

# INHALED nebulised unfractionated HEParin for the treatment of hospitalised patients with COVID-19 (INHALE-HEP): Protocol for an investigator-initiated international meta-trial of randomised studies

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## Abstract

Introduction Inhaled nebulised unfractionated heparin (UFH) has a strong scientific and biological rationale and warrants urgent investigation of its therapeutic potential for COVID-19. UFH has antiviral effects and prevents the SARS-CoV-2 virus' entry into mammalian cells. In addition, UFH has significant anti-inflammatory and anti-coagulant properties, which limit progression of lung injury and vascular pulmonary thrombosis. Methods and intervention This meta-trial is a prospective collaborative individual patient data meta-analysis of randomised controlled trials and early phase studies. Individual studies are conducted in multiple countries. Adult patients admitted to the hospital with confirmed SARS-CoV-2 infection, who do not require immediate mechanical ventilation, are randomised to inhaled nebulised UFH or standard care. All studies collect a minimum core dataset. The primary outcome is intubation (or death, for patients who died before intubation) at day 28, assessed in a time-to-event analysis. The secondary outcomes are oxygenation, clinical worsening and mortality, assessed in time-to-event analyses. Individual studies may have specific outcome measures in addition to the core set. Ethics and dissemination: The meta-trial is registered at ClinicalTrials.gov, ID NCT04635241. Results of this study will be shared with the WHO, published in scientific journals and presented at scientific meetings.

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Authors’ contributions: FvH drafted the manuscript. All authors contributed to revision and finalisation of the manuscript. All authors read and approved the final draft for submission.

Meta-trial Protocol date and version: 1 December 2020, INHALE-HEP meta-trial version

Sponsor meta-trial: INHALE-HEP Collaborative Research Group (CRG). Each individual investigator of every contributing trial is a member of the INHALE-HEP CRG.

Role sponsor: The INHALE-HEP CRG’s executive committee is responsible for the meta-trial’s study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication. Investigators from individual trials have ownership of their trial data. A collaboration and data sharing agreement between investigators facilitates and governs the collecting and meta-analysing of de-identified individual patient data from individual trials and sets out eligibility for authorship.

## Declarations

Funding: No funding has been obtained for the meta-trial. Individual contributing studies/countries are responsible for their own funding.

Availability of data and material: The datasets used for the current manuscript are available from the corresponding author on reasonable request.

Competing interests:

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## Highlights

- This meta-trial is a prospective individual patient data meta-analysis of randomised trials and early phase studies, to determine whether a biologically plausible intervention improves outcomes in hospitalised patients with COVID-19.
- In previous pre-pandemic clinical trials, nebulised unfractionated heparin limits lung injury progression and the development of ARDS and accelerates recovery in mechanically ventilated patients with, or at risk of, acute respiratory distress syndrome.

- The pragmatic pre-specified meta-analysis design effectively deals with recruitment difficulties that could occur in individual studies given the uncertainties of the international dynamics of the COVID-19 pandemic. It also allows smaller feasibility/safety studies to be included in this analysis.
- Individual studies contributing to the meta-trial are conducted in multiple countries, which improves effect size estimates across different conditions as well as the external validity of the results.

## Abstract

### Introduction

Inhaled nebulised unfractionated heparin (UFH) has a strong scientific and biological rationale and warrants urgent investigation of its therapeutic potential for COVID-19. UFH has antiviral effects and prevents the SARS-CoV-2 virus' entry into mammalian cells. In addition, UFH has significant anti-inflammatory and anti-coagulant properties, which limit progression of lung injury and vascular pulmonary thrombosis.

### Methods and intervention

This meta-trial is a prospective collaborative individual patient data meta-analysis of randomised controlled trials and early phase studies. Individual studies are conducted in multiple countries. Adult patients admitted to the hospital with confirmed SARS-CoV-2 infection, who do not require immediate mechanical ventilation, are randomised to inhaled nebulised UFH or standard care. All studies collect a minimum core dataset. The primary outcome is intubation (or death, for patients who died before intubation) at day 28. The secondary outcomes are oxygenation, clinical worsening and mortality, assessed in time-to-event analyses. Individual studies may have specific outcome measures in addition to the core set.

Ethics and dissemination: The meta-trial is registered at ClinicalTrials.gov, ID NCT04635241. The Brazilian study protocol was approved by the Institute of Biomedical Sciences (ICB) Ethics Committee, Sao Paulo (ID 38660320.0.0000.5467). The Argentinian study protocol was approved by the Independent Ethics Committee for Clinical Pharmacology Trials, Buenos Aires (ID N 3183). The Egyptian study protocol was approved by the Ethics committee, Faculty of Medicine, Alexandria University (ID 2158\_11456\_4737). Each contributing study is also registered individually as follows: PACTR202007606032743 (Egypt), NCT04530578 (Argentina). Registration and ethics approval are pending in other countries. Results of this study will be shared with the WHO, published in scientific journals and presented at scientific meetings.

### Introduction

In December 2019, a novel coronavirus (severe acute respiratory syndrome coronavirus 2, SARS-CoV-2) emerged in China and has since spread globally. Nearly 20% of patients with coronavirus disease 2019 (COVID-19) experience hypoxaemia, which is the primary reason for hospitalisation.[1] A significant proportion of patients admitted to hospital for COVID-19 develop acute respiratory failure, with 12-24% requiring intubation for invasive mechanical ventilation.[2-6]

The pathophysiology of COVID-19 associated lung injury is summarised in Figure 1A and is characterised by diffuse alveolar damage, hyperinflammation, coagulopathy, DNA neutrophil extracellular traps (NETs), hyaline membranes and microvascular thrombosis. The scientific rationale and current pre-clinical and clinical evidence for the use of nebulised unfractionated heparin (UFH) as a treatment for COVID-19 has been outlined previously.[7, 8] Nebulised UFH has anti-viral, anti-inflammatory, anticoagulant, and mucolytic effects. The SARS-CoV-2 Spike S1 protein receptor binding domain attaches to UFH and undergoes conformational change that prevents it from binding to the Angiotensin Converting Enzyme 2 (ACE-2) receptor.[9, 10] It was recently demonstrated that spike protein binding to human epithelial cells requires engagement of both cell surface heparan sulphate (HS) and ACE-2, with HS acting as a co-receptor for ACE-2 interaction, and UFH blocked the binding and infectivity of SARS-CoV-2 to human bronchial epithelial cells.[11] The inhibition of SARS-CoV-2 infection of Vero E6 cells by an UFH preparation was found to be concentration dependent, occurred at therapeutically relevant concentrations and is significantly stronger compared to low molecular weight heparins (LMWHs).[12] The anti-inflammatory effects of inhaled UFH are thought to reduce pulmonary hyperinflammation and the generation of DNA NETs, both of which contribute to COVID-19 lung

injury. The anticoagulant actions of nebulised UFH limit fibrin deposition, hyaline membrane formation and microvascular thrombosis, which are also important features of COVID-19. The effects of nebulised UFH in COVID-19 are summarised in Figure 1B.

Animal studies of nebulised UFH in different acute lung injury models have consistently shown a positive effect on pulmonary coagulation, inflammation and oxygenation.[7] Small human studies indicate that nebulised UFH limits pulmonary fibrin deposition, attenuates progression of acute lung injury and hastens recovery.[7] Early-phase trials in patients with acute lung injury and related conditions found that nebulised UFH reduced pulmonary dead space, coagulation activation, microvascular thrombosis, improved lung injury and increased time free of ventilatory support.[13-17] A multi-centre randomised double-blind placebo-controlled trial of nebulised heparin in 256 patients with or at risk of developing ARDS demonstrated reduced progression of lung injury, fewer cases of ARDS and accelerated recovery with more survivors at home by day 60.[18]

We hypothesise that treatment with inhaled nebulised UFH of hospitalised patients with COVID-19 limits progression to acute respiratory failure requiring intubation, reduces the risk of death, reduces the risk of clinical worsening, and improves oxygenation. The collective goal of the proposed meta-trial is to reach a conclusion about the efficacy of inhaled UFH in COVID-19 as quickly as possible by pooling information from multiple clinical trials not originally configured as a network.[19]

## Objectives

The primary objective of the meta-trial is to demonstrate that inhaled nebulised UFH in hospitalised patients with COVID-19 who do not require immediate invasive mechanical ventilation, significantly reduces the proportion of patients who are intubated to receive invasive mechanical ventilation at day 28, compared to standard care alone.

## Methods and analysis

This protocol manuscript has been prepared in accordance with the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) 2013 guideline (Appendix 1).[20]

## Study concept and design

The meta-trial employs prospective pooling of individual patient data from ongoing individual clinical trials and early phase studies.[19] The term “meta-trial” refers to a prospective meta-analysis planned to streamline data collection from multiple individual trials, allowing for faster accumulation of data for major clinical endpoints during the pandemic.[21] This meta-trial is designed as a collaborative prospective individual patient data meta-analysis of investigator-initiated, randomised studies of inhaled nebulised UFH in addition to standard care compared to standard care alone in hospitalised patients with confirmed COVID-19. The primary outcome of the meta-analysis is intubation (or death, for patients who died before intubation) after randomisation, assessed in a time-to-event analysis. Primary outcomes for individual studies may be different clinical or biochemical endpoints and are listed in the individual trial protocols.

## Study setting

This meta-trial will include hospitalised patients with COVID-19 pneumonia who do not immediately require invasive mechanical ventilation in participating institutions. A full list of participating institutions will be available in each individual trial record on respective trial registries. Studies from other countries may be added to this meta-trial after publication of the meta-trial protocol, provided the studies meet the criteria for the meta-trial (patient eligibility criteria, intervention, core set of outcome measures).

## Eligibility criteria

### *Inclusion criteria*

To be eligible, a patient must satisfy all these inclusion criteria:

- Age 18 years or older
- Currently admitted to hospital for COVID-19
- Modified ordinal clinical scale 3-5. The modified ordinal scale is shown in Table 1 and is based on the WHO minimal common outcome measure set for COVID-19.[22]
- There is a PCR positive sample for SARS-CoV-2 within the past 21 days. The sample can be a nasal or pharyngeal swab, sputum, tracheal aspirate, bronchoalveolar lavage, or another sample from the patient.

#### *Exclusion criteria*

To be eligible, a patient must have none of these exclusion criteria:

- Intubated and on mechanical ventilation or requiring immediate intubation as per the treating clinician's assessment
- Enrolled in another clinical trial that is unapproved for co-enrolment
- Heparin allergy or heparin-induced thrombocytopenia (HIT)
- APTT > 120 seconds and this is not due to anticoagulant therapy
- Platelet count < 20 x 10<sup>9</sup> per L
- Pulmonary bleeding, which is frank bleeding in the trachea, bronchi or lungs with repeated haemoptysis or requiring repeated suctioning
- Uncontrolled bleeding
- Pregnant or might be pregnant. Females aged 18-49 years are excluded unless there is documented hysterectomy or a pregnancy test was performed and is negative.
- Myopathy, spinal cord injury, or nerve injury or disease with a likely prolonged incapacity to breathe independently e.g. Guillain-Barre syndrome
- Acute brain injury that may result in long-term disability
- Death is imminent or inevitable within 24 hours
- Treatment limitations in place, i.e. not for resuscitation, not for ICU admission, not for invasive mechanical ventilation
- Clinician objection
- Refusal of participant (person responsible) consent

#### **Recruitment**

Due to the rapidly evolving pandemic situation, we have a strong uncertainty about the pace of enrolment. There is likely to be considerable variation in the number of COVID-19 infections requiring hospitalisation in different regions. The pragmatic pre-specified meta-analysis design overcomes recruitment difficulties that could occur in the individual studies given the international dynamics of the COVID-19 pandemic.

Research coordinators and investigators at each site and for each study will work with clinicians to identify potential candidates for enrolment. Logs will be maintained of patients who met the inclusion criteria but were not enrolled, with the reason for exclusion recorded on the log.

#### **Interventions**

Participants assigned to “nebulised UFH” receive nebulised UFH in addition to the standard care required as determined by the treating team. The dose, frequency, duration and delivery method differ between participating studies as follows:

- Brazil, USA, UK and Australia: 25,000 IU UFH every 6 hours using a vibrating mesh nebuliser (Aerogen Solo), for a maximum of 21 days or until the modified ordinal scale is 1 or 2 (Table 1)
- Egypt: 1000 IU/kg predicted body weight UFH every 6 hours for 7 days using a compressed air nebuliser (Beurer IH18)
- Argentina: 5000 IU UFH every 8 hours for 7 days using a Venturi system connected to a full-face mask (Free Breath) fitted with an HMF anti-viral expiratory filter

Participants assigned to ‘standard care’ will receive the standard care required as determined by the treating team and will *not* be treated with nebulised heparin.

Nebulised UFH will be stopped if the patient has been intubated and commenced on invasive mechanical ventilation.

Nebulised UFH will be withheld if any of the following occurs:

- The treating physician deems that there is a clinically unacceptable increase in APTT
- The treating physician deems that there is excessive bloodstaining of respiratory secretions
- There is pulmonary bleeding, major bleeding or suspected or confirmed heparin-induced thrombocytopenia (HIT)

Nebulised UFH will be recommenced if:

- Having been withheld because the APTT was unacceptably prolonged, the APTT becomes acceptable
- Having been withheld because there was excessive bloodstaining of upper or lower respiratory secretions, the bloodstaining of the respiratory secretions has resolved
- Having been withheld for pulmonary bleeding or major bleeding, the bleeding is definitively controlled
- Having been withheld for suspected HIT, the patient is found not to have this condition

### **Relevant concomitant care permitted or prohibited during the trial**

Treatment with any or all of the following therapies is permitted during the study and not a reason to withhold study medication: deep vein thrombosis prophylaxis with UFH or low molecular weight heparin (LMWH); ‘full’ therapeutic dose UFH or LMWH for a recognised clinical indication; non-heparin anticoagulants; anti-thrombotic medications; protamine; prone positioning; and inhaled nitric oxide. There are no prohibitions during the trial.

### **Provisions for post-trial care**

Post-trial care will be standard care through the standard healthcare system from each institution and jurisdiction.

### **Outcomes**

#### *Primary outcome*

The primary outcome is intubation (or death, for patients who died before intubation) after randomisation.

#### *Secondary outcomes*

Secondary efficacy outcomes are as follows:

- Survival to day 28; Survival to day 60; and Survival to hospital discharge, censored at day 60
- Daily ratio of oxygen saturation by pulse oximetry to the fraction of inspired oxygen (SpO<sub>2</sub>/FiO<sub>2</sub> ratio, highest and lowest levels)
- Daily change in modified ordinal score from baseline to day 14

- Number of patients showing worsening on the modified ordinal scale (see Table 1)

at 3, 7 and 14 days. Worsening is defined as an increase by at least 1 point among patients who had been receiving supplemental oxygen at baseline (modified ordinal scale 4 or 5) or at least 2 points among those who had not been receiving supplemental oxygen at baseline (modified ordinal scale 3).

### *Safety outcomes*

Safety outcomes are as follows:

- Number who record major bleeding. Major bleeding is defined as: bleeding that results in death and/or; bleeding that is symptomatic and occurs in a critical area or organ (intra-cranial, intra-spinal, intra-ocular, retroperitoneal, intra-articular, or intramuscular with compartment syndrome) and /or; bleeding that results in a fall in haemoglobin of 20g/L or more, or results in transfusion of two or more units of whole blood or red cells.
- Number who record pulmonary bleeding. Pulmonary bleeding is frank bleeding in the lungs, trachea or bronchi with repeated haemoptysis or requiring repeated suctioning *and* associated with acute deterioration in respiratory status.
- Number who record epistaxis.
- Number who record HIT. HIT is defined as an unexplained fall in platelet count *and* a positive heparin antibody test.
- Number who record other adverse events and reactions. Adverse events and reactions are those that, in the site Principal Investigator's judgement, are not part of the expected clinical course *and* could be related (at least possibly) to the study *and* were medically significant or had serious sequelae for the patient.

### *Process of care assessments*

Process of care assessments are as follows:

- Time from hospital admission to randomisation
- Total cumulative dose of nebulised heparin
- Days of treatment with nebulised heparin
- Mean daily APTT among all participants, and among those treated with intravenous or subcutaneous unfractionated heparin, and among those not treated with intravenous or subcutaneous unfractionated heparin
- Highest APTT among all participants, and among those treated with intravenous or subcutaneous unfractionated heparin, and among those not treated with intravenous or subcutaneous unfractionated heparin
- Days of treatment with each of the following therapies while in the study: unfractionated heparin, IV and SC; LMWH, IV and SC; lopinavir-ritonavir; remdesivir; hydroxychloroquine; interferon- $\beta$  interleukin antagonists; oseltamivir, laninamivir, zanamivir or peramivir; macrolide; non-macrolide antibacterial; antifungal; corticosteroid; inotrope or vasopressor infusion; and renal replacement.

### *Other Outcomes*

Individual studies have various other primary and secondary outcomes, which are listed in the individual study protocols.

## **Data Collection**

Data will be collected by trained staff at each site under the supervision of the site principal investigator using a case report form and data dictionary. Data will be collected at baseline, from day 0-14 (blood tests, SpO<sub>2</sub>/FiO<sub>2</sub> ratio, modified ordinal scale); on day 28 and day 60 (invasive mechanical ventilation status, vital

status, discharge status). The detailed list of collected data items and the schedule for data collection are provided in the individual study protocols.

Where paper records are used, they will be stored in locked rooms accessible only to authorised personnel. Electronic information will be kept on password protected computers accessible only to authorised personnel. All study material, including case report forms and the study database, will be stored for a minimum period of 15 years after the conclusion of the study or for a period as required by local laws and regulations. Any paper study material that requires disposal will be shredded using a commercial grade shredder or other means that preserves the confidentiality of participants. Any electronic data requiring disposal will be thoroughly erased from its electronic media. Each participating centre will maintain a log of enrolled patients that includes patient identifiers. Patient identifiers are not transferred to the study coordinating centres, nor to the meta-trial coordinating centre, but it must be possible to reidentify patients by each participating centre to allow future audit against source documents.

### **Data Safety Monitoring Board**

There is no independent Data Safety Monitoring Board (DSMB) for the meta-trial. DSMBs are recommended for individual studies and will be specified in the protocols of the individual studies if applicable. The INHALE-HEP executive committee and the trial statisticians are responsible for Bayesian monitoring of the meta-trial.

### **Data management and statistical analysis plan**

#### *Randomisation, allocation concealment and blinding*

Each individual study will be randomised. At randomisation each participant will be assigned to nebulised heparin or standard care. There is a one-to-one allocation ratio. Randomisation is stratified at the individual study level. Allocation concealment is performed at the level of each study. All studies are open label by design. The data analysts are blinded. Unblinding is permissible when pre-specified Bayesian stopping rules for efficacy or safety have been met.

#### *Quality Assurance Monitoring*

Conduct and progress of this meta-trial will be monitored on an ongoing basis by the meta-trial steering committee.

#### *Sample Size*

To demonstrate a clinically important reduction in the primary outcome, a sample size of 712 is required, assuming a decrease in the proportion of patients receiving invasive mechanical ventilation from 12% to 6%, with power 80% and a two-sided significance level of 0.05.

We plan to perform frequent monitoring and analysis of the accumulating data, with use of Bayesian stopping rules that allow timely decisions without the penalties for multiple data looks and alpha spending associated with the classic randomised controlled trial monitoring approach.[19, 23, 24] At each interim analysis, the posterior distribution of the proportion of patients who were intubated will be reported and the prespecified stopping criteria will guide the recommendations of the CRG's executive committee.

#### *Statistical Analysis*

This meta-analysis will be carried out on studies which are conducted in multiple countries, which increases effect size estimates across different conditions as well as the external validity of the results. We plan a prospective meta-analysis of individual patient data. Common variables from all datasets will be combined to conduct the analysis. Analyses will follow the intention-to-treat principle, considering all participants in the treatment group to which they were assigned.

We will use a Bayesian approach for analysis of the pooled individual patient data and for frequent monitoring of the accumulating data for efficacy and safety. We also plan to undertake additional subgroup analyses of

the following variables: protocol individual studies (which includes dose and nebuliser system), severity of COVID-19 (according to the PaO<sub>2</sub>/FiO<sub>2</sub> or SpO<sub>2</sub>/FiO<sub>2</sub> ratio and the modified ordinal scale), duration of intervention, time from admission to start of intervention, time from development of symptoms to start of intervention, influence of other therapies, age of the patients. We describe our detailed statistical analysis plan in a separate manuscript in this journal. [reference to SAP]

### **Ethics and dissemination**

All contributing studies and the meta-trial will be performed in accordance with the ethical principles of the Declaration of Helsinki. Approval of the protocols and related documents will be obtained from the relevant Human Research Ethics Committee (HREC) or Institutional Review Board (IRB) prior to the commencement of each individual study. These authorisations will include data inclusion in the meta-analysis. The investigators of the individual studies will ensure that all HREC/IRB conditions for the conduct of each study are met and that all requisite information is submitted to the responsible HREC/IRB. Any protocol modifications will be communicated timely to relevant parties, including investigators and HREC/IRBs.

Patients will be asked for their consent to participate in the trial.

The results of this study will be provided to the WHO, published in peer-reviewed medical journals and presented to the medical community and other stakeholders.

### **Study status**

At the time of submitting for publication, the studies in Argentina, Egypt and Brazil are recruiting patients. Preparations for the studies in the USA, UK and Australia are underway.

### **Conclusion**

Nebulised UFH has a strong scientific and biological rationale, and warrants urgent investigation of its therapeutic potential, for COVID-19. This investigator-initiated international individual patient data meta-trial of randomised controlled trials and early phase studies investigates the efficacy and safety of nebulised UFH, on relevant outcomes in patients who are hospitalised for COVID-19.

### **Legend**

Table 1: Modified Ordinal Clinical Scale for COVID-19

Figure 1:

Panel A: Lung injury in coronavirus disease 2019 (COVID-19). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) binds to angiotensin-converting enzyme 2 (ACE-2) primarily on type II alveolar cells. After endocytosis of the viral complex, surface ACE-2 is down-regulated, resulting in unopposed angiotensin II accumulation. SARS-CoV-2 further causes lung injury through activation of residential macrophages, lymphocyte apoptosis, and neutrophils. The macrophages produce cytokines and chemokines, resulting in a cytokine storm. Inflammatory exudate rich in plasma borne coagulation factors enters the alveolar space, followed by expression of tissue factor by alveolar epithelial cells and macrophages and the formation of fibrin and the hyaline membrane. Neutrophils in the alveoli cause formation of NETs, composed of extracellular DNA, cytotoxic histones and neutrophil elastase, which cause further lung injury. COVID-19 also induces microvascular endothelial damage leading to increased permeability, expression of tissue factor with coagulation activation and thrombus formation.

Panel B: Proposed effects of inhaled nebulised unfractionated heparin (UFH) in COVID-19 lung injury. UFH prevents SARS-CoV-2 from binding to ACE-2 and from entering the alveolar cells. UFH reduces formation of the hyaline membrane and microvascular thrombosis, counteracts the hyperinflammation and the formation of NETs, increases NO release with vasodilation, and also has mucolytic properties.

NETs = neutrophil extracellular traps, SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2, ACE-2 = angiotensin-converting enzyme 2, COVID-19 = Coronavirus disease 2019

Appendix: Reporting checklist for a protocol, based on the SPIRIT guidelines

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