and digital post-boxes). Furthermore, the potential participant is asked if he/she wishes to schedule an appointment for the oral information visit (see section 0) and/or a screening visit (see section 0). The potential participant can notify interest and give telephone number to the investigator via a link to a REDCap survey or he/she may telephone the project manager directly.

**Oral information telephone call and informed consent**

It has been decided that the oral information, consent and screening is provided over phone or Skype, Facetime, Microsoft Teams etc. to reduce unnecessary spread of the virus. The oral information telephone call will be organised with an investigator (or his/her delegate) (see section 0 and appendix). Potential participants have the right to have next of kin or another person of the participant’s choice to join him/her on the call.

The information will include that

- Participation in the trial is voluntary
- Participants have the right to 24 hours reflection time before deciding to sign the informed consent or not.
- Participants can at any time and without giving any reason, withdraw from the trial without affecting the potential participant’s right to current or future treatment.

Further, the oral information will include: aim, procedures, potential benefits and risks when participating in the trial, procedures for random findings during the project, procedures for securing the participants privacy and data protection, information on the trial organisation, funding, as well as contact information on the primary investigator and other key investigators.

The investigator will make sure that participants have received and understood the information given to them. Furthermore, the investigator will make sure that potential participants are aware that they have the right to minimum 24 hours reflection time before signing the informed consent.

However, if the participant expresses that he/she does not want to wait for 24 hours, consent may be signed without further reflection time. The written information material will be provided through e-Boks. Signed informed consent will be collected, by sending a REDCap survey to the participants E-box.
Screening call
Prior to the telephone screening call, written informed consent has been collected from the participant. The screening procedures will only be done upon signed informed consent.
At the screening visit the following procedures will be done in this order:
1. Provision of signed informed consent (see section 0)
2. Assessment of in- and exclusion criteria (see section 0)
Patients who meet all inclusion criteria and who do not have exclusions will be continued to a baseline assessment.

Baseline visit
During the baseline visit (by phone call), the following will be completed (see section 0 for detailed descriptions):
- Demographic information and health status self-assessment including height, weight, smoking and alcohol consumption
- CAT-score
- Randomisation
- Scheduling of treatment visits (calls) (including handout of a PEP-flute according to allocation) and follow-up visits (calls)

Day 30 call (primary outcome assessment)
At the day 30 visit the following will be completed (see section 0 for detailed descriptions):
- CAT-score
- Compliance assessment (intervention group only)
- Weight
- Smoking

3-month call
At the 3-month visit the following will be completed (see section 0 for detailed descriptions):
- CAT-score
- Compliance assessment (intervention group only)

6-month call
At the 6-month visit the following will be completed (see section 0 for detailed descriptions):
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- CAT-score
- Compliance assessment (intervention group only)

Study completion
The end-of-study is defined as the date of the last participant's last scheduled visit or the actual date of follow-up contact, whichever is longer.

DETERMINATION OF SAMPLE SIZE AND STATISTICAL ANALYSIS PLAN

Determination of Sample Size
Determination of sample size is based upon reported symptom scores in a previous study of community-acquired pneumonia (6). On a 0-100 value scale (higher values indicate more symptoms), the mean symptom score at time of diagnosis was 51.7 (SD 20.1). If we use these scores as reference, we assume the following values of CAT-score in the trial (the CAT scale runs from 0 (no symptoms) to 40 (maximum all symptoms):

Baseline T0 mean CAT 20 (± 10)

A minimal clinical reported difference (MCID) of 2 on the CAT scale has been reported on clinical studies of COPD rehabilitation (12). Based on this MCID and the assumed means scores of respiratory affections at baseline, we have estimated a need of including n > 141 in each group, Figure XX:

Follow-up 30 days T30 Intervention: mean CAT 10 (± 6)
                      Control: mean CAT 12 (± 6)

Significance level 5 %
Level of power 0.8

With consideration to potential dropouts in a heterogenous sample, we assess that inclusion of 200 participants in each intervention arm will be an adequate number.
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Sample size estimation based on programme found at:
http://powerandsamplesize.com/Calculators/Compare-2-Means/2-Sample-Equality

Study participants description

Disposition of participants
The number of randomized patients will be summarized as total and by site using counts and percentages. The number of patients either completing or permanently discontinuing the study will be summarized using counts and percentages.

Analysis Population Sets

Equivalence assessment
For the assessment of equivalence, we will use the per-protocol population (defined below) in the primary analysis, as it is the most conservative approach.
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Superiority assessment
For the assessment of superiority, we will use the intention-to-treat-protocol population in the primary analysis, as it is the most conservative approach.

Study Population definitions

As-observed population (AO)
The AO population consists of participants who has the outcome of interest assessed at any given time point of interest (i.e. no imputation of missing data will be done).

Per-protocol population (PP)
The PP population is defined as the AO population participants that adhere to this protocol, defined by the following criteria to the two groups:

Intervention:
- Have a baseline measure of outcome, AND
- Have a follow-up measure of outcome at the primary assessment call (day 30), AND
- Have complied to the intervention for as long as respiratory symptoms are still reported in the CAT-score
- Have no major protocol violations

Control:
- Have a baseline measure of outcome, AND
- Have a follow-up measure of outcome at the primary assessment call (day 30), AND
- Have no major protocol violations i.e. have not reported the use of a PEP-flute or treatment (related to the respiratory system) from a physiotherapist

Intention to treat population (ITT)
The ITT population consist of all randomized patients irrespective of whether the patient actually received study intervention or the patient’s compliance with the study protocol, in the treatment group to which the participant was assigned at randomisation. A patient will be considered randomised as soon as a treatment is assigned by according to the allocation sequence.

General statistical approach
A statistical analysis plan that describes the details of the planned statistical analyses will be produced before last patients last visit.
Assessments of changes from baseline and construction of CIs for continuous measures will be based on analysis of covariance (ANCOVA; including group as the main factor and baseline measure of outcome as covariate).

Superiority will be claimed if the computed 95% confidence interval of the estimated group difference in [primary outcome] does not include 0 in the ITT population.

All statistical tests will be two-sided and statistical significance will be claimed if the computed p-value is equal to or less than 0.05.

**Subgroup comparisons**

Interactions between intervention status and baseline participant groupings i.e. gender and age (and comorbidity, and BMI at entry, if possible) will be prioritised as a priori subgroup analyses for the primary and secondary outcomes. Age and BMI will be analysed as categorical variables to avoid assumptions of linearity (or the complexity of fitting polynomial terms) and for ease of presentation. These pre-planned analyses will be conducted by the inclusion of appropriate interaction terms in the regression models. Where interactions are significant, results for each subgroup will be reported. This will mitigate the problems of multiple testing due to many subgroup comparisons. We recognise that statistical power in subgroups will vary with sample size and provide plots of detectable effect size versus sample size for reference.

**Research Ethics**

**General considerations**

Prior to screening, all potential trial participants are informed, both orally and in writing, about the purpose of this trial, its process and potential risks, as well as costs and benefits of participation. In addition, the leaflet 'Rettigheder som forsøgsperson i et sundhedsvidenskabeligt forskningsprojekt' and 'Før du beslutter dig' will be sent to the e-Boks alongside the written information material. All participants are informed of their rights to withdraw from the study at any time without this impacting on any future investigations and/or treatments at any site or by some of the members of the study group. After the information is delivered, read and understood, voluntary informed consent is given by the participant by signing a consent form before trial participation can take place.
Oral information
When a potential participant contacts the trial, an appointment for an information interview is made. It will be stressed that the investigator is asking the participant to consider participation in the trial, and that the potential trial participant has the right to bring a companion to the information interview. The written information material has already been to the potential trial participant thus initiating a period of reflection as to participation. Additional reflection time of at least 4 hours is suggested to the potential participant.

The oral information is based on the written information and will be given in a language easily understood without technical or value-laden terms. The information will be given in a considerate way that is tailored to each potential trial participants. The aim is that the conversation takes place without interference. It is the responsibility of the interviewer to ensure that the potential trial participant has understood the information. The information interview is performed by the investigator or in his/her absence by a designated delegate. Guidelines for the oral information will be prepared and attached this protocol as an Appendix (document in Danish).

Written information
All written information material is prepared and attached this protocol as an Appendix (document in Danish).

Informed consent
Consent to participation in the trial is given based on the written and oral information.

An informed consent form (ICF; Appendix; document in Danish) has been prepared. The form must be signed and dated by the participants prior to participation in the trial by formula in REDCap. A copy of the form is provided to the participants i.e. sent to e-Boks. The investigator or his/her designated delegates can receive copy of the signed consent form. Prior to consent, it must be ensured that a potential participant has been given enough time to consider his or her participation. The source documentation and CRFs (case record forms) will document for each participant that informed consent was obtained prior to participation in the study. The signed ICF must remain in each participant’s study file and must be available for verification by study monitors at any time.
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Research ethics - the interventions

Regular use of PEP flute in combination with standard care
As mentioned in section 8.3.1, the use of PEP flute is considered a low risk intervention with no expected side effect. Since the interventions will be delivered in combination with standard treatment and we will be closely monitoring potential side effects, we anticipate no ethical issues. The intervention is considered justifiable in a health research ethics perspective.
An inquiry about the study has been directed to the Danish Medicines Agency, because the PEP flute is classified as a medical device. No approval from the Agency is needed since the flute is used for a purpose within the CE-classification (Agency reference number: 2020051572).

Standard care only
The intervention is considered justifiable in a health research ethics perspective.

Research ethics – the outcomes

Self-reported CAT score
CAT scores are measured through a very short questionnaire, with little burden to the participants. The intervention is considered justifiable in a health research ethics perspective.

Hospital admissions and use of antibiotics
Obtained from the Danish National Patient Registry and the Danish National Prescription Registry with no burden to the participants. The intervention is considered justifiable in a health research ethics perspective.

Health Research Ethical approval
This protocol, the informed consent form, written patient information, any anticipated advertising materials, and relevant supporting information will be submitted to the health research ethical committee system, by the Sponsor, prior to study initiation. The study will be conducted in accordance with Danish law, the Helsinki declaration, and health research ethics committee requirements.
The Sponsor is responsible for keeping the committee informed of amendments or changes to the protocol, and the progress of the study, as appropriate.
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## Source Documents and Case Report Forms

**Source Documents**

Source documents are defined as original documents, data and records. This may include hospital records, clinical and office charts, laboratory data/information, participants' diaries or evaluation checklists, health professionals’ records or charts, and other records, recorded data from automated instruments, mobile phone apps and images.

The investigator(s)/institution(s) will permit study-related monitoring, audits and regulatory inspection(s), providing direct access to source data documents.

**Case Report Forms**

The study will use electronic case report forms (eCRF) using an on-line web-based clinical trial management application (REDCap).

REDCap allows individual patients to supply data from home and clinical and data can be entered by staff who has been granted permissions. At the end of the trial, all data will be merged and stored in the REDCap application. The REDCap application meets all regulatory standards and allow management of all activities related to clinical trials that ensures optimal resource use and safety according to good clinical practice and data protection legislation.

## Regulatory Standards

**Participant confidentiality**

Participant medical information obtained by this study is confidential, and disclosure to third parties other than those noted below is prohibited.

With the participant’s permission, medical information may be shared with his or her personal physician or with other medical personnel responsible for the participant’s welfare.

If the data from this study are published, the presentation format will not include names, recognizable photos, personal information or other data which compromises the anonymity of participating participants.

**Data Protection Act and General Data Protection Regulation**

The study will be conducted in accordance with the Data Protection Act and follow the General Data Protection Regulation. The study data management and data security procedures are approved by the Regional Knowledge Centre on Data Protection Compliance (Videnscenter for
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Dataanmeldelser i Region Hovedstaden) on behalf of the Danish Data Protection agency (file no.: XXXX).

Quality assurance
All data will be entered into a study database for analysis and reporting. Any data captured electronically will be stored electronically in a separate database according to standard procedures at The Parker Institute. Upon completion of data entry, the databases will be checked to ensure acceptable accuracy and completeness. System backups and record retention for the study data will be consistent with The Parker Institute standard procedures.

Individuals involved in study evaluations will be trained to perform the efficacy and safety evaluations described in the protocol.

Financing and insurance information
The study is initiated by Annette Mollerup who has received funding from Innovation Fund Denmark (1,003,138 kr.) and The Danish Nursing Council (30,000 kr.) to complete this study. Moreover, the Parker Institute is supported by a core grant from the Oak Foundation. None of the investigators have conflicts of interests related to the funding of this study. This information is disclosed to all participants in the written information material.

All sources of support (including technical and financial support) provided for this study is disclosed in the written information material and in publication of the study results.

The participants are insured by the Danish Patient Insurance Association. Financing and insurance issues are addressed in the written information material.

Publication
Development of the core publication will be coordinated by the executive committee, whose membership includes investigators who provided significant input into study design, implementation, conduct and interpretation. Authors of publications related to this trial include the members of the executive committee and possibly other key study personnel (to be agreed upon by the executive committee) who has contributed significantly to the implementation and conduct of the study and non-site personnel who contribute substantially to the design, interpretation or analysis of the study and fulfil the requirements for authorship as recommended by the international committee of medical journal editors (ICMJE).
Development of secondary and/or sub-study publication(s) will be coordinated by the executive committee. A named author approach will be utilized (authors to be agreed upon by executive committee) under the criteria recommended by ICMJE as above. In accordance with the principles of the Helsinki declaration, all results of the study, positive as well as negative and inconclusive will be published.

Activities that alone (without other contributions) do not qualify a contributor for authorship include but are not limited to: acquisition of funding; general supervision of a research group or general administrative support; and writing assistance, technical editing, language editing, and proofreading. Those whose contributions do not justify authorship may be acknowledged individually or together as a group under a single heading (e.g. "Clinical Investigators" or "Participating Investigators"), and their contributions will be specified (e.g., "served as scientific advisors," "critically reviewed the study proposal," "collected data," "provided and cared for study patients", "participated in writing or technical editing of the manuscript"). Written permission to be acknowledged from all acknowledged individuals will be collected prior to submission of a manuscript for publication.

**COMPLETION OF THE STUDY**

The end-of-study is defined as the date of the last participant's last scheduled visit or the actual date of follow-up contact, whichever is longer.

**LIST OF APPENDICES**

- A Deltagerinformation
- B Screening ved inklusion
- C Informeret samtykke
- D Baseline spørgeskema
- E Skema til daglig selvrapportering (kontrol + intervention)
- F Follow-up spørgeskema (kontrol + intervention)
- G Rekrutteringsmateriale til opslag på www. Forsøgsperson.dk og/eller annoncer
- H Protokolresumé
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Testing for SARS-CoV-2 in Denmark

Testing is a key aspect of the fight against COVID-19 in Denmark. As other parts of the Danish health care service, SARS-CoV-2 testing is tax financed and free of charge for all citizens. Since the spring 2020, surplus testing capacity has been available for all citizens, which has generally ensured easy access to testing without having to provide an explanation or present a doctor’s referral. In the phase of active recruitment for the project, the period 6th October 2020 to 28th February 2021, the test capacity in Denmark with 5.7 million inhabitants had been up to 400,000 daily tests with both PCR tests and antigen tests. In the study were only included individuals with a positive PCR test.

The testing is organized in a ‘health care track’ as well as in a ‘societal track’. Individuals having symptoms of COVID-19 and in need of health care assessment are prioritised in the health care track, whereas the societal track in general is available for all individuals regardless of symptoms. All participants included in this study have been tested in one of the facilities at the “health care track”.

Oropharyngeal swabs were collected by health-care workers and the swabs were sent to the departments of Clinical Microbiology within six hours. The samples were subject to RNA extraction and real-time RT-PCR to detect the presence of SARS-CoV-2. In brief: The nucleic acids in the sample were extracted together with an internal RNA control, using magnetic silica particles, and transferred to a specific RT-PCR, targeting two separate gene segments.

Results from the PCR tests are reported electronically within 24 hours from the laboratory information systems to the official portal for the public Danish Healthcare Services, thus also to the individual. At the same time, the national body for COVID-19 contact tracking, the Danish Patient Authority, is electronically informed. Healthcare professionals can access all laboratory results through electronic healthcare systems. In the study, trial information and invitation to participate were sent to the individual’s e-Boks only after the positive test result had been sent through the abovementioned channels.
**Trial intervention and video-instructions**

The trial intervention was the regular use of a PEP flute as add-on to usual care. A kit of two PEP flutes and three airway resistances (yellow; 2·5 mm, blue; 3·0 mm, green; 3·5 mm, equivalent to a resistance of 10-20 cm H₂O) were handed to the participants by personal deliverance or day-to-day mail.

Steered by co-author, Linette M. Kofod, two videos were produced by the Communication Unit at Copenhagen University Hospital Hvidovre. Linette was also the health care professional, specialised in respiratory physiotherapy, who participated in the videos. Production took place at Hvidovre Hospital and was finalised around September 1st, 2020. The videos were sent to the participants in the PEP flute-selfcare group by e-Boks immediately after randomization and allocation to the intervention arm. Both videos had subtitles in Danish.

Video 1 (4:00 minutes) gave instructions as to the rationale and how to use the PEP flute including how to choose the suitable resistance according to respiratory functioning. The participants were instructed to use the PEP flute at least three times daily with the morning session being emphasised as particularly essential. Ideally, each session should consist of 10-15 breaths (for approximately one minute) and then repeated. The participant was advised to be sitting at an upright position.

Video 2 (1:19 minutes) instructed to hygienic maintenance, advised to be daily because of the manifest SARS-CoV-2 infection. Ordinary detergents for dishwashing were recommended, to be succeeded by disinfection with boiling water. For the purpose, a dishwasher could be used instead.

In the active intervention period, a designated hotline staffed by corresponding author, Annette Mollerup, was available to the participants 7 days a week throughout daytime. Participants from the PEP flute-self-care group calling because of experiencing PEP-related dizziness were advised to decrease their respiratory volume in the sessions by reducing the number of respirations in each session, as the dizziness presumably was consequential to momentarily hyperventilation. To compensate, they could then increase the number of sessions throughout the day.
Screenshots from the videos: lower row left from Video 2, otherwise photos are from Video 1.
COVID-19 related health care service in Denmark
According to COVID-19, individuals having been tested positive of SARS-CoV-2 are advised to self-isolate and pay extra attention to hygiene and cleaning. Through pamphlets from the Danish Health Authority, the individual is advised to contact by phone the doctor (general practitioner (GP) or medical helpline outside GP’s opening hours) if needed, in case of COVID-19 symptoms and feeling of illness. In these cases, the doctor can then assess whether an examination on the same day is needed. In such circumstances, this will take place at special, local COVID-19 assessment units.

The term, urgent care visits, used in this report, then refers to hospital admissions, check-ups at acute care departments or designated COVID-clinics i.e. the local COVID-19 assessment units. As part of follow-up at day 30, the participants were asked: Have you been admitted to the hospital because of COVID-19? If yes to hospital admission, the participants were asked: How many days did you stay in hospital?
STATISTICAL ANALYSIS PLAN

PEP flute-selfcare to prevent respiratory deterioration and hospitalization among COVID-19 patients: a randomized trial

Trial Registration

Clinicaltrials.gov Trial registration identifier: NCT04530435

Protocol Version and Date

This document has been written based on information contained in the study protocol version 3.0, 14/07/2020.

Statistical Analysis Plan Version and Date

Version 1.0
Date: March 30, 2021

Statistical Analysis Plan Authors

Annette Mollerup
Marius Henriksen
Sofus C. Larsen
Berit L. Heitmann
**CHANGE HISTORY**

| Protocol version | Updated SAP version | Section Number Changed | Description of and reason for change | Date changed |
|------------------|---------------------|------------------------|--------------------------------------|---------------|
|                  |                     |                        |                                      |               |
|                  |                     |                        |                                      |               |
|                  |                     |                        |                                      |               |
SIGNATURES

Approved by Study Investigator and Data Analyst Marius Henriksen

Marius Henriksen, Prof, PhD  
The Parker Institute  
Signature  
Date  
30-03-2021

Approved by Study Investigator and Trial Manager Annette Mollerup

Annette Mollerup, PhD  
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30-03-2021

Approved by Principal Investigator Berit L. Heitmann

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Approved by Study Investigator and Data Analyst Sofus C. Larsen

Sofus C. Larsen, PhD  
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PURPOSE
This statistical analysis plan (SAP) describes detailed aspects of data preparation and analysis and was set up before starting the final analysis. The SAP is based on the final trial protocol (Version 3.0, 14/07/2020).

STUDY SYNOPSIS

| Background and rationale: | Infection with severe respiratory syndrome Corona Virus 2 (SARS-CoV-2) may progress to severe pulmonary disease Corona Virus Disease 2019 (Covid-19). In case of a critical course a median time of 7-12 days from symptom onset to severe hypoxemia and intensive care unit (ICU) admission has been reported. Little is known of measures to alter the course of early-stage disease i.e. to prevent the need of hospitalisation. Positive expiratory pressure (PEP) by use of a PEP flute has been shown to prevent pneumonia among patients with leukaemia without any adverse events. This trial has been designed to compare the effects of a PEP flute-selfcare intervention to usual care on respiratory symptoms and hospital admissions in individuals infected with SARS-CoV-2. |
|---|---|
| Objectives: | **Primary objective:** To examine the effectiveness on respiratory symptoms by regular use of PEP flute among SARS-CoV-2 infected, non-hospitalized individuals with Covid-19 symptoms as compared to no intervention.  
**Secondary objectives:** To compare development in self-rated health and prevalence of symptoms related to Covid-19 between the intervention group and the usual care group |
| Outcomes: | **Primary outcome:** Change from baseline in the overall COPD Assessment Test (CAT) and in the respiratory subscale of the CAT-score at 30 days.  
**Secondary outcomes:** Self-reported hospitalisation related to Covid-19 infection. Change from baseline in Self-rated health (from habitual value) and presence of Covid-19 related symptoms at 30 days. |
| Study design: | The trial is a randomised, controlled, open-label trial with two parallel groups comparing the PEP flute-selfcare intervention and self-monitoring of symptoms with usual care and self-monitoring of symptoms. |
| Statistical considerations: | Primary analyses will be based on an intention-to-treat principle. Continuous scores will be analysed using repeated measures mixed linear models adjusted for baseline values of the scores, taking randomization stratification factors into account. Missing data will not be imputed. Dichotomous scores will be analysed using logistic regression models.  
Sensitivity analyses will be done on the per-protocol population and using data sets with missing data replaced using multiple imputation, and baseline observation carried forward.  
Adverse events will be presented in a descriptive way for both groups. |

For further details regarding the trial design, please see the protocol version 3.0, July 14, 2020.
INTRODUCTION

Background and rationale

In brief, infection with severe respiratory syndrome Corona Virus 2 (SARS-CoV-2) may progress to severe pulmonary disease Corona Virus Disease 2019 (Covid-19). In case of a critical course a median time of 7-12 days from symptom onset to severe hypoxemia and intensive care unit (ICU) admission has been reported [1]. Little is known of measures to alter the course of early-stage disease i.e. to prevent the need of hospitalisation. Positive expiratory pressure (PEP) by use of a PEP flute has been shown to prevent pneumonia among patients with leukaemia without any adverse events [2].

Most research about SARS-CoV-2 and Covid-19 relate to screening measures, development of vaccines and optimising treatment of hospitalised patients. It is likely that we may have this pandemic for some years until we have reached a high level of immunity in the population through vaccination. There is a need of measures to help the SARS-CoV-2 infected individual at home to overcome the course of disease with less symptoms and strain. Potentially less severe courses of Covid-19 may also reduce the overall burden of the health care system and the society. This trial has been designed to compare the effects of a PEP flute-selfcare intervention to usual care on respiratory symptoms and hospital admissions in individuals infected with SARS-CoV-2.

For more details about the background, rationale and evidence base of the trial, please see the protocol version 3.0, July 14, 2020.

STUDY METHODS

Trial Design

The trial is a single centre, randomised, parallel-group, open-label, superiority trial comparing a selfcare intervention (daily use of a PEP flute and self-monitoring of symptoms) as add-on to usual care with combined self-monitoring and usual care.

The trial is conducted among individuals with confirmed SARS-CoV-2 infection. Participants are at home without undergoing a medical examination at trial entry, but they all have Covid-19 related symptoms at inclusion. A planned total of 400 patients are randomly assigned to the intervention group testing PEP flute-selfcare or the usual care group. Both groups self-monitor their symptoms throughout the 30-day active intervention period.
**Study Objectives**

The primary objective of this trial is to examine the effect of PEP flute use among SARS-CoV-2 infected, non-hospitalized individuals with Covid-19 related symptoms on self-reported change in COPD Assessment Test (CAT)-score [3] during 30 days of follow-up.

The key secondary objectives are to compare the development in hospitalisation in the intervention group and the control group during the follow-up period. It was planned to retrieve hospitalisation from the Danish National Patient Registry to ensure data completeness despite attrition. However, due to difficulties with being granted access to the Danish National Patient Registry, these objectives will not be part of this statistical analysis plan. Instead, we have surveyed the participants about hospitalisation related to the Covid-19 infection, which we will report as a secondary outcome. Registry-based hospitalisation and mortality may be reported separately later if/when access to the registry is granted.

Our other secondary objectives include between-group differences of self-rated health and presence of Covid-19 related symptoms.

**Randomisation**

Randomisation is equal, i.e. on a 1:1 basis. Each randomisation is via minimisation incorporating a random element stratified for the following baseline conditions:

| Stratification factors | Criterion |
|------------------------|-----------|
| Sex                    | A binary variable (male vs female) according to the unique Danish personal identification number (odd number = male; even number = female) |
| Age                    | < 60 years and ≥ 60 years |

**Blinding**

This is an open-label trial. Participants and clinicians who deliver the interventions are not blinded. The statistical analyses will be performed blinded for group allocation.

**Sample Size and Power**

This trial was designed as a superiority trial. The sample size was calculated to test the superiority of PEP flute-selfcare versus usual care in the assessment of change in CAT-score from randomisation to the end of the active intervention period, 30 days after allocation.
Determination of sample size was based upon reported symptom scores in a previous study of community-acquired pneumonia [4]. On a 0-100 value scale (higher values indicate more symptoms), the mean symptom score at time of diagnosis was 51.7 (SD 20.1). We used these scores as reference and assumed the mean baseline CAT-score in the trial (the CAT scale runs from 0 (no symptoms) to 40 (maximum all symptoms) to equate to 20 (shown in table below).

Hypothesised CAT-scores at baseline and at 30-day follow-up

|                                | Intervention group | Usual care group |
|--------------------------------|--------------------|------------------|
| CAT-score at baseline $T_0$    | mean (SD)          | 20.0 (10.0)      | 20.0 (10.0)      |
| CAT-score at follow-up $T_{30\text{days}}$ | mean (SD)          | 10.0 (6.0)       | 12.0 (6.0)       |

**Statistical power calculation for potential superiority claim**

We assume that the intervention group will improve in CAT-score to a mean value of 10 (SD 6.0) after 30 days of active intervention. A minimal clinical important difference (MCID) of 2 on the CAT scale has been reported on clinical studies of COPD rehabilitation [5]. Based on the assumed means scores at baseline, we have estimated a need of including n > 141 in each group to detect this MCID at a significance level of $p < 0.05$ and level of power of 80%.

In the absence of a feasibility study and only sparse evidence of Covid-19 symptom severity at early-stage disease, and with consideration to potential dropouts in a heterogenous sample, we assessed that inclusion of 200 participants in each intervention arm would be an adequate number. Sample size estimation was calculated on programme found at http://powerandsamplesize.com/Calculators/Compare-2-Means/2-Sample-Equality.

**Framework**

This is a superiority trial.

**Statistical Interim Analyses and Stopping Guidance**

No statistical interim analysis has been planned. In the trial design, there was no guidance for stopping the trial. However, because of successful epidemic control in Denmark the number of SARS-CoV-2 infected individuals was considerably reduced from the beginning of 2021. Hence, enrolment to the trial was stopped by February 28, 2021 after including 378 participants. We chose this approach because at this point, the estimated sample size of n > 141 in each group as regards the primary outcome had been reached.
Timing of Final Analysis

Final analysis will take place in two steps: The first (and main) report/publication of the trial will be prepared for the between-group comparison when every trial participant has completed the day 30 assessment and data for the self-reported secondary outcomes have been cleaned (April 2021). High research activity related to the ongoing pandemic generates latency in the deliverance of register data from the Danish Health Authorities. Hence, this statistical analysis plan only relates to the primary and secondary outcome data, self-reported by participants. The second publication of results based on the key secondary outcomes will be prepared for the between-group comparison when register data have been received and cleaned (anticipated to be May 2021).

Timing of Outcome Assessments

The schedule of study procedures and visit windows are given in the table below.

| Day                          | Screening | Baseline | Daily self-report | Follow-up |
|------------------------------|-----------|----------|-------------------|-----------|
| Positive PCR test            | ●         |          |                   |           |
| Written information via electronic mailbox | ●         |          |                   |           |
| Eligibility criteria         | ●         | ●        |                   |           |
| Informed consent             | ●         |          |                   |           |
| Randomization                | ●         |          |                   |           |
| Outcomes                     |           |          |                   |           |
| CAT score                    | ●         | ●        | ●                 |           |
| Self-reported hospitalization for Covid-19 | ●         |          | ●                 |           |
| Symptoms within last week    | ●         |          | ●                 |           |
| Self-reported overall health | ●         |          | ●                 |           |
OUTCOMES

Considerations of Covid-19 related symptom severity in course of disease

Eligible participants in the trial are individuals having a confirmed SARS-CoV-2 infection and symptoms of Covid-19. We do not conduct a medical examination as part of screening or baseline assessments. Days from symptom onset to time of screening are asked for and should not exceed more than 14 days to correspond to the purpose of prevention and not rehabilitation. At least one respiratory and one general symptom known to be related to Covid-19 are needed to be included in the trial.

Covid-19 is a new disease thus no symptom severity measurements were accessible and applicable in the design phase of the trial. We assess symptom severity by use of the CAT-score although this measurement is validated for COPD assessment only. This calls for special attention in the analytic phase. At inclusion, the CAT-score is assessed but no cut-off level of CAT-score has been determined prior to study initiation. We expect the study population to be heterogeneous as regards symptom severity thus some participants may deteriorate throughout the 30-day follow-up period while others may recover entirely shortly after trial enrolment.

Primary outcome

The primary outcome is assessed at 30-day follow-up as change from baseline in the COPD Assessment Test (CAT) score. The CAT-score is widely used in treatment of COPD both as a telemonitoring tool and as a means to stratify the patients into groups based upon the severity of symptoms. Even among patients in the most unstable phase of COPD, the CAT-score is considered quick and easy for patients to use [6,7].

Secondary outcomes

The following outcomes are assessed as other secondary outcomes:

- Self-reported hospitalisation for Covid-19 during the study participation (assessed at day 30).
- Presence of Covid-19 related symptoms at day 30
- Change from baseline (habitual prior to symptom onset) in self-rated health at day 30
Exploratory outcomes

As an explorative approach in the absence of a validated Covid-19 symptom severity score, four items covering symptoms from the respiratory organ system will constitute a specific respiratory subscale of the CAT-score.

Definition of outcome variables

COPD Assessment Test (CAT)-score

The COPD Assessment Test (CAT) is an instrument designed to evaluate symptoms in COPD-patients [3]. The CAT-score consists of 8 items covering both respiratory symptoms, namely, *Cough, Phlegm* (mucos), *Chest tightness* (pain) and *Dyspnoea* (shortness of breath) and limitations of functioning, namely, *Limitations of activities at home, Feeling safe at home despite symptoms* (modified for PEP-CoV trial from ‘Confidence at leaving home despite symptoms’), *Sleep Quality* and *Energy*. The CAT uses a six-point differential scale scoring system with each item ranging from 0 (no symptom/limitation) to 5 (most severe). Thus, the CAT-score has a range from 0 to 40, with higher scores representing worse health.

In development and validation of the CAT, special attention was given towards possible floor and ceiling effects [3]. A floor effect was defined as > 25% of patients indicating that they did not experience the symptom or health state. Of the eight items in the CAT, the item ‘I am confident leaving my home despite my lung condition’ demonstrated a high floor effect (42%). Because this item would not be relevant to assess in a course of Covid-19 with the individual obliged to stay in self-quarantine at home, we modified it for the PEP-CoV trial. In the analysis, we will thoroughly assess if a similar floor effect is seen despite the change in wording. An interpretation of such a floor effect would be that the item discriminates better in cases of higher symptom severity [3]. As regards the conceptual properties of this item, we consider the item still to measure the experienced psychological impact imposed by the overall symptom severity.

In the validation study, a ceiling effect was defined as > 25% responding to the most severe impact of the item on the scale. The item ‘Breathlessness when walking up a hill or one flight of stairs’ tended to show a high ceiling effect suggesting that this item has greatest discriminant power for participants with milder symptom severity [3]. In the analysis, we will thoroughly assess if an equivalent ceiling effect is seen as regards this item.

In COPD assessment, the CAT must always be used in its entirety. According to guidelines on missing data, if more than two responses are missing, a score cannot be calculated. When one or two items are missing, their scores can be set to the average of the non-missing item scores [3,7]. This approach will be applied in the data analysis of the PEP-CoV trial. Because of the uncertainty
inherent using a measurement validated for other purposes, we will both calculate the primary outcome, the overall CAT score, as well as a subscale consisting of the four items Cough, Phlegm (mucos), Chest tightness (pain) and Dyspnoea (shortness of breath) i.e. a respiratory symptom-score. This respiratory symptom-score will range from 0 (no symptoms) to 20 (highest severity) and will be treated as an exploratory outcome in this trial.

**Self-reported hospitalisation**

As part of the survey used to collect other outcome data at day 30, we asked the participants if they have been hospitalised for covid-19 since start of the trial.

The exact phrase is in Danish, but the authors own translation of the question is: “Have you been admitted to a hospital for your Covid-19 disease?” with answer options “Yes”, “No”, “Do not wish to answer”.

If the participants answer yes, they are asked to provide the length of the stay.

The exact phrase is in Danish, but the authors own translation of the question is: “How many days were you admitted?”

**Presence of Covid-19 related symptoms**

At baseline as part of the eligibility screening, participants are asked about the presence of Covid-19 related symptoms within the last week. The symptoms are divided in two categories i.e. respiratory symptoms and other symptoms and at least one symptom from each category is requested to be included in the trial. The symptoms list are based upon surveillance data from the Danish Health Authorities, the COVIDmeter, a digital platform for the citizens’ voluntary reporting of symptoms throughout the pandemic [8]. Respiratory symptoms include chest pain, runny/stuffy nose, sneezing, sore throat, shortness of breath, and cough. Other symptoms are nonspecific and include chills, headache, muscle/joint pain, fatigue, lost/ altered sense of taste, lack of appetite, nausea/loathing for food, vomiting, diarrhoea, abdominal pain, pain at micturition, reddened or itchy eyes, lost/ altered sense of smell, fever or fever sensation, and feeling of illness [8]. The symptoms are asked for at the 30-day follow-up. The outcome then is the between-group difference in prevalence of symptoms at follow-up.

**Self-rated health**

Self-rated health is measured by one single item on a five-point Likert scale (*In general, would you say that your health is excellent-very good-good-fair-poor?*) as a baseline score (habitual self-rated...
health), assessed retrospectively as the health state prior to the SARS-CoV-2 infection. The self-rated health state is reassessed at the 30-day follow-up as current health state.

**Adverse and serious adverse events**

The investigators monitor each participant for evidence of adverse events (AEs) and serious adverse events (SAEs) throughout the study. As part of the trial information, the participants allocated to the intervention group are encouraged to contact the project via a designated hotline in case of any event or user uncertainty related to the PEP flute use. The participants in both the usual care group and the intervention group are contacted via telephone or text messages approx. day-15 as a check-up regarding their health status.

The investigator will assess and record any AE and SAE in detail including the date of onset, description, severity, duration and outcome, relationship to study treatment, and any action(s) taken.

The project manager will adjudicate all reported AEs and SAEs and confer with the physician having the medical responsibility in the trial, if needed.

**DATA MANAGEMENT**

**Data validation**

All variables used in the database, including derived variables, will be checked for missing values, outliers and inconsistencies. We do not expect many faulty data points because error checks and warnings were implemented into the eCRF and database system and all data are handled electronically. The trial is internally monitored.

The primary outcome, CAT-score, is assessed at day-30 both as a self-reported value and as part of the 30-day follow-up call. The latter outcome assessment can be taken within + 1 week for the scheduled visit.

**Data preparation**

**Changes from baseline**

The primary outcome is change from baseline in CAT at day 30. This will be calculated for each participant as the day 30 value subtracted from the baseline value:

\[
\text{CAT}_{\text{change, day30}} = \text{CAT}_{\text{day30}} - \text{CAT}_{\text{baseline}}
\]
Thus, a negative change value indicate that the baseline value is greater than the day-30 value, which suggest an improvement in the CAT-score (= reduced symptom severity).

The SARS-CoV-2 pandemic causes Covid-19, which is a new disease meaning that we do not fully understand the trajectory of the disease. Participants may be enrolled in the trial at different points during their course of disease. The abovementioned calculation of change in primary outcome is based upon the assumption that participants are enrolled at the time of highest symptom severity. It is, however, likely that many participants will be included in the trial very early in the disease trajectory thus their symptom severity may peak after enrolment.

TRIAL POPULATIONS

Participant flow

A CONSORT participant flow diagram will be drawn following the CONSORT standards (see 12.1 Shell Figure 1).

The flow diagram will be used to summarise the number of patients who were:

- tested positive for SARS-CoV-2 infection in the Capital Region and Region Zealand from Oct 6, 2020 to Feb 28, 2021 (according to the surveillance data from the Danish Health Authorities)
- sent an invitation to possible trial participation
- requesting contact and further information about the trial
- assessed for eligibility at screening by telephone
- ineligible at screening*
- eligible and randomised
- allocated to each intervention
- received the randomly allocated intervention
- did not receive the randomised allocation*
- discontinued the intervention*
- lost to follow-up at day 30*
- randomised and included in the intention-to-treat population
- randomised and included in the per-protocol population

*reasons will be provided.
Intention-To-Treat population

The Intention-To-Treat (ITT) population consist of all randomised participants irrespective of whether the patient actually received study intervention or the patient’s compliance with the study protocol, in the treatment group to which the participant was assigned at randomisation (i.e. referring to the ITT principle). A participant will be considered randomised as soon as a treatment is assigned according to the allocation sequence.

The participant demographics and baseline data for the ITT population will be summarised in a table (shell Table 1). Participants will be described with respect to baseline age, sex, height, body mass index, CAT-score at baseline, respiratory symptoms within last week, other symptoms within last week, days from onset of symptoms to baseline, self-rated health prior to Covid-19, smoking habits and alcohol intake, separately for the two groups.

Continuous data will be summarised by means and SDs. Categorical data will be summarised by numbers and percentages. Tests of statistical significance will not be undertaken for baseline characteristics; rather the clinical importance of any imbalance will be noted.

Per Protocol Population

The per protocol (PP) population consists of all participants in the ITT population who did not have any major protocol deviations that could make the interpretation of analyses on the ITT population difficult.

The following are pre-defined major protocol violations with a direct bearing on the primary outcome:

- Not adherent to the allocated intervention (see below for definition of adherence) unless non-adherence is due to adverse reactions\(^\text{a}\) to study treatment
- For the intervention group: non-adherent to the intervention for as long as respiratory symptoms are still reported in the CAT-score
- For the usual care-group: reported the use of a PEP-flute or treatment (related to the respiratory system) from a physiotherapist
- 30-day CAT-score not self-reported or 30-day follow-up not completed within + 7 days of the specified time window

\(^{a}\) an adverse reaction is defined as an adverse event that is deemed related to the study treatment by an investigator.
Midst in the trial period, the vaccines from Pfizer-Biontech, Moderna, and later, AstraZeneca were launched in Denmark. As these vaccinations all have side-effects similar to Covid-19 related symptoms, the participants are asked about their vaccination status (binary variable, yes/no) at follow-up day-30. Participants having had vaccinations within the 30-day follow-up period are not considered part of the per protocol population.

The number (and percentage) of patients with major protocol deviations will be summarised by treatment group with details of type of deviation provided. The number of randomised participants in each group will be used as the denominator to calculate the percentages. No formal statistical testing will be undertaken.

**Adherence**

For each trial participant allocated to the PEP flute intervention, adherence is assessed based on the self-reported daily number of PEP flute-sessions. The PEP flute is recommended to be used at least three times daily for as long as respiratory symptoms are present. Ideally, each session consists of 10-15 breaths repeated twice. However, ≥ one daily session of PEP flute use while still having respiratory symptoms will be considered as adherence to intervention allocation. Due to technical irregularities in the planned automated text messages meant to prompt daily self-reports of CAT-scores and PEP flute-usage, adherence to treatment allocation in the intervention group will also be evaluated based on participants’ response to a question of frequency of PEP flute use at the 30-day follow-up (several times a day, at least one time daily, less than one time daily, less than one time weekly, have tried the PEP flute but not used it regularly, never used).

The participants in the usual care group are asked specifically about any use of a PEP flute during the active intervention period whether this has been self-initiated or prescribed as part of medical treatment, as a binary variable.

Descriptive statistics on the percent compliance will be summarized. Also, the number and % of participants non-adherent to allocation group will be presented by treatment group.

**Safety population**

The safety population consists of all participants in the ITT population who have used the PEP flute at least once (PEP flute group) and all the participants in the usual care group.

**STATISTICAL ANALYSES**
**General considerations**

The statistical analyses will be performed blinded to the treatment allocation.

**Missing Data and Robustness**

Our primary (efficacy) analyses will be based on the ITT population, including all randomised participants with available data at baseline. For the continuous primary outcome, missing data will be handled indirectly by statistically using Mixed Linear models based on the repeated-measurements framework. These models are generally considered valid if data are ‘Missing at Random’ (MAR); i.e. where “Any systematic difference between the missing values and the observed values can be explained by differences in observed data”. Contrasts between groups will be estimated based on repeated-measures mixed linear models across all timepoints (i.e., with explicit estimates derived at day 30 from baseline).

Robustness is a concept that refers to the sensitivity of the overall conclusions to various limitations of the data, assumptions, and analytic approaches to data analysis. Robustness implies that the treatment effect and primary conclusions of the trial are not substantially affected when analyses are carried out based on alternative assumptions or analytic approaches.

Loss to follow-up and missing data for various reasons is difficult to avoid in randomized trials and in particular in pragmatic trials. We will apply an analysis framework in which missing data related to the ITT approach depend on making plausible assumptions about the missingness of the data and including all participants in subsequent sensitivity analyses; we will:

1. Attempt to follow up all randomized participants, even if they withdraw from allocated treatment (i.e., contact all individuals unless they explicitly stated that they had withdrawn their consent)
2. Perform a main analysis of all observed data that are valid under a plausible assumption about the missingness of the data (i.e., Model-based: data as observed; using linear mixed models, assuming that data are ‘Missing at Random’ [MAR])
3. Perform sensitivity analyses to explore the effect of departures from the assumption made in the main (#2) analysis (i.e., conservative imputation methods of missing data; these models will potentially be informative even if data are ‘Missing Not At Random’ [MNAR])
4. Account for all randomized participants, at least in the main and sensitivity analyses (covered by #2 and #3 above, plus the corresponding analyses based on the per protocol population).
For sensitivity analyses (see below) we will use multiple imputation techniques for repeated replacement of missing data at day 30. We will use baseline variables, outcome measures at day 30, and group allocation as predictors in the imputation models.

The primary analysis will be repeated based on participants in the ITT population, but by imputing missing postbaseline observations at the day 30 follow-up (where the outcome was scheduled to be measured) using a multiple imputation procedure. The imputation will be performed according to the following steps:

1. Missing values are imputed based on observed data using a Markov Chain Monte Carlo method where 5 copies of the dataset will be generated;
2. For each of the 5 copies, missing values at day 30 will be analysed using an ANCOVA model including treatment group, and stratification factors as fixed factors and the baseline level as covariates;
3. From this repeated standard ANCOVA model, estimated differences between groups in each of the imputed datasets will differ (at least slightly) because of the variation introduced in the imputation of the missing values, and they are only useful when averaged together to give overall estimated associations. The corresponding standard errors will be calculated using Rubin’s rules, which take account of the variability in results between the imputed datasets, reflecting the uncertainty associated with the missing values. Valid inferences are obtained because we will be averaging over the distribution of the missing data given the observed data.

**Primary analysis**

Our primary analysis population will be all participants with available data at baseline, statistically modelled using repeated-measures linear mixed models (see above). These models will be valid if data are ‘MAR’.

The primary analyses will be conducted according to the ITT principle. The ITT principle asserts the effect of a treatment policy (that is, the planned treatment regimen), rather than the actual treatment given (i.e., it is independent of treatment adherence). Accordingly, participants allocated to a treatment group will be followed up, assessed and analysed as members of that group, irrespective of their adherence to the planned course of treatment (i.e., independent of withdrawals and cross-over phenomena). The primary and continuous secondary outcomes will be assessed using mixed linear models adjusted for baseline values and stratification factors.
Primary analysis of primary outcome

The primary outcome analysis will be a superiority analysis based on the ITT population, asking whether the PEP flute group is superior to the control group regarding change from baseline in CAT scores at the end of the treatment period (day 30). We will use a repeated measures linear mixed model regression analysis model adjusted for stratification factors and the baseline score of the CAT score. An interaction for time and group will be included.

\[
\text{CAT}_{\text{change}} = \text{GROUP} + \text{DAY} + \text{GROUP} \times \text{DAY} + \text{Stratification} + \text{CAT}_{\text{baseline}}
\]

Analyses will include all collected data, and effects will be estimated at day 30; missing data will be handled implicitly via the mixed methods (maximum likelihood) approach. From this model the observed differences in the change from baseline in CAT scores between groups at day 30 will be estimated together with the associated 95% confidence interval (and the p-value) corresponding to the test of the hypothesis of no difference between treatments.

Superiority will potentially be claimed if the computed 95% confidence interval of the estimated group difference in the change from baseline in the CAT scores at day 30 excludes the null.

Primary analysis of secondary outcomes

The primary analyses of the continuous secondary outcomes will be superiority analyses using the ITT population. Missing data will not be imputed but handled in mixed linear models. Continuous secondary outcomes will be analysed identically to the primary outcome and adjusted for the stratification factors and the respective baseline value if available. We will compute differences with unadjusted two-sided 95% confidence intervals interpreted based on the equivalence paradigm.

Dichotomous outcomes and handling of missing data: Categorical changes for dichotomous outcomes (i.e. self-reported hospitalisation and presence of symptoms) will be analysed with the use of logistic regression with the same fixed effects as the respective mixed linear models explained above; however, the models will only include data from the before-and-after setting (i.e. without a repeated measures analysis element) at day 30. For ease of interpretation the corresponding Odds Ratios will be converted back into Adjusted Risk Ratios and/or Risk Differences based on the number of observations in the control group.

Handling of missing data for the dichotomous outcomes: We will use a fixed-set multiple imputation framework for our missing dichotomous outcomes. For these binary outcomes, we will a use a methodology that imputes the extreme displays that reveal the effects of all outcomes for each randomised individual, using combinations of the values of missing data in the first arm (PEP flute group) and the second arm (control group); the imputation technique is based on the idea of 'tipping-point' (TP) analysis. We enhance this idea by formalizing the process of robust estimation
using a more detailed display in conjunction with multiple imputation (MI) of missing data. We will semi-automatically generate 5 individual data sets based on the following approach:

- Data as observed (including missing data)
- Worst \((Y_{Mis} = 0)\) AND Worst \((Y_{Mis} = 0)\) case imputation in each group, respectively
- Best \((Y_{Mis} = 1)\) AND Best \((Y_{Mis} = 1)\) case imputation in each group, respectively
- Worst \((Y_{Mis} = 0)\) AND Best \((Y_{Mis} = 1)\) case imputation in each group, respectively
- Best \((Y_{Mis} = 1)\) AND Worst \((Y_{Mis} = 0)\) case imputation in each group, respectively.

From these 4 simulated (complete) data sets as well as the original data set (with missing data), 5 different sets of the point and variance estimates will be computed. Using Rubin’s rules, which take account of the variability in results between the imputed datasets, reflecting the uncertainty associated with the missing values, we will combine these results and generate valid and robust statistical inference about the multiply imputed Odds Ratio, as well as the proportions responding in each group.

**Secondary analyses**

The interpretation of the corresponding statistical measures of uncertainty of the treatment effect and treatment comparisons will involve consideration of the potential contribution of bias to the 95% confidence intervals and of the inference in general. Therefore, we will perform sensitivity analyses for the primary and key secondary outcomes.

First, we will repeat the primary analyses on the Per Protocol population that includes only participants who adhered to the allocated treatment without major protocol violations as defined above. This analysis will be conducted without imputation of missing data.

Secondly, we will perform an analysis of covariance of the continuous primary and secondary outcomes at day 30 on the ITT population, with multiple imputation of missing data at day 30 (see above) adjusted for stratification factors and the baseline values

\[
\text{VARIABLE}_{\text{change}, \text{day}30} = \text{GROUP} + \text{Stratification}_i + \text{VARIABLE}_{\text{baseline}}
\]

Thirdly, we will repeat the analysis of covariance of the continuous primary and secondary outcomes at day 30 on the ITT population, with a baseline observation carried forward imputation of missing data at day 30 adjusted for stratification factors and the baseline values

\[
\text{VARIABLE}_{\text{change}, \text{day}30} = \text{GROUP} + \text{Stratification}_i + \text{VARIABLE}_{\text{baseline}}
\]
If the sensitivity analyses are in agreement, and the sensitivity analyses and the primary analysis lead to essentially the same conclusions, confidence in the results is increased.

The dichotomous secondary outcomes (self-reported hospitalisation and presence of symptoms) will not undergo the latter two sensitivity analyses as multiple imputation has already been done (see section 10.3.2). Rather we will present the estimates from the 4 imputed datasets (as described above) separately together with the other sensitivity analyses.

Assessment of statistical assumptions

For the linear models of the primary and continuous secondary outcomes, we will check for the normality of residuals by visual inspection of residual plots.

Statistical Software

The analysis will be carried out using the statistical software SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). Linear mixed-effect models will be fitted using the MIXED procedure (proc mixed). Logistic regression models will be fitted using the LOGISTIC procedure (proc logistic).

Harms

Analyses of AEs and SAEs will be performed on the Safety Population (see section 9.5).

The number (and percentage) of patients experiencing AEs and SAEs will be presented for each treatment arm categorised by severity.

No formal statistical testing will be undertaken. The AEs/SAEs will be presented descriptively.
DEVIATIONS FROM THE PROTOCOL

The following details in this SAP represents deviations from trial protocol version 3.0

| Header in protocol | Deviation | Reason |
|--------------------|-----------|--------|
| 7.4 Exclusion criteria | At screening, eligible participants are specifically asked about recent or planned Covid-vaccination | As of ultimo December 2020, especially health care professionals have been vaccinated thus presenting with new Covid-like symptoms although already remitted. |
| 7.6 Blinding | Outcome assessors are not blinded to treatment allocation | Not possible in REDCap to blind the allocation status in the actual survey because the survey is administered by telephone interview. The branching logic in REDCap with certain parts of the survey being omitted work when surveys are sent out to participants e.g. by email |
| Blinding efficacy is not tested by questions to outcome assessors about presumed treatment arm | |
| 8.1.1/8.2.1 Treatment [in both intervention and standard of care] | Day-15 telephone calls by project manager to avoid attrition changed to contact by use of text message | To many ‘lost’ calls. Asynchronous contact more successful and less disturbing to the participants |
| 9.0 Outcome assessment/variables | The presence of Covid-19 related symptoms in general and self-rated health at day 30 is included | |
| 9.2 Secondary outcomes | Hospital admissions and use of antibiotics were described as secondary outcomes. For this analysis plan we focus on self-reported hospital admission for Covid-19 | It was planned to retrieve hospitalisation from the Danish National Patient Registry to ensure data completeness despite attrition. However, due to difficulties with being granted access to the Danish National Patient Registry, these objectives will not be part of this statistical analysis plan. Instead, we have surveyed the participants about hospitalisation related to the Covid-19 infection, which we will report as a secondary outcome. |
| 10.1 Self-reported CAT score | Four items of the overall CAT-score constitute a respiratory CAT sub-score to specifically measure PEP flute efficacy | Covid-19 appears to have many and long-lasting symptoms also systemically. The PEP flute intervention is hypothesized to affect primarily respiratory symptoms |
| 13.3 Pre-screening procedures and recruitment | Recruitment period extended from 4 months to almost 5 months (Oct 6, 2020 - Feb 28, 2021) | The daily number of eligible participants reduced significantly halfway because of a general lockdown of societal activities. Enrolment terminated at n = 378 instead of the expected 400 participants |
12 MANUSCRIPT OUTLINE

12.1 Shell Figure 1 (Flow Diagram)

Figure X: CONSORT flow diagram

Total population of SARS-CoV-2 infected individuals in the Capital Region and the Region Zealand from October 6, 2020 – February 28, 2021 (n=)

Invitation to contact project sent to individuals assessed for eligibility by screening of age and requisition status; data provided from KMA* (n=8,386)

Assessed for eligibility by telephone among individuals requested contact and consented to trial participation (n=)

Not eligible, n=

Day 0 (Baseline) Randomisation (n=378)

Allocation

PEP flute-selfcare (n=190)
Received allocated intervention (n=)
Did not receive allocated intervention (n=)
  ▪ Consent withdrawal (n=)

Usual care (n=188)
Received allocated usual care (n=)
Did not receive allocated usual (n=)
  ▪ Consent withdrawal (n=)

Discontinued intervention (n=)
  ▪ Adverse event (n=)
  ▪ Consent withdrawal (n=)

Discontinued usual care (n=)
  ▪ Adverse event (n=)
  ▪ Consent withdrawal (n=)

Follow-up

Day 30
  ▪ Lost to follow-up (n=)

Analysis

Analysed ITT (n=) (PP, n = )

Day 30
  ▪ Lost to follow-up (n=)

Analysed ITT (n=) (PP, n = )
# 12.2 Shell Table 1 (Baseline)

Table 1: Demographics and Baseline Characteristics

| Demographics                                      | PEP flute | Usual care |
|---------------------------------------------------|-----------|------------|
| Age, years                                        | xx.x (xx.x) | xx.x (xx.x) |
| Female sex, n[%]                                  | xx (xx.x%) | xx (xx.x%) |
| Body mass, kg                                     | xx.x (xx.x) | xx (xx.x%) |
| Height, m                                         | xx.x (xx.x) | xx (xx.x%) |
| Body Mass Index, kg/m2                            | xx.x (xx.x) | xx (xx.x%) |
| COPD Assessment Test (CAT) score (0-40)           | xx.x (xx.x) | xx.x (xx.x) |
| CAT-respiratory symptoms subscore (0-20)          | xx.x (xx.x) | xx.x (xx.x) |
| **Overall no. Covid-19 related symptoms within last week** | xx (xx.x%) | xx (xx.x%) |
| Respiratory symptoms within last week             |           |            |
| Chest pain, n[%]                                  | xx (xx.x%) | xx (xx.x%) |
| Runny/stuffy nose, n[%]                           | xx (xx.x%) | xx (xx.x%) |
| Sneezing, n[%]                                    | xx (xx.x%) | xx (xx.x%) |
| Sore throat                                       | xx (xx.x%) | xx (xx.x%) |
| Shortness of breath                               | xx (xx.x%) | xx (xx.x%) |
| Cough                                             | xx (xx.x%) | xx (xx.x%) |
| Other symptoms within last week                   |           |            |
| Chills, n[%]                                      | xx (xx.x%) | xx (xx.x%) |
| Headache, n[%]                                    | xx (xx.x%) | xx (xx.x%) |
| Muscle/joint pain, n[%]                           | xx (xx.x%) | xx (xx.x%) |
| Fatigue, n[%]                                     | xx (xx.x%) | xx (xx.x%) |
| Lost/altered sense of taste, n[%]                 | xx (xx.x%) | xx (xx.x%) |
| Lack of appetite, n[%]                            | xx (xx.x%) | xx (xx.x%) |
| Nausea/loathing for food, n[%]                    | xx (xx.x%) | xx (xx.x%) |
| Vomiting, n[%]                                    | xx (xx.x%) | xx (xx.x%) |
| Diarrhoea, n[%]                                   | xx (xx.x%) | xx (xx.x%) |
| Abdominal pain, n[%]                              | xx (xx.x%) | xx (xx.x%) |
| Pain at micturition, n[%]                         | xx (xx.x%) | xx (xx.x%) |
| Reddened/itchy eyes, n[%]                         | xx (xx.x%) | xx (xx.x%) |
| Lost/altered sense of smell, n[%]                 | xx (xx.x%) | xx (xx.x%) |
| Fever or fever sensation, n[%]                    | xx (xx.x%) | xx (xx.x%) |
| Feeling of illness, n[%]                          | xx (xx.x%) | xx (xx.x%) |
| **Days from onset of symptoms to trial inclusion** | xx.x (xx.x) | xx.x (xx.x) |
| **Self-rated health prior to Covid-19 (0-4)**      | xx.x (xx.x) | xx.x (xx.x) |
| **Smoking habits including e-cigarettes**          |           |            |
| Everyday smoker                                   | xx.x (xx.x) | xx.x (xx.x) |
| Weekly/social occasions smoker                    | xx.x (xx.x) | xx.x (xx.x) |
| Quit smoking/never smoked                         | xx.x (xx.x) | xx.x (xx.x) |
| **Alcohol intake**                                |           |            |
| Units of alcohol per week in average              | xx.x (xx.x) | xx.x (xx.x) |
### 12.3 Shell Table 2 (Primary analysis day-30)

Table 2: Change from baseline in Primary and Secondary Outcomes at day-30 in the ITT population. CI denotes 95% confidence interval.

|                                | PEP flute (N=) | Usual care (N=) | Estimated treatment difference | P-value |
|--------------------------------|----------------|-----------------|-------------------------------|---------|
|                                | LS Mean (SE)   | LS Mean (SE)    | Δ LS Mean (95% CI)             |         |
| **Primary outcome:**           |                |                 |                               |         |
| Change in CAT-score (0-40)     | xx.x (xx.x)    | xx.x (xx.x)     | xx.x (xx.x to xx.x)           | 0.xxx   |
| **Secondary Outcomes:**        |                |                 |                               |         |
| Self-reported hospitalisation for Covid-19, n(%) | xx (xx.x %) | xx (xx.x%) | ARD xx.x (xx.x to xx.x) | 0.xxx   |
| Length of stay, days           | xx.x (xx.x)    | xx.x (xx.x)     | xx.x (xx.x to xx.x)           | 0.xxx   |
| Change in self-rated health (0-4) | xx.x (xx.x)    | xx.x (xx.x)     | xx.x (xx.x to xx.x)           | 0.xxx   |
| Change in CAT-respiratory symptoms subscore (0-20) | xx.x (xx.x) | xx.x (xx.x) | xx.x (xx.x to xx.x) | 0.xxx   |

|                                | N(%)            | N(%)            | Adjusted Risk Diff (95% CI)   | P-value |
|                                | N(%)            | N(%)            | Adjusted Risk Diff (95% CI)   | P-value |
| No. of overall Covid-19 related symptoms, no. (%) | xx (xx.x %) | xx (xx.x%) | xx.x (xx.x to xx.x) | 0.xxx   |
| **Respiratory symptoms within last week** |                |                 |                               |         |
| Chest pain, no. (%)            | xx (xx.x %)     | xx (xx.x%)      | xx.x (xx.x to xx.x)           | 0.xxx   |
| Runny/stuffy nose, no. (%)     | xx (xx.x %)     | xx (xx.x%)      | xx.x (xx.x to xx.x)           | 0.xxx   |
| Sneezing, no. (%)              | xx (xx.x %)     | xx (xx.x%)      | xx.x (xx.x to xx.x)           | 0.xxx   |
| Sore throat, no. (%)           | xx (xx.x %)     | xx (xx.x%)      | xx.x (xx.x to xx.x)           | 0.xxx   |
| Shortness of breath, no. (%)   | xx (xx.x %)     | xx (xx.x%)      | xx.x (xx.x to xx.x)           | 0.xxx   |
| Cough, no. (%)                 | xx (xx.x %)     | xx (xx.x%)      | xx.x (xx.x to xx.x)           | 0.xxx   |
| **Other symptoms within last week** |                |                 |                               |         |
| Chills, no. (%)                | xx (xx.x %)     | xx (xx.x%)      | xx.x (xx.x to xx.x)           | 0.xxx   |
| Headache, no. (%)              | xx (xx.x %)     | xx (xx.x%)      | xx.x (xx.x to xx.x)           | 0.xxx   |
| Muscle/joint pain, no. (%)     | xx (xx.x %)     | xx (xx.x%)      | xx.x (xx.x to xx.x)           | 0.xxx   |
| Fatigue, no. (%)               | xx (xx.x %)     | xx (xx.x%)      | xx.x (xx.x to xx.x)           | 0.xxx   |
| Lost/altered sense of taste, no. (%) | xx (xx.x %) | xx (xx.x%) | xx.x (xx.x to xx.x) | 0.xxx   |
| Lack of appetite, no. (%)      | xx (xx.x %)     | xx (xx.x%)      | xx.x (xx.x to xx.x)           | 0.xxx   |
| Nausea/food loathing, no. (%)  | xx (xx.x %)     | xx (xx.x%)      | xx.x (xx.x to xx.x)           | 0.xxx   |
| Vomiting, no. (%)              | xx (xx.x %)     | xx (xx.x%)      | xx.x (xx.x to xx.x)           | 0.xxx   |
| Diarrhoea, no. (%)             | xx (xx.x %)     | xx (xx.x%)      | xx.x (xx.x to xx.x)           | 0.xxx   |
| Abdominal pain, no. (%)        | xx (xx.x %)     | xx (xx.x%)      | xx.x (xx.x to xx.x)           | 0.xxx   |
| Pain at micturition, no. (%)   | xx (xx.x %)     | xx (xx.x%)      | xx.x (xx.x to xx.x)           | 0.xxx   |
| Reddened/itchy eyes, no. (%)   | xx (xx.x %)     | xx (xx.x%)      | xx.x (xx.x to xx.x)           | 0.xxx   |
| Lost/altered sense of smell, no. (%) | xx (xx.x %) | xx (xx.x%) | xx.x (xx.x to xx.x) | 0.xxx   |
| Fever or fever sensation, no. (%) | xx (xx.x %) | xx (xx.x%) | xx.x (xx.x to xx.x) | 0.xxx   |
| Feeling of illness, no. (%)    | xx (xx.x %)     | xx (xx.x%)      | xx.x (xx.x to xx.x)           | 0.xxx   |

ARD: Adjusted Risk Difference
12.4 Shell Figure 2 (CAT-score trajectories)

**Figure X**: Exemplar (hypothetical) trajectories for our primary efficacy outcome measure (i.e. primary endpoint, change from baseline in CAT-score) in the ITT population. Based on repeated measures mixed linear models, where missing data is modelled implicitly.
13 REFERENCES

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Highest number of PEP-sessions reported in one day as registered by daily self-reports in the PEP selfcare-group (n=185)

Among the daily reports of PEP flute use, the highest number of sessions registered for one day is shown according to frequencies of number of participants (n=185). This means, that 103 participants in the intervention group registered using the PEP-flute in three sessions at least one day. Thirty-four participants reported using the PEP-flute in four sessions at least one day, 21 participants reported five sessions at least one day and so forth. Data from five participants in the intervention group are omitted as the numbers reported appeared to be based upon misunderstandings of how to register (registration of number of respirations instead of sessions).
Mean PEP-use and CAT-scores by intervention day

The figure above shows the mean reported number of PEP-sessions (circular dots and scaled as the left-side y-axis) in relation to the average CAT-score in the intervention group (squared dots and scaled as the right-side y-axis). Note, that the PEP-flute was delivered at a mean of 1.6 days and that the participants responded to the question: ‘How many times did you use the PEP-flute yesterday’ i.e. the self-reported number of PEP-sessions is missing at day-30 because the daily automated surveys ceased after day-30.

Supplementary analysis of self-reported urgent care visits

In the usual care-group, 12 participants reported urgent care visits (at the hospital or at a COVID-19 assessment unit) within the 30-day follow-up lasting from few hours up to six days. In the PEP selfcare-group, 16 participants reported urgent care visits lasting from two hours up to three days. At least one hospital admission in the PEP selfcare-group was related to dehydration rather than respiratory deterioration. Further analysis of hospital admissions alongside hospital procedures and medication throughout the period awaits the provision of register data.
Sensitivity analyses day-30: Change from baseline in Primary and Secondary Outcomes at day-30 in the ITT population with missing data modelled implicitly by the linear mixed model adjusted for sex and age as continuous variable.

|                      | PEP-flute (N=152) | Usual care (N=172) | Estimated treatment difference |
|----------------------|-------------------|--------------------|-------------------------------|
| **Primary outcome:** |                   |                    |                               |
| Change in CAT-score (0-40) | -8.7 (0.4)       | -7.6 (0.4)         | -1.2 (-1.2 to 0.02)           | 0.017 |
| Change in self-rated health (1-5) | 0.4 (0.1)     | 0.5 (0.1)          | -0.2 (-0.4 to 0.1)           | 0.041 |
| Change in CAT-respiratory symptoms subscore (0-20) | -3.9 (0.2)     | -3.5 (0.2)         | -0.4 (-0.9 to 0.1)           | 0.16  |

LSMean, Least Squares Mean. SE, Standard Error. CI, Confidence Interval. CAT, COPD Assessment Test. *
Adjusted for stratification factors: Sex and Age (continuous variable).

Sensitivity analyses day-30: Change from baseline in Primary and Secondary Outcomes at day-30 in the per protocol population with missing data modelled implicitly by the linear mixed model.

|                      | PEP-flute (N=152) | Usual care (N=172) | Estimated treatment difference |
|----------------------|-------------------|--------------------|-------------------------------|
| **Primary outcome:** |                   |                    |                               |
| Change in CAT-score (0-40) | -8.3 (0.5)       | -7.2 (0.4)         | -1.1 (-1.2 to 0.05)           | 0.040 |
| Change in self-rated health (1-5) | 0.4 (0.1)     | 0.5 (0.1)          | -0.1 (-0.3 to 0.1)           | 0.43  |
| Change in CAT-respiratory symptoms subscore (0-20) | -3.7 (0.2)     | -3.4 (0.2)         | -0.3 (-0.9 to 0.2)           | 0.27  |

LSMean, Least Squares Mean. SE, Standard Error. CI, Confidence Interval. CAT, COPD Assessment Test. *
Adjusted for stratification factors: Sex and Age ≥ 60 years.
Per protocol in PEP-flute group to determine whether the PEP-flute was used while having respiratory symptoms (CAT > 5): based on sets of at least seven days with aligned registrations of CAT-scores and PEP-flute use. Otherwise according to protocol.
Per protocol in usual care group: no protocol violations i.e. no use of a PEP-flute during the 30-day period.
Methodological considerations and perspectives on future research

A concern in our trial is the lack of validation of the CAT-score in covid-19. In order to inform future research in self-reported symptom severity of Covid-19, we will discuss the use of CAT as outcome measure in Covid-19 more thoroughly.

As no Covid-19 symptom severity scale for self-reporting of symptoms existed when the PEP-CoV trial was designed, we chose the CAT because of overlaps in reported symptoms between COPD and Covid-19 and because the CAT is widely used to assess symptom status as part of telemedicine. A recent brief publication by Daynes et al. (COPD assessment test for the evaluation of COVID-19 symptoms. *Thorax* 2021;76:185-187) concludes, that the CAT can be used to guide treatment of Covid-19 but further research is needed to identify impact of interventions. In our trial, we found that the use of PEP-selfcare had statistically significant beneficial effects on CAT_dyspnoea and CAT_tightchest as well as CAT_vigour and CAT_activities. At the same time, analyses of the individual items showed higher scores in the PEP-group in CAT_phlegm and no differences as regards CAT_cough. Several participants (n=56) from the intervention group stated that PEP-usage provoked to coughing of phlegm and in many cases, the participants were not aware of having this phlegm until using the PEP-flute. As the CAT-score is a composite score of eight items, it is reasonable to deduce, that the higher scores of CAT_phlegm in the PEP-group alongside the equally distributed scores between groups in CAT_cough add to a smaller reported effect when only looking at the overall CAT-score. The physiological rationale is illustrated below.

PEP-use $\rightarrow$ loosening of secretions $\rightarrow$ coughing $\rightarrow$ less dyspnoea/chest tightness

$\uparrow$ scores of CAT_phlegm  $\uparrow$ scores of CAT_cough  $\downarrow$ scores of CAT_dyspnoea/tightchest

In fact, this means that we induce reports of relative higher severity scores in the intervention group by asking the participants to use the PEP-flute and the higher scores on CAT_phlegm and CAT_cough can be viewed as intermediate effects on the pathway to less respiratory symptoms as regards dyspnoea and tightness of the chest.

Another challenge of using the CAT as a composite outcome measure in Covid-19 interventional trials may be that the disease trajectory is different from COPD. As Covid-19 is an acute infectious disease, several participants in a Covid-19 trial will recover spontaneously and very early in the course of disease. Although, this is likely to be the case in both the intervention and the usual care group, this will potentially dilute the effect of the intervention.

In the future design of a similar trial, it could be considered to have as primary outcome measure a numeric rating scale assessing the overall respiratory symptoms on a scale from 0-10 (e.g. 0=no respiratory symptoms to 10=very bad respiratory symptoms). Then the individual CAT-items on a daily basis could be supplemental and qualify assessment of the intervention by adding to the insight on specific mechanisms of the PEP-selfcare. It could also be considered to conduct this as a cluster-randomised trial as has recently been reported related to the impact of community masking on Covid-19 ([https://www.poverty-action.org/sites/default/files/publications/Mask_RCT___Symptomatic_Seropositivity_083121.pdf](https://www.poverty-action.org/sites/default/files/publications/Mask_RCT___Symptomatic_Seropositivity_083121.pdf)). Hence, objective data on clinical progression according to WHO clinical progression scale (e.g. hospital admission, use of mechanical ventilation, mortality) could potentially constitute the primary outcome with adequate power.