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Hyperbaric oxygen for treatment of long COVID-19 syndrome (HOT-LoCO): protocol for a randomised, placebo-controlled, double-blind, phase II clinical trial

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ABSTRACT

Introduction Long COVID-19, where symptoms persist 12 weeks after the initial SARS-CoV-2-infection, is a substantial problem for individuals and society in the surge of the pandemic. Common symptoms are fatigue, postexertional malaise and cognitive dysfunction. There is currently no effective treatment and the underlying mechanisms are unknown, although several hypotheses exist, with chronic inflammation as a common denominator. In prospective studies, hyperbaric oxygen therapy (HBOT) has been suggested to be effective for the treatment of similar syndromes such as chronic fatigue syndrome and fibromyalgia. A case series has suggested positive effects of HBOT in long COVID-19. This randomised, placebo-controlled clinical trial will explore HBOT as a potential treatment for long COVID-19. The primary objective is to evaluate if HBOT improves health-related quality of life (HRQoL) for patients with long COVID-19 compared with placebo/sham. The main secondary objective is to evaluate whether HBOT improves endothelial function, objective physical performance and short-term HRQoL.

Methods and analysis A randomised, placebo-controlled, double-blind, phase II clinical trial in 80 previously healthy subjects debilitated due to long COVID-19, with low HRQoL. Clinical data, HRQoL questionnaires, blood samples, objective tests and activity metre data will be collected at baseline. Subjects will be randomised to a maximum of 10 treatments with hyperbaric oxygen or sham treatment over 6 weeks. Assessments for safety and efficacy will be performed at 6, 13, 26 and 52 weeks, with the primary endpoint (physical domains in RAND 36-Item Health Survey) and main secondary endpoints defined at 13 weeks after baseline. Data will be reviewed by an independent data safety monitoring board.

Ethics and dissemination The trial is approved by the Swedish National Institutional Review Board (2021–02634) and the Swedish Medical Products Agency (5.1-2020-36673). Positive, negative and inconclusive results will be published in peer-reviewed scientific journals with open access.

Trial registration number NCT04842448.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ Randomised, placebo-controlled, double-blind, parallel-groups clinical trial in compliance with International Council for Harmonisation–Good Clinical Practice.
⇒ Evaluation of safety and efficacy, including objective and explanatory endpoints.
⇒ New syndrome with unknown mechanisms.
⇒ Power calculation is based on similar syndromes.
⇒ Selection bias as patients are enrolled from the same post-COVID clinic.

INTRODUCTION/BACKGROUND

In the wake of the first wave of the SARS-CoV-2 pandemic, a new set of often debilitating postinfectious symptoms have arisen. Such symptoms that persist for more than 3 months, even after mild SARS-CoV infection, have become a major burden for individuals affected, healthcare providers and society in general.[1] The prevalence of long COVID-19 is difficult to determine due to a plethora of symptoms and different definitions.[2] A recent estimation from a UK cohort of 508 707 patients suggests that more than 30% had experienced at least one symptom with 'significant impact on my daily life', giving an overall prevalence of 1.72%.[3] Most patients experiencing lingering symptoms are women, of which many have experienced only mild, if any, respiratory symptoms, and

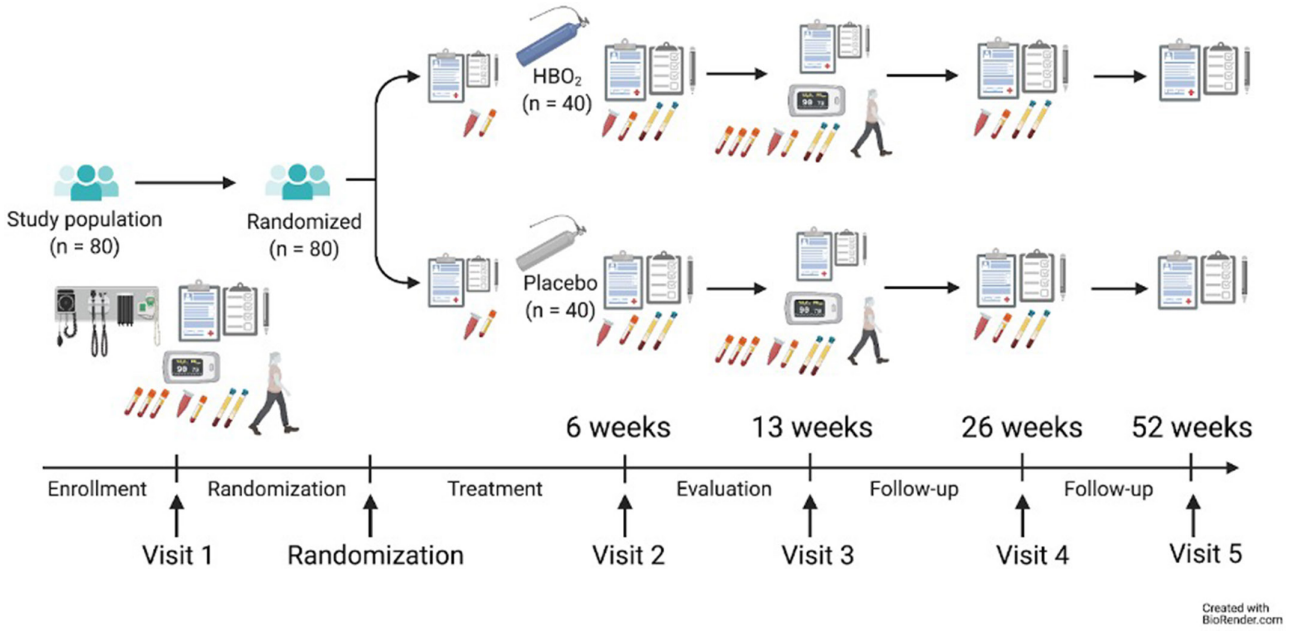

Figure 1 Trial flowchart.

seldom required hospital care during the acute phase of their SARS-CoV-2 infection.[4] Reported long-term symptoms include shortness of breath, fatigue, postexertional malaise and cognitive dysfunction, frequently leading to reduced working capability.[2] Some patients are also diagnosed with autonomic dysfunction, including postural orthostatic tachycardia syndrome (POTS) and inappropriate sinus tachycardia.[5 6]

As the pandemic continues to spread, with new mutations and resulting variants of SARS-CoV-2 appearing, effective treatments are needed to quell infection rates as well as mitigate lingering long-term symptoms. There is still no uniform definition or name of the syndrome, but postacute COVID-19 syndrome, post-COVID-19 syndrome or long COVID-19 are commonly used.[7] An attempt to achieve a global definition of post-COVID-19 condition, the name suggested by WHO, has recently been made by a Delphi consensus process.[8] Post-COVID-19 condition is previously listed in International Classification of Diseases, 10th Revision (ICD-10) with code U09.9, which includes all commonly used names. Experts in the field have recently suggested management guidelines for monitoring and follow-up, but to date, there is no effective treatment.[9] The underlying mechanisms are not understood, but several hypotheses including endothelial dysfunction, oxidative stress and chronic inflammation have been proposed.[10 11] In fact, a recent study demonstrated persistent microvascular endothelial dysfunction for 4 months following COVID-19 infection.[12]

Hyperbaric oxygen therapy (HBOT) is administered by delivering 100% oxygen at raised pressure to patients in a hyperbaric chamber. HBOT has previously been used as an adjunctive treatment for COVID-19, resulting in faster recovery in prospective trials, case series[13] and a randomised controlled trial (RCT),[14] with additional RCTs ongoing.[15] The rationale for treatment of COVID-19 with HBOT is the treatment's well-established anti-inflammatory effects.[16 17] Furthermore, a small retrospective cohort study has shown promising results in alleviating symptoms of long COVID-19 in patients treated with HBOT.[18] The safety profile of HBOT is well established and is considered both safe and effective for the treatment of several chronic inflammatory diseases such as soft tissue radiation injury.[19] HBOT has been shown to improve symptoms and quality of life in other syndromes associated with chronic fatigue.[20 21] We explore HBOT administered within a randomised, placebo-controlled clinical trial as a potential treatment for patients suffering from long COVID-19. The purpose of this article is to provide a summary of our protocol that complies with International Council for Harmonisation–Good Clinical Practice (ICH-GCP), with a detailed description and rationale for the primary and main secondary endpoints, including patient-reported outcomes (PROs), in line with Standard Protocol Items: Recommendations for Interventional Trials-PRO Extension Guidelines.[22]

Hypothesis and objectives

The overall hypothesis to be evaluated is that HBOT reduces oxidative stress and chronic inflammation, improves endothelial dysfunction and thereby alleviates symptoms associated with long COVID-19.

The primary objective is to evaluate whether HBOT improves health-related quality of life (HRQoL) for patients compared with placebo. The main secondary objectives are to evaluate whether HBOT improves endothelial dysfunction, objective physical performance and improvement of short-term HRQoL. Other secondary objectives are to evaluate if HBOT improves autonomic dysfunction and restorative sleep, and to evaluate the

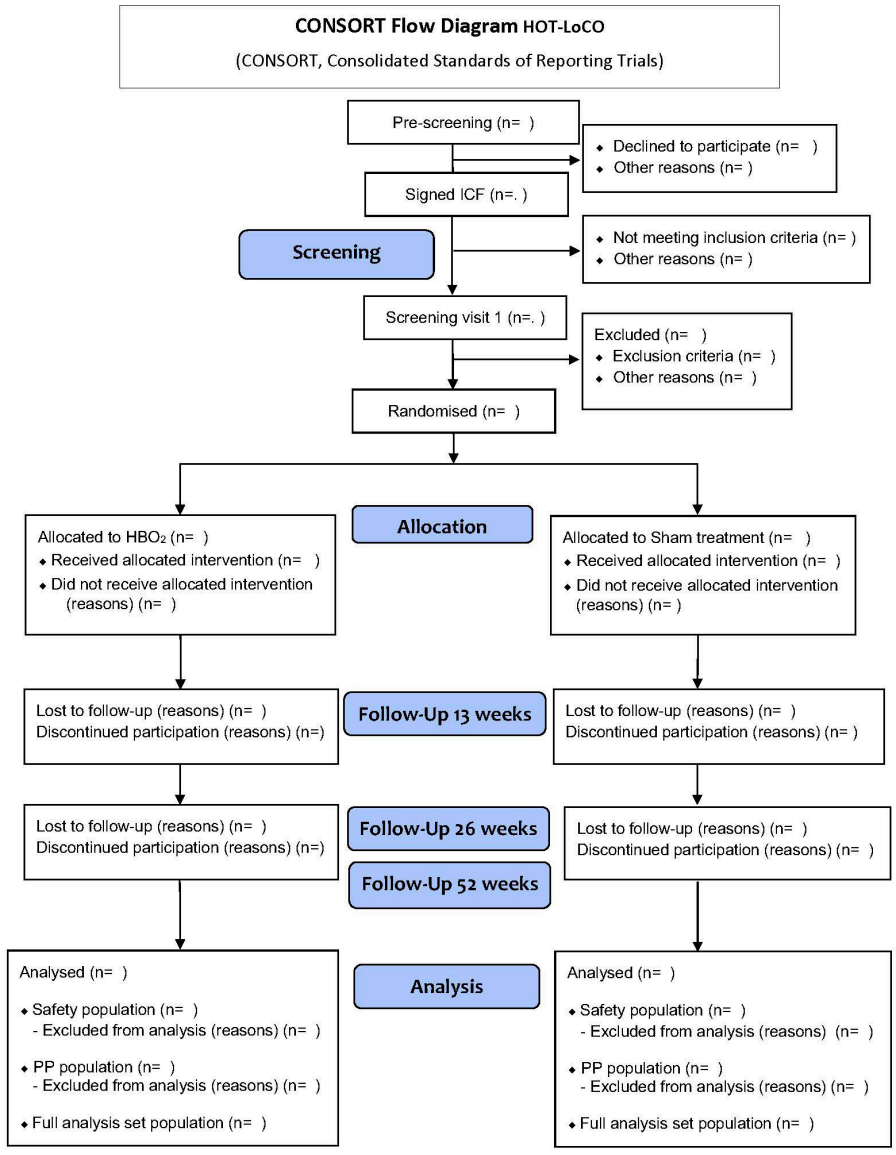

Figure 2 CONSORT flow diagram. CONSORT, Consolidated Standards of Reporting Trials; HOT-LoCO, hyperbaric oxygen for treatment of long COVID-19 syndrome; ICF, informed consent form; PP, per protocol.

health-economic benefits of the treatment and the biomarkers for the HBO effect on inflammation and chronic hypoxia. Furthermore, we aim to evaluate the safety profile of HBOT for patients with long COVID-19.

METHODS AND ANALYSIS

Trial design

The trial is designed as a prospective, randomised, placebo-controlled, double-blind, phase II clinical trial. The trial consists of five visits for 52 weeks. At visit 1, participant eligibility will be established, and baseline data will be collected. Block randomisation will be performed, stratified by gender and disease severity as determined by the RAND 36-Item Health Survey (RAND-36) questionnaire. Eligible subjects are randomised for a maximum of 2 weeks before the first treatment and will receive a maximum of 10 treatments over 6 weeks from randomisation. Treatment is conducted by designated staff not involved in assessment or data collection; subjects and investigators are blinded to the treatment allocation. The randomisation and blinding process is described in a standard operating procedure (SOP) (see online supplemental file 1). Visit 2 is conducted on the day of the last treatment. The primary and main secondary endpoints will be assessed at 13 weeks from baseline at visit 3. Visits 4 and 5 are long-term follow-up. Subjects will also be asked to participate in a post-trial follow-up over 4 years. A flowchart of the trial design is depicted in figure 1. Moreover, the Consolidated Standards of Reporting Trials flow diagram is depicted in figure 2.

Patient and public involvement

The trial design and consent form were discussed with and approved by a patient representative. We thank Svenska Covidföreningen through chairman Åsa Kristofferson-Hedlund for their support.

Setting

The trial is investigator initiated and will take place in a single centre. The sponsor is Region Stockholm via the Karolinska University Hospital in collaboration with Karolinska Institutet, both in Stockholm, Sweden. Patients will be recruited through the post-COVID outpatient clinic and/or advertisement. Measurements and treatments will take place at the hyperbaric unit. If included in the trial, all patients, regardless of intervention or control, will be treated at the hyperbaric treatment facility, staffed by anesthesiologists and intensivists, as well as nurses specifically trained in HBOT. All personnel involved in the trial are designated to specific duties and trained in ICH-GCP. As per protocol (PP) at Karolinska University Hospital, each treatment in the hyperbaric chambers must be overseen by a minimum of two staff members. Local, national and international guidelines for clinical trials and HBOT during the COVID-19 pandemic will be followed.

Trial population

Eighty patients aged 18–60, previously generally healthy (defined as American Society of Anesthesiologist classes I and II), will be recruited. They must have had symptoms consistent with long COVID-19 for a minimum of 12 weeks, as well as a long COVID-19 diagnosis with ICD-10 code U09.9. Subjects must have been working or studying before the diagnosis. An HBOT-specific questionnaire with focus on HBOT contraindications will be filled in by all subjects; contraindications include pregnancy, claustrophobia, obstructive lung disease and history of spontaneous pneumothorax. All inclusion and exclusion criteria are listed in table 1. Subjects who are diagnosed with long COVID-19 through the Karolinska University Hospital post-COVID outpatient clinic will be evaluated by a multidisciplinary team consisting of an infectious disease specialist, a pulmonologist, a cardiologist as well as a physiotherapist. All patients will be assessed with a battery of questionnaires, physical tests, laboratory tests and radiology, including MRIs.

Treatment/interventions

The HBOT group will undergo HBOT at 2.4 atmospheres absolute (ATA), approximately 240 kPa for 90 min with two airbrakes (see online supplemental file 2), with a maximum of 10 treatments within 6 weeks of randomisation. The placebo group will undergo 'sham treatment' with air breathing at 1.34 ATA, approximately 134 kPa (see online supplemental file 3) to equate the sensation of HBOT, and airbrakes will be simulated. They will undergo a maximum of 10 treatments within 6 weeks of randomisation.

The hyperbaric chambers to be used are designed for a single patient (monoplace chamber) or for multiple patients (multiplace chamber). In the case of the monoplace chamber, it is pressurised with 100% oxygen, and staff and equipment are located outside the chamber. However, multiplace chambers are pressurised with air, allowing staff and equipment to be inside the same chamber where the patient breathes oxygen through a mask. The latter is suitable for patients requiring a high level of medical care or groups of patients who can sit in a chair for 90 min, whereas the monoplace chamber is more comfortable but requires the patient to be fully alert and stable.

Procedures

The patients will be informed about the trial orally and in writing and will be given the chance to ask questions. If they agree to participate, an informed consent form will be signed by the patient and an investigator before any study-specific procedures occur. Subjects will then be scheduled for a screening visit (visit 1) where baseline data will be collected and inclusion/exclusion criteria are verified. Subjects eligible for inclusion in the trial will subsequently enter the trial, be randomised and allocated to treatment. After the treatment period of 6 weeks, the subjects will be scheduled for follow-up visits at 13±2 weeks and 26 and 52 weeks±4 weeks after randomisation.

All procedures in the trial are described in detail in the full protocol (See online supplemental file 4). For

Table 1 Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
► Aged 18–60 years. ► Healthy or mild systemic disease (ASA classes I and II) prior to COVID-19. ► Symptoms consistent with long COVID-19 for at least 12 weeks. ► Diagnosed with long COVID-19, postacute COVID-19 syndrome and post-COVID-19 syndrome (ICD-10 U09.9). ► Working or studying prior to COVID-19. ► Documented informed consent according to ICH-GCP and national regulations.	► Known pregnancy or positive pregnancy test in women of childbearing age. ► ASA class III or more from other cause than long COVID-19. ► Score above 70 in RAND-36 domains role limitation physical health or physical functioning. ► Diabetes mellitus. ► Diagnosed with hypertension prior to COVID-19. ► Contraindication for HBOT treatment according to local guidelines. ► Participation or recent participation in a clinical trial with an investigational product. ► Mental inability, reluctance or language difficulties that result in difficulty understanding the meaning of trial participation.

ASA, American Society of Anesthesiologists; HBOT, hyperbaric oxygen therapy; ICD-10, International Classification of Diseases, 10th Revision; ICH-CGP, International Council for Harmonisation–Good Clinical Practice; RAND-36, RAND 36-Item Health Survey.

Table 2 List of procedures

Activity	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5
Week	0	6	13	26	52
Signed informed consent form	**X**				
Inclusion/exclusion criteria	**X***				
Randomisation	**X**				
Medical history	**X**	X†	X†	X†	X†
Sociodemographics	**X**	X‡	X‡	X‡	X‡
Concomitant medications	**X**	**X**	**X**	X	X
RAND-36	**X**	X	**X**	**X**	**X**
EuroQol-5 Dimensions Questionnaire	**X**	**X**	**X**	**X**	**X**
Reactive Hyperaemia Index	**X**		**X**		
6-Minute Walk Test	**X**	X	**X**	X	X
30/60-Second Chair-Stand Test	**X**	X	**X**	X	
Nexfin	**X**		**X**		
Treatment (HBOT/placebo)		**X** (1–10)			
Treatment planned		**X** (1–10)			
Adverse event/adverse drug reaction	**X**	**X**	X	X	X
Trial-specific biochemistry	**X**	**X**	**X**	X	X
Biobanking (PBMC, plasma and EPR)	**X**	**X**, **X**	**X**	**X**	
Activity metre	**X**	**X**	**X**	**X**	**X**

Trial-specific procedures are marked with bold X. Data collected from medical records are marked with narrow X).
*Exclusion criteria include a pregnancy test (if applicable), RAND-36 questionnaire, an HBOT specific questionnaire, review of medical records and a medical examination if needed.
†Medical history includes COVID-19 specific history, routine blood tests, questionnaires, physical tests and radiology; medical records will be reviewed and recorded.
‡Sociodemographics that may change over time, such as sick leave, weight, activity and smoking habits.
EPR, Electron Paramagnetic Resonance Spectroscopy; HBOT, hyperbaric oxygen therapy; PBMC, Peripheral Blood Mononuclear Cells; RAND-36, RAND 36-Item Health Survey.

treatments, blinding procedures and assessments, SOPs will be followed. A list of procedures is depicted in table 2.

Assessments/measurements

Prior to inclusion, subjects will have undergone extensive tests, including radiology with different modalities such as CT, MRI, dual-energy CT, cardiac ultrasound and chest X-rays, and objective physical measurements such as handgrip strength, spirometry and head-up tilt test and questionnaires used in clinical practice to confirm the diagnosis and to rule out any differential diagnosis. These data will be obtained from medical records.

Blood-based biochemical values for kidney function, liver function, cardiac enzymes, haematology and blood glucose will be obtained from patients' medical records. Trial-specific biochemistry will include ferritin, D-dimer, Lactate Dehydrogenase (LDH), troponin T and a pregnancy test for any woman of childbearing age; blood for biobanking will be collected from fasting subjects.

During the screening visit (visit 1), subjects will fill out the RAND-36, EuroQol-5 Dimensions Questionnaire (EQ-5D) and undergo physical tests including the 6-Minute Walk Test (6MWT) and the 30/60-Second Chair-Stand Test (CST), and other objective evaluations including endothelial function with pulse amplitude tonometry (PAT), measurements of cardiac function and activity, heart rate variability and sleep patterns with an activity metre.

PRO measures

RAND 36-Item Health Survey

RAND-36 is a self-reporting questionnaire that contains 36 items that measure eight concepts of health in general terms, at present and past 4 weeks: physical functioning (10 items), role limitations due to physical health (4 items), role limitations due to emotional problems (3 items), energy/fatigue (4 items), emotional well-being (5 items), social functioning (2 items), pain (2 items) and general health (5 items). It also includes a single item that provides an indication of perceived change in health over the last year. Scoring RAND-36 is a two-step process. First, numerical values from the survey are coded so that all items are scored from 0 (lowest score) to 100 (highest possible score). Scores then represent the percentage of total possible score achieved. In step 2, items in the same scale are averaged together to create the eight-scale

Table 3 HOT-LoCO: trial endpoints

Primary endpoints	Mean change from baseline to 13 weeks in RAND-36 domains RP and PF, respectively
Main secondary efficacy endpoints	► Mean change from baseline to 13 weeks in Reactive Hyperaemia Index. ► Mean change from baseline to 13 weeks in the CST. ► Mean change from baseline to 13 weeks in the 6MWT. ► Mean change from baseline to 13 weeks in EQ-5D scores. ► Proportion of subjects with a normalisation* of levels in RAND-36 domains RP and PF, respectively, at 13 weeks.
Other efficacy endpoints	► Mean change in other RAND-36 domains at 13, 26 and 52 weeks compared with baseline. ► Mean change in EQ-5D at 6, 26 and 52 weeks compared with baseline. ► Mean change in physical activity using an activity metre at 6, 13 and 26 weeks compared with baseline. ► Mean change in HRV using an activity metre at 6, 13 and 26 weeks compared with baseline. ► Mean change in sleeping pattern using an activity metre at 6, 13 and 26 weeks compared with baseline.
Explorative/descriptive endpoints	► Mean change from baseline in hypoxia pathways in PBMCs evaluated by RNA sequencing at 6, 13 and 26 weeks. ► Mean change from baseline in inflammatory PBMCs evaluated by RNA sequencing at 6, 13 and 26 weeks. ► Mean change from baseline of reactive oxygen species in red blood cells measured by EPR at 6 and 13 weeks. ► Mean change from baseline of microRNA in plasma at 6 and 13 weeks. ► Mean change from baseline in trial-specific clinical biochemistry at 6 and 13 weeks. D-dimer. Ferritin. LDH. Troponin T. ► Long-term post-trial follow-up of HRQoL using EQ-5D as variable up to 4 years post trial.
Safety and compliance endpoints	► Number of subjects, proportion of subjects and number of adverse events at 13 weeks. ► Number of subjects and proportion of subjects that have completed planned treatments after 6 weeks.

*According to Swedish normative data.[23]
CST, 30/60-Second Chair-Stand Test; EQ-5D, EuroQol-5 Dimensions Questionnaire; HOT-LoCO, hyperbaric oxygen for treatment of long COVID-19 syndrome; HRQoL, health-related quality of life; HRV, Heart Rate Variability; 6MWT, 6-Minute Walk Test; PF, physical functioning; RAND-36, RAND 36-Item Health Survey; RP, role limitations due to physical health.

scores. Items that are left blank (missing data) are not considered when calculating the scale scores. Hence, scale scores represent the average for all items in the scale that the respondent answered. RAND-36 is well documented in terms of reliability and variability also for Swedish translation.[23] National gender and age normative data are available for comparison.[23] The questionnaire will be sent out digitally to the subjects on the day of the visit and uploaded to the medical records when filled out. The dimensions in RAND-36 are presented separately, and we have chosen the physical domains role limitations due to physical health (RP) and physical functioning (PF) as primary endpoints for two reasons:

- The physical domains seem to be severely affected in conditions associated with chronic fatigue and POTS.[24 25]
- We expect the physical domains to be least affected by placebo.

EuroQol-5 Dimensions Questionnaire

EQ-5D is a widely used patient-reported questionnaire aimed at measuring five different dimensions of present health with three or five levels of severity: no problems, some/moderate problems and severe/extreme problems. The five different dimensions are mobility, self-care, usual activities, pain/discomfort and anxiety/depression. It also uses a visual analogue scale of 0–100 for quantifying measures of overall health. EQ-5D is a well-validated tool, and the index that is calculated from the dimensions gives an estimate of quality-adjusted life years, with a low index indicating a low HRQoL.[26] We will use five levels of severity of EQ-5D in our trial. One of the strengths of EQ-5D is that gender and age normative data for the Swedish population are available for use in health economic evaluation,[27] and the index can be used to predict ability to work or study. The questionnaire will be sent out digitally to the subjects on the day of the visit and when filled out, uploaded to the medical records.

The rationale for choosing RAND-36 is that it is well validated and used in previous studies with similar methodology to enable power calculations. EQ-5D was chosen to provide an evaluation of HRQoL in a shorter perspective, as it is easier to fill in and may therefore be a better

option for long-term follow-up, to enable a simple health economic evaluation.

Physical tests

6-Minute Walk Test

The 6MWT will be performed in a corridor with a measured distance of 30 m, with markings for every metre. The subject will carry a pulse oximeter with a probe attached to their forehead. The test will be monitored by an experienced instructor recording parameters every minute, the total number of metres walked in 6 min, the subject's graded and subjective feeling of leg fatigue and dyspnoea according to the Borg CR-10 scale, as well as the feeling of general exertion according to the Borg-Rating of Perceived Exertion (RPE) scale, both at baseline and at the end of the tests.[28]

30/60-Second Chair-Stand Test

Here the subject will stand up straight and sit down completely as many times as possible for 30/60 s. An instructor will record the number of times the subject manages to perform the movement, as well as the subject's graded and subjective feeling of general exertion according to the Borg-RPE scale, and dyspnoea and leg fatigue according to the Borg CR-10 scale at baseline and the end of the test. The rationale for recording 30/60 s is that some subjects may not be able to perform the full 60 s test.

Objective measurements

Nexfin

The *Nexfin* monitor will be connected to a fasting subject. This is a non-invasive measurement of cardiovascular indices, with a beat-to-beat pulse wave analyser. The Nexfin device (ClearSight, Edwards Lifesciences) is placed on the middle phalanx of the middle finger on the right hand. The Nexfin device comprises a pneumatic plethysmograph that provides advanced haemodynamic parameters and continuous non-invasive blood pressure from a finger cuff, with a redesigned self-coiling mechanism that reconstructs the clinical standard brachial arterial waveform from the finger arterial pressure waveform; it has been validated towards invasive measurements in several clinical trials.[29]

Reactive Hyperaemia Index (RHI)

Endothelial function will be determined in fasting state using an *EndoPAT 2000* device (Itamar Medical, Caesarea, Israel). The subjects will be connected to the PAT device for non-invasive determination of digital endothelial function. The PAT device comprises a pneumatic plethysmograph that allows measurements of pulse amplitude at baseline and during hyperaemia following a 5 min arterial occlusion of the forearm.[30] The change in the PAT signal is used for calculating the RHI, which has been shown to reflect microvascular endothelial dysfunction, reduced Nitric Oxide (NO) bioavailability and to predict cardiovascular events.[31]

Activity meter

The *OURA ring* (Oura Health Oy) will be used as an activity tracker that registers heart rate variability, body temperature, physical activity and sleep patterns. Subjects will wear the ring for at least 1 week before and after each visit with data being synced in OURA's smartphone application, which subsequently will be uploaded to an encrypted database.[32] The weekly mean of each variable will be collected.

Randomisation

Subjects who meet the inclusion criteria will be randomised using a digital tool, Randomizer.at V.2.0.0 (Institute for Medical Informatics, Statistics and Documentation, Medical University of Graz). The system has a complete electronic audit trail for all activities involved with the randomisation. Randomisation is stratified for gender and 'illness severity'. Illness severity is determined as the mean of RAND-36 score for RP and PF into three strata: (1) <30, (2) 30–50 and (3) >50. Investigators access the randomisation system through a web portal with access control. Staff designated to treatment allocation have user-specific access to the unblinded treatment schedule. Study treatment is allocated according to protocol, 10 treatments over 6 weeks, a maximum of 2 weeks after randomisation.

Subjects as well as all personnel participating in assessments of symptoms and any objective findings will be blinded to the treatment. The placebo sham treatment protocol is well established and even experienced divers cannot differ between sham treatment and HBOT.[33] Designated personnel, experienced in HBOT and trained in Good Clinical Practice (GCP) and the specific protocols, will administer the assigned treatments. All subjects will furthermore be asked during the first week of treatment whether they believe they received the placebo treatment or HBOT to validate the blinding process.

Trial endpoints

The primary endpoints are the mean change from baseline to 13 weeks in RAND-36 domains RP and PF, respectively. The main secondary endpoints are mean change from baseline to 13 weeks in RHI, 6MWT, CST, EQ-5D and proportion of subjects with a normalisation of levels in RAND-36 domains RP and PF respectively, at 13 weeks. Primary, main secondary, selected other and safety endpoints are listed in table 3.

Safety and adverse events (AEs)

Collection of AEs and serious adverse event (SAE) data will start directly after inclusion and will be recorded until visit 3. Only SAE will be collected outside the treatment period (visit 2). Ongoing AE and SAE at the end of visit 3 will be followed up during long-term follow-up until the subject's last visit. The definition, handling, follow-up and reporting of AEs are defined in the original protocol (p.34–38). The safety endpoints will be evaluated by an independent data safety monitoring board (DSMB) in

the context of the trial design and currently existing information about long COVID-19 and HBOT. The DSMB is composed of three experts in their respective disciplines of medicine, clinical trial methodology and conduct. The DSMB will review the data at the predetermined interim analyses and at the end of trial, a charter delineating their guidelines for operating and stopping rules for terminating individual subjects, a portion or all the trial prematurely, was drawn up and agreed on before the trial started. The members of the DSMB, meeting plan and responsibilities are specified in the original protocol (p.6 and 44).

Statistical analyses

This section is a short summary of the planned statistical analyses of the most important endpoints including primary and main secondary endpoints. A longer summary is available in the full protocol (p.38–42). A more technical and detailed elaboration of the principal features will be written in a separate statistical analysis plan (SAP). The SAP will be finalised prior to database lock.

Analysis population

Full analysis set (FAS), PP and safety population will be employed. The FAS population will be defined as all randomised subjects who were exposed at least once to the study intervention.

Sample size calculation

The primary endpoint is mean change from baseline to week 13 in the RAND-36 score. A 10-point higher mean change in the HBOT group compared with the placebo group is considered as a clinically relevant difference. Sample size calculation was performed using t-test for independent groups, with an 80% power), and a type I error rate of 0.05 (5%), assuming a common SD of 15 from prior studies, to detect a 10-unit difference between groups. Power calculations indicate that at least 37 subjects per group are needed. Subsequently, we aim to recruit 80 subjects. nQuery V.7 was used for sample size calculation.

Hypothesis testing and adjustment for multiplicity

Hypothesis testing will be controlled at the type I error rate of 0.05 and adequately adjusted for multiplicity in the two primary endpoints. However, there will be no adjustment for multiplicity in main secondary endpoints as this is an exploratory study, but nominal p values will be presented, and results will be interpreted as exploratory findings. All hypothesis tests will be two-sided. Details of the multiplicity adjustment in terms of the selection of endpoints to include in the testing sequence and the criteria for rejecting (or not rejecting) individual hypotheses will be provided in the SAP.

Subgroups

Subgroup analysis will be done and presented for gender and disease severity defined as the mean of RAND-36 RP and PF and divided into 'RP and PF below 30', 'RP and PF 30–50' and 'RP and PF above 50'.

Statistical methodology

Primary and secondary endpoints will be evaluated using the FAS population and sensitivity analyses performed using the PP population. The primary objective of the study is to confirm a superior efficacy for the active treatment compared with placebo in the primary endpoints. The null hypothesis to be tested is that there is no difference between HBO treatment and placebo; that is, the mean change in (HBOT)=mean change (placebo). The same statistical hypothesis will be used for key secondary endpoints.

All continuous variables will be described using standard statistical measures, that is, number of observations, mean and median value, SD, and minimum and maximum values. All categorical variables will be summarised in frequency tables.

In general, for continuous outcome variables including the primary endpoint, they will be analysed using analysis of covariance, unless otherwise specified, including stratification factors and treatment as fixed factors in the model. Estimates will be presented using least square means for differences between treatment arms. In addition, continuous endpoints measured repeatedly over time, such as EQ-5D and RAND-36 domains, the change from baseline will be analysed using a linear mixed-effect model including baseline, treatment group, sex, symptom severity, visit and treatment group by visit interaction, and subjects as random effects, in the models. An unstructured covariance matrix will be assumed.

Analysis for categorical data in terms of binary data (yes/no) will be presented as the proportion of subjects with the frequency of presence or absence, by treatment group of the characteristics of interest and analysed using the Cochran-Mantel-Haenszel chi square test (CMH) χ^2 test including stratification factors, where the parameter used for the statistical hypothesis testing will be the OR, as a measure of the relative difference in odds between treatment arms. An OR of >1 indicates efficacy in favour of HBOT compared with placebo.

Missing data will be adequately imputed for all subjects in the FAS population. In addition, the observed cases population will be evaluated as a sensitivity analysis.

An interim safety analysis will be performed when 20 subjects have available data for the safety endpoints, and a second interim analysis will be performed when 40 subjects have data available for primary endpoint to adjust the sample size if needed. The trial will also be evaluated for futility regarding the primary endpoints, that is, the predictive probability of success at the end of the trial.

Safety analysis

The number and percentage of patients reporting AEs and the number of AEs reported will be presented. The events will be tabulated by system organ class and preferred term by treatment group. In addition, summaries by

relationship to trial drug and severity will be presented. AEs will also be presented in separate tabulations.

The number of patients experiencing an AE will be compared descriptively between groups. All patients with AEs will be listed individually with the patient number in addition to the type of event, start and stop time, duration, seriousness, severity, any action taken, relationship to trial drug and outcome of AE.

DISCUSSION

This article presents the trial design and rationale for the hyperbaric oxygen for treatment of long COVID-19 syndrome trial. The trial is conducted in compliance with ICH-GCP to protect the safety and well-being of the subjects as well as the integrity and validity of the data. HBOT has been used for almost a century for other chronic inflammatory conditions with well-documented safety profiles for accepted indications.[34] However, the intervention is not without risk. The nature of the disease, which provokes multiple symptoms, and a low quality of life make the risk group a 'vulnerable group', and it is important to make sure that the subjects are not unduly influenced by the expectation or benefits associated with participation.

The randomised, double-blinded design is gold standard, and thus is a strength considering primary endpoints being PRO. The trial design involves multiple exploratory and descriptive endpoints, which may provide valuable data regarding the disease regardless of clinical outcomes. Should HBOT prove clinically effective for the efficacy endpoints, the trial design also allows further investigation into possible causal mechanisms.

LIMITATIONS

The current trial has some important limitations. Long COVID-19 is a novel disease with unknown mechanisms. The prevalence is continuously being revised and it is not known how symptoms and best practice treatment will evolve over time. The treatment protocol in this trial is novel and thus considered a limitation. Normally, HBOT is administered 5 days a week, with 30–40 sessions over 6–8 weeks. The protocol in this trial is based on experience from severe COVID-19 where five treatments seem to be sufficient. However, more research on the dose is needed. Further limitations lie in the possible selection bias of patients being referred through the same outpatient clinic; most patients are severely debilitated (a prerequisite for referral was at least 50% sick leave), and due to long waiting times, most patients have been ill for more than 1 year. The power calculation for the primary endpoint is extrapolated from studies of similar design and diseases with similar symptoms but have not been based on a pilot trial and thus are considered as an increased risk of type II error. However, interim analyses will be performed when 20 patients have data available for safety endpoints, and when 40 patients have available for primary endpoint to minimise the risk of an underpowered trial. Furthermore, sham treatment may have up to 58% efficacy.[35] We did not take this into account when we performed our power calculation, which could result in the trial being underpowered. Both EQ-5D and RAND-36 are the most widely used PRO measures for HRQoL and have been used in the setting of long COVID-19 and similar conditions such as Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) and fibromyalgia, but due to the novelty of the condition, we do not know what to expect from our population and our 'clinically relevant' estimation may be set too high. Three to five points have been proposed as a minimally clinically important difference for RAND-36 when used in health economic evaluation.[36] This assumption in our power calculation may also cause a type II error.

ETHICS AND DISSEMINATION

The trial is conducted in accordance with the Declaration of Helsinki, ICH-GCP, and local and national regulations. The trial was approved by the Swedish Ethical Review board (EPM no 2021–02634, amendment 2021–04572; approval 2021-05-25 and 2021-09-22) and the Swedish Medical Products Agency (LV number 5.1-2020-36673, approval 2021-07-06). The trial was registered online (EudraCT number 2021-000764-30) before the start of the trial.

The trial is monitored by the Karolinska Trial Alliance before the trial started, during the trial and after trial completion. A designated monitor will monitor the randomisation and blinding process. The monitoring is performed to ensure that the trial is conducted in compliance with the protocol, detailed in a separate monitoring plan, and that data are handled according to ICH-GCP.

The first publication will report the results of the interim safety analysis to help other researchers in trial designs and healthcare providers in decision making. The main publication will report the primary and main secondary endpoints together with the full safety and compliance report at 13 weeks. Separate publications will report exploratory endpoints: (1) descriptive results from the Oura ring; (2) health economic analysis; (3) exploratory biomarkers and biochemical analyses; (4) descriptive results from medical history that are collected during the trial; (5) depending on the outcome of the primary endpoint at 13 weeks, follow-up on HRQoL at 26 and 52 weeks; and (6) long-time, post-trial follow-up on HRQoL, 4 years.

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Contributors AK was the principal investigator who wrote the hypothesis and developed most of the protocol together with PL. AK and PL wrote the applications to Swedish institutional review board and MPA. LA-H drafted the manuscript together with AK. AH, SEG, SA-E and EB were subinvestigators who enrolled and evaluated the subjects and collected the data. MN-B, JB, MS and MR were trial chairs involved in writing the protocol and applications. JHK wrote the statistical analysis plan together with AK and designed the randomisation. All authors including CJS, KAR-W, SBC, XZ, KM and JP contributed to the current submission and critically reviewed the manuscript. AK is corresponding author for this work and attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

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Competing interests AK and PL disclose funding from Swedish Heart-Lung Foundation (HLF) and Stockholm Health Council for the present trial. AK disclose funding from Oura Health Oy with complimentary hardware and software for the Oura rings. MS discloses funding from Swedish Research Council and Dysautonomia International during the trial and previously from HLF. MS also disclose consulting fees from the Swedish Agency for Health Technology Assessment of Social Services, speaker honoraria from Orion Pharma, Werfen, and has filed a patent for pharmacological treatment in post-COVID postural orthostatic tachycardia syndrome. JK declares consulting fee for statistical work in this trial. LA-H, AH, SEG, SA-E, EB, CJS, JP, KM, KRW, XZ, SBC, MR, JB and MN-B declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability statement Data are available upon reasonable request. The full trial protocol, statistical plan and consent form will be publicly available. Data will be available on patient level; data will be pseudonymised, the full dataset and statistical code will be available upon request. All publications will be made available on Open Access. Source data will be described in a Meta-data repository. A full description of the intended use of the data must be sent to the corresponding author for review and approval. Participant consent for data sharing is conditioned and new ethics approval may be required.

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REFERENCES

1 Goërtz YMJ, Van Herck M, Delbressine JM, *et al*. Persistent symptoms 3 months after a SARS-CoV-2 infection: the post-COVID-19 syndrome? *ERJ Open Res* 2020;6:00542-2020.
2 Deer RR, Rock MA, Vasilevsky N, *et al*. Characterizing long COVID: deep phenotype of a complex condition. *EBioMedicine* 2021;74:103722.
3 Whitaker M. *Persistent symptoms following SARS-CoV-2 infection in a random community sample of 508,707 people*, 2021. Available: https://spiral.imperial.ac.uk/handle/10044/1/89844 [Accessed 09 Jan 2022].
4 Davis HE, Assaf GS, McCorkell L, *et al*. Characterizing long COVID in an international cohort: 7 months of symptoms and their impact. *EClinicalMedicine* 2021;38:101019.
5 Johansson M, Ståhlberg M, Runold M, *et al*. Long-Haul Post-COVID-19 symptoms presenting as a variant of postural orthostatic tachycardia syndrome: the Swedish experience. *JACC Case Rep* 2021;3:573–80.
6 Ståhlberg M, Reistam U, Fedorowski A, *et al*. *Post-COVID-19* tachycardia syndrome: a distinct phenotype of post-acute COVID-*19* syndrome. *Am J Med* 2021;134:1451–6.
7 Venkatesan P. Lancet Respir Med. In: *Nice guideline on long COVID*, 2021.
8 Soriano JB, Murthy S, Marshall JC, *et al*. A clinical case definition of post-COVID-19 condition by a Delphi consensus. *Lancet Infect Dis* 2022;22:e102-e107.
9 Shah W, Hillman T, Playford ED, *et al*. Managing the long term effects of covid-19: summary of NICE, sign, and RCGP rapid guideline. *BMJ* 2021;372:n136.
10 Ferraro E, Germanò M, Mollace R, *et al*. Hif-1, the Warburg effect, and Macrophage/Microglia polarization potential role in COVID-19 pathogenesis. *Oxid Med Cell Longev* 2021;2021:1–10.
11 Chang R, Mamun A, Dominic A, *et al*. SARS-CoV-2 mediated endothelial dysfunction: the potential role of chronic oxidative stress. *Front Physiol* 2020;11:605908.

12 Mahdi A*et al*. *Erythrocytes Induce Vascular Dysfunction in COVID-19*. In: Institutet K, ed. *Basic to translational science*. SSRN: JACC, 2021.
13 Oliaei S, SeyedAlinaghi S, Mehrtak M, *et al*. The effects of hyperbaric oxygen therapy (HBOT) on coronavirus disease-2019 (COVID-19): a systematic review. *Eur J Med Res* 2021;26:96.
14 Cannellotto M, Duarte M, Keller G, *et al*. Hyperbaric oxygen as an adjuvant treatment for patients with COVID-19 severe hypoxaemia: a randomised controlled trial. *Emerg Med J* 2022;39:88-93.
15 Kjellberg A, Douglas J, Pawlik MT, *et al*. Randomised, controlled, open label, multicentre clinical trial to explore safety and efficacy of hyperbaric oxygen for preventing ICU admission, morbidity and mortality in adult patients with COVID-19. *BMJ Open* 2021;11:e046738.
16 Kjellberg A, De Maio A, Lindholm P. Can hyperbaric oxygen safely serve as an anti-inflammatory treatment for COVID-19? *Med Hypotheses* 2020;144:110224. and.
17 De Maio A, Hightower LE. COVID-19, acute respiratory distress syndrome (ARDS), and hyperbaric oxygen therapy (HBOT): what is the link? *Cell Stress Chaperones* 2020;25:717–20.
18 Robbins T, Gonevski M, Clark C, *et al*. Hyperbaric oxygen therapy for the treatment of long COVID: early evaluation of a highly promising intervention. *Clin Med* 2021;21:e629–32.
19 Oscarsson N, Müller B, Rosén A, *et al*. Radiation-Induced cystitis treated with hyperbaric oxygen therapy (RICH-ART): a randomised, controlled, phase 2-3 trial. *Lancet Oncol* 2019;20:1602–14.
20 Efrati S, Golan H, Bechor Y, *et al*. Hyperbaric oxygen therapy can diminish fibromyalgia syndrome--prospective clinical trial. *PLoS One* 2015;10:e0127012.
21 Akarsu S, Tekin L, Ay H, *et al*. The efficacy of hyperbaric oxygen therapy in the management of chronic fatigue syndrome. *Undersea Hyperb Med* 2013;40:197–200.
22 Calvert M, Kyte D, Mercieca-Bebber R, *et al*. Guidelines for inclusion of patient-reported outcomes in clinical trial protocols: the SPIRIT-PRO extension. *JAMA* 2018;319:483–94.
23 Orwelius L, Nilsson M, Nilsson E, *et al*. The Swedish RAND-36 Health Survey - reliability and responsiveness assessed in patient populations using Svensson's method for paired ordinal data. *J Patient Rep Outcomes* 2018;2:4.
24 Hardt J, Buchwald D, Wilks D, *et al*. Health-Related quality of life in patients with chronic fatigue syndrome: an international study. *J Psychosom Res* 2001;51:431–4.
25 Bagai K, Song Y, Ling JF, *et al*. *Sleep disturbances and diminished quality of life in postural tachycardia syndrome*. *Journal of Clinical Sleep Medicine* 2011;07:204–10.
26 Dolan P. Modeling valuations for EuroQol health states. *Med Care* 1997;35:1095–108.
27 Burström K, Sun S, Gerdtham U-G, *et al*. Swedish experience-based value sets for EQ-5D health states. *Qual Life Res* 2014;23:431–42.
28 Enright PL. The six-minute walk test. *Respir Care* 2003;48:783–5.
29 Ameloot K, Van De Vijver K, Broch O, *et al*. Nexfin noninvasive continuous hemodynamic monitoring: validation against continuous pulse contour and intermittent transpulmonary Thermodilution derived cardiac output in critically ill patients. *ScientificWorldJournal* 2013;2013:1–11.
30 Hamburg NM, Benjamin EJ. Assessment of endothelial function using digital pulse amplitude tonometry. *Trends Cardiovasc Med* 2009;19:6–11.
31 Alexander Y, Osto E, Schmidt-Trucksäss A, *et al*. Endothelial function in cardiovascular medicine: a consensus paper of the European Society of cardiology working groups on atherosclerosis and vascular biology, aorta and peripheral vascular diseases, coronary pathophysiology and microcirculation, and thrombosis. *Cardiovasc Res* 2021;117:29–42.
32 Altini M, Kinnunen H. The promise of sleep: a Multi-Sensor approach for accurate sleep stage detection using the Oura ring. *Sensors* 2021;21:4302.
33 Lansdorp CA, van Hulst RA. Double-Blind trials in hyperbaric medicine: a narrative review on past experiences and considerations in designing sham hyperbaric treatment. *Clin Trials* 2018;15:462–76.
34 Heyboer M, Sharma D, Santiago W, *et al*. Hyperbaric oxygen therapy: side effects defined and quantified. *Adv Wound Care* 2017;6:210–24.
35 Redberg RF. Sham controls in medical device trials. *N Engl J Med* 2014;371:892–3.
36 Samsa G, Edelman D, Rothman ML, *et al*. Determining clinically important differences in health status measures: a general approach with illustration to the health Utilities index mark II. *Pharmacoeconomics* 1999;15:141–55.