Nebulised heparin in COVID-19-related ARDS patients undergoing non-invasive ventilation with helmet cPAP:

A prospective, randomised, double blind, placebo-controlled, multicentre study

Date: 07/04/2021, Version 1.2

Protocol name: HelmetHeparin

Sponsor: ASST Fatebenefratelli Sacco

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Signature

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	7-14-2021
Signature	**Date**

1. INTRODUCTION

SARS-Cov-2 coronavirus infection causes a variety of clinical pictures ranging from few minor symptoms to bilateral interstitial pneumonia requiring ventilator support and intensive care treatment (1-3). A full-blown Acute Respiratory Distress Syndrome (ARDS) occurs in around 10% of symptomatic patients and is partly related to an increase in lung vascular permeability leading to capillary oedema and a reduction in ventilated lung parenchyma (4). This is mainly due to an excessive inflammatory response that activates a cytokine storm leading to the activation of the coagulation cascade and microembolism (5, 6). Mortality may be as high as 66%, notably exceeding that from ARDS related to other etiologies (3).

Autopsy studies on COVID-19 patients demonstrated prominent fibrin deposition and hyaline membrane formation in the alveoli together with microthrombi in the lung vascular bed (7). These data suggest a possible role for nebulised unfractionated heparin (UFH) to prevent pulmonary microembolism and there are now a number of clinical studies that are already ongoing (Table 1).

A number of UFH properties theoretically support its use in this setting, namely its antiviral, anti-inflammatory, anticoagulant and mucolytic effects. Heparin is a glycosaminoglycan, present on many biological surfaces such as the endothelium (8), therefore it is an attractive target for viruses.

In humans, heparin is produced by mast cells and plays a major immunological role independent from its anticoagulative properties (9). There is evidence that heparin exerts a competitive effect against another proteoglycan – heparan sulphate – impairing the adhesion on the inner vascular surfaces of many pathogens, such as *Pseudomonas aeruginosa*, *Burkholderia cenocepacia*, *Burkholderia pseudomallei*, *Legionella pneumophila*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, respiratory syncytial virus and influenza A virus (10-12).

A recent study demonstrated a high affinity of heparin with the SARS-CoV2 spike1 protein, impairing its bond with angiotensin converting enzyme receptor-2 (ACE-2) receptor, which typically occurs during coronavirus infections. (13). This is markedly more prominent for UFH than for low-molecular weight heparins (14).

The anti-inflammatory effect of heparin is also connected with its action on endothelial surfaces. In fact, heparin competes with different cytokines, reducing the inflammatory response and favouring nitric oxide release, which improves lung function (15). Moreover, nebulised heparin reduces the concentration of pro-inflammatory cytokines in the lung parenchyma and the NF-kB and TGF-β expression in the alveolar macrophages (16). Animal studies show how the favourable effects of heparin on the inflammatory cascade are much more evident for UFH than for low molecular weight heparins (17). The anti-inflammatory effects of heparin have been known since 1935 (18).

In comparison to its systemic administration, nebulised heparin acts locally on inflammation and fibrin deposits. This allows for higher dosages and may provide a significant effect without increasing the

haemorrhagic risk (19). Indeed, the inhalation route presents low systemic absorption and may be used together with systemic anticoagulation without increasing the risk of haemorrhage (20).

Several studies in patients with acute exacerbations of chronic obstructive airways disease have shown a reduction in ventilator free days following inhaled UFH (25,000 UI every 6 hours) (22, 23, 24). Clinical benefit has been observed in patients with ARDS following UFH at doses up to 75,000 IU twice daily (bid) (25,26). Notably none of these studies have reported any adverse effects from bleeding following inhaled UFH at 25,000 IU every 6 hours, suggesting this may be a relatively safe way of treating COVID-19 patients (27).

The primary endpoint of our prospective, randomised, double-blind, placebo-controlled, multicentre study is the evaluation of the effect of nebulised UFH on patients with COVID-19 undergoing non-invasive continuous positive airway pressure (cPAP) helmet ventilation. The ventilatory support provided by cPAP is meant to "buy time" during the COVID acute phase by improving the alveolar function. Except for patients for whom cPAP is stopped because of need of tracheal intubation or death, the time spent on cPAP is an indicator of the duration of the acute phase. Hence we measure the primary outcome effect as the time on cPAP in patients who survive and do not need tracheal intubation.

Our secondary endpoints are the need for cPAP helmet ventilation, the need for mechanical ventilation, the duration of mechanical ventilation itself, mortality and safety.

1.1 Dose Justification for Nebulised Heparin

Heparin sodium 25,000 IU/5 mL (125 mg) every 6 hours for 10 days.

Since the discovery of heparin in 1916, many toxicological studies of nebulised UFH in rodents and other small animals have been conducted indicating the safety of this approach. Lung tissue from dogs that received intrapulmonary heparin (10 to 15 mg/kg by intratracheal instillation) for a year showed no signs of haemorrhage, anaemia or ulcerative lesions (28). Heparin has also been instilled intratracheally in rabbits using a microspray device every 48 hours in escalating doses of 0.2, 2, 20, 100 and 200 mg/kg, showing signs of bleeding only in the top 2 doses (29).

No acute or chronic toxicological effects have been associated with the inhaled route for any dose of heparin in man. In healthy human subjects, 60,000 to 300,000 UI of heparin was administered as an aerosol with no sign of toxicity (29). No incidence of pulmonary haemorrhage associated with nebulised UFH was reported in any study in a total of 536 patients with smoke inhalation injury, acute lung injury, asthma and allergy (31). Not even pulmonary lavage with intratracheal instillation of 200 to 250 mg/kg of heparin to treat alveolar proteinosis induced bleeding into the lungs or was associated with lung damage a year later (29). Nebulised UFH does not readily cross the bronchial mucosa. In dogs, mice, rats, and man, doses of intrapulmonary heparin > 8mg/kg are required for detectable intravenous (IV) anticoagulation

(29, 30). Furthermore, nebulised UFH did not induce allergic reactions in any study, not even when followed up over long periods of up to 485 days. In a study of the effect of nebulised UFH in patients with idiopathic pulmonary fibrosis, 750 mg (750,000 UI) as the nebuliser fill dose was deemed to be the threshold dose above which effects on systemic coagulation could first be detected (32). In a compassionate phase of the study, patients continued to use nebulised UFH for up to 100 weeks, without side effects. Previous studies (35) have indicated inhalation of heparin treats local inflammation, mucus hypersecretion and lung injury, without systemic anticoagulation (20 studies, 536 patients) (31) and is safe and effective in patients with smoke inhalation injury, acute lung injury, asthma and allergy, cystic fibrosis (33) and ARDS. In another clinical study in patients with moderate to severe ARDS 8% of the loading dose of UFH (150,000 IU) was delivered to the lungs (60 mg) significantly improving lung function, exercise capacity and dyspnoea with no evidence for toxicity or adverse side effects at this dose (26).

For COVID-19, inhaled UFH may offer benefit for patients to reduce the complications arising from a cytokine storm and to prevent the magnitude of the effects of a cytokine storm in patients with moderate to severe COVID-19 driven disease. The recent data suggest that inhaled UFH may also bind the Spike 1 protein that the COVID-19 virus uses to enter cells and may provide an additional anti-viral effect in patients with COVID-19. Those with pneumonic consolidation and consequent increased sputum production will be treated by the mucolytic effect of nebulised UFH (26, 34).

2. MATERIALS AND METHODS

2.1 Screening procedures

Investigators at each site will identify potential patients for enrolment for a total of 160: patients with respiratory failure due to interstitial SARS-CoV2 pneumonia - confirmed with molecular test on naso-pharingeal swab and CT scan - and candidates to helmet cPAP according to the current criteria (21), i.e. the presence of at least one of the following signs during O_2 therapy with O_2 mask reservoir-bag 15 L/min:

- $SpO_2<92\%$,
- respiratory rate > 26/min and/or evident respiratory distress.

After enrolment, helmet cPAP will be applied with PEEP = 5 cmH_2O and FiO_2= 50%.

After 1 hour, the patient will be re-evaluated. If SpO2 will be ≥ 92% and the respiratory rate will be ≤ 26/min without respiratory distress (i.e. if the parameters prompting non-invasive ventilation have shown an improvement) the patient will be randomised and will enter the study. Otherwise, the patient will not enter the study and will be treated according to the attending Anaesthesiologists' indications.

A log will be maintained of patients who met the inclusion criteria but were not enrolled, with the reason

for exclusion recorded on the log.

2.2 Randomisation and allocation concealment

Randomisation will be produced by random number generation from a dedicated software (R: see below in the "Statistics" section). Twenty-patients randomisation blocks will be generated and each participating centre will receive one randomisation block. Closed envelopes will transmit the randomisation number to investigators. Allocation concealment will be maintained by use of a central secure randomisation process hosted at the Fatebenefratelli Hospital, Milan. A randomisation number will be used to ensure allocation concealment cannot be violated by deciphering the sequence near the end of each block. At randomisation each participant is assigned to nebulised heparin or placebo. There is a one to one allocation ratio.

2.3 Study treatment

The study drug will be provided by the Fatebenefratelli Pharmacy in kits of 800 identical 5 ml ampoules of either 25.000 IU heparin sodium without preservative (Veracer 25.000 IU/5ml solution for injection, Medic Italia) or sodium chloride 0.9% (sodio cloruro (fisiologica) 0.9% 5 ml solution for injection, Monico S.p.A.) with labels indicating only the randomisation number.

The study drug will be administered as a nebulised aerosol dose of 25,000 IU heparin or placebo every 6 hours using the Aerogen Solo vibrating mesh nebuliser (Aerogen®). The study drug will be administered in 10-25 minutes and low flow oxygen will be administered using a 6-7 L/min oxygen flow. Helmet cPAP ventilation will be interrupted.

Doses will be made up immediately before use as follows: one 5 ml ampoule of heparin sodium 25.000 UI without preservative or placebo will be added to the nebuliser chamber. A facemask will be applied to the patient which will be connected to the Aerogen Ultra, a valved collection chamber and the Aerogen Solo nebuliser (Figure 1). The study drug should never be mixed with another drug or administered concurrently.

Nebulised heparin sodium 25,000 IU will be delivered using a 6-7 L/min oxygen flow (36) in 10-25 minutes every six hours daily for 10 days or until the patient has no respiratory symptoms.

Randomisation and vials will be delivered to each participant centre's Principal Investigator (PI).

Patients will be randomised to 2 groups.

1. Heparin (study) group; 80 patients.
   Nebulised UFH 25,000 UI (5 ml) will be administered every 6 hours throughout the duration of cPAP ventilation up to a maximum of 10 consecutive days.
2. Placebo (control) group; 80 patients.
   Sodium chloride 0.9% (5 ml) will be administered every 6 hours throughout the duration of cPAP ventilation up to a maximum of 10 consecutive days.

All patients will receive standard COVID-19 therapy, in particular:

- Dexamethasone 6 mg/24 h IV;
- Enoxaparine 4,000 IU/24 h SC, but if there is evidence of thrombosis or the D-dimer raises > 1,000 ng/ml, the dose will be increased to 4,000 IU/12 h (for real body weight < 50 Kg) or 6,000 IU/12 h (for real body weight ≥50 Kg);
- Antibiotic therapy leaded by microbiological evidence.

No other specific COVID-19 treatment is admitted. All other ongoing concomitant medication of the patient is allowed.

Patient's posturing different from the supine will be registered, together with any ventilation parameter changes.

Nebulised heparin administration will stop on the occurrence of one of the following outcomes:

- Positive outcome

  The patient is weaned from helmet cPAP, since he/she is supine or seated with:

  - SpO_2 consistently >94% for at least 12 consecutive hours
  - PEEP ≤ 5 cmH_2O
  - FiO_2 = 40%
  - Respiratory rate ≤ 20/min without respiratory distress

- Negative outcome

  The patient dies or is intubated and mechanically ventilated. The maximum cPAP setting after which intubation is required is PEEP = 12 cmH_2O and FiO_2 = 60%.

The nebulised heparin administration will be stopped after 10 days even if none of the outcomes mentioned above occur.

The duration of helmet cPAP (primary endpoint) will be measured in hours.

Arterial blood gas analysis, PaO2/FiO2, heart rate, arterial pressure, respiratory rate, SpO2, cPAP parameters, PT/PTT, platelet count, type of sedation, and type of posture will be recorded daily. cPAP removal and intubation will be recorded until 10 days after randomisation. If intubation is needed, the number of days of mechanical ventilation will be recorded.

Any adverse event will be registered until 28 days after the last dose of treatment. In particular the reintroduction of cPAP after weaning will be noted.

Mortality will be monitored until 28 days after starting cPAP. A lung CT scan will be obtained 28 days after randomisation – if possible – and according to each participating centre follow-up.

The end of the trial is considered the last visit of the last subject (28 days after the last dose of treatment of the last patient who started cPAP).

2.4 Study Flow Chart

Assessment	V1 Screening and Baseline – D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	V2 Follow-up
Days	1	2	3	4	5	6	7	8	9	10	28-38
Informed consent	X										
Demography	X										
Comorbidities	X										
Concomitant medications (a)	X	X	X	X	X	X	X	X	X	X	
Vital signs (b)	X	X	X	X	X	X	X	X	X	X	
BMI	X										
Eligibility	X										
Randomisation	X										
IMP administration	X	X	X	X	X	X	X	X	X	X	
cPAP	X	X	X	X	X	X	X	X	X	X	
Type of sedation	X	X	X	X	X	X	X	X	X	X	
Type of posture	X	X	X	X	X	X	X	X	X	X	
AEs monitoring	X	X	X	X	X	X	X	X	X	X	X
Mortality monitoring	X	X	X	X	X	X	X	X	X	X	X
Intubation monitoring	X	X	X	X	X	X	X	X	X	X	X
Lung CT											X

(a) Oxygen, enoxaparin, lopinavir-ritonavir, remdesevir, hydroxychloroquine, interferon-ß, interleukin antagonists, oseltamivir, laninamivir, zaninamivir or peramivir, macrolide, non-macrolide antibacterials, antifungal, corticosteroid, vasopressor infusion and renal replacement

(b) Arterial blood gas analysis, PaO2/FiO2, heart rate, arterial pressure, respiratory rate, SpO2, PEEP, PT/PTT

2.5 Safety assessments: adverse events

Nebulised UFH is not approved for any indication. IV heparin is approved and is generally well-tolerated. Adverse events reported for the approved labelling for IV heparin include haemorrhage, heparin-induced thrombocytopenia (HIT) and heparin-induced thrombocytopenia and thrombosis (HITT), thrombocytopenia, heparin resistance and hypersensitivity; however, these events have not been reported for nebulised UFH due to reduced systemic absorption. Recent studies (37) indicate that the association between subcutaneous LMWH and nebulised heparin is safe therefore only clinical monitoring and daily PT/PTT/platelet count to monitor bleeding adverse events are necessary.

Patients receiving nebulised UFH can expect to expectorate larger volumes of sputum, reflecting the pharmacological effect of a mucolytic.

Moreover, possible adverse effects associated with nebulised UFH include:

- Headache
- Epistaxis
- Systemic anticoagulation (this is not expected at the inhaled dose used)
- Haemoptysis (although for all the reasons given above this is unlikely to be associated with nebulised UFH and is as likely to be seen in the placebo group)

2.5.1 Definitions

Adverse event (AE):

An AE can be defined as any untoward medical event which occurs in a patient or clinical investigation subject who has been administered a medicinal product and which does not necessarily have a causal relationship with the treatment.

Serious adverse event (SAE)

A SAE can be defined as any untoward medical occurrence or effect that at any dose results in any and following outcomes:

- death,
- life-threatening,
- inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant disability or incapacity,
- a congenital anomaly/birth defect,
- an intervention to prevent permanent impairment or damage,
- important and significant medical event that may not be immediately life-threatening or resulting in death or hospitalization, but based upon appropriate medical judgment, may jeopardize the participant or may require intervention to prevent one of the other outcomes listed above.

Non-Serious Adverse Event (non-SAE)

A non-SAE can be defined as event that does not meet the definition of a SAE.

Suspected Unexpected Serious Adverse Reaction (SUSAR)

A SUSAR refers to an adverse reaction to an investigational product of which nature and severity are inconsistent with the respective clinical study information, including Investigator's Brochure in the pre-approval experience and Summary of Product characteristic (SPC) in post-approval experience.

2.5.2 Classification of AE

2.5.2.1 Severity of AE

The severity of an AE should be judged based on the following terms:

- *mild*: awareness of sign(s) or symptom(s) which is/are easily tolerated;
- *moderate*: enough discomfort to cause interference with normal daily activity;
- *severe*: incapacitating or causing inability to work or to perform daily activity.

Please note: a severe AE is not the same as a serious AE. Seriousness of an AE serves as a guide for defining regulatory reporting obligations.

2.5.2.2 Causal relationship to investigational product

The assessment of whether there is a reasonable possibility of a causal relationship between investigational product and AE is usually made by the Investigator. A comprehensive scale of common use to categorise an event is:

Term	Description
Certain	A clinical event or laboratory test abnormality, with plausible time relationship to drug administration, and which cannot be explained by disease or other drugs. The response to withdrawal of the study intervention should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.
Probably related	A clinical event or laboratory test abnormality, with reasonable time sequence to drug administration, and which unlikely to be attributed to disease or other drugs, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfil this definition.
Possibly related	A clinical event or laboratory test abnormality, with a reasonable time relationship to drug intake, but which could also be explained by concurrent disease or other drugs.
Unlikely to be related	A clinical event or laboratory test abnormality, with a time to drug administration that makes a relationship improbable, and in which disease or other drugs provide a plausible explanation.
Not related	The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another aetiology. There must be an alternative, definitive aetiology documented by the clinician.

2.5.2.3 Expectedness

The Sponsor is responsible for assessing whether an AE is expected or unexpected. An AE will be considered unexpected if its nature, severity and/or frequency is inconsistent with the information in the Reference Safety Information (e.g., the documents including the Investigator's Brochure).

2.5.3 Recording of AE

Any AE occurred in a patient who has been provided Informed Consent, regardless of the assumption of casual relationship, must be documented on the eCRF.

For each AE, the Investigator will provide the following information: event description, time to onset, severity, start date and stop date (if applicable), seriousness, action taken.

The AE should be documented from the time the subject signs the Informed Consent until 28 days after the last dose of investigational product. If a subject has an ongoing AE at the time of study completion, the ongoing AE must be followed up and appropriate medical care must be provided until the events resolve or stabilise.

2.5.4 SAE reporting

Every SAE, regardless of the causal relationship to the investigational product, occurring after the patient signed Informed Consent, until 28 days after the patient stopped the study treatment, must be reported by the Investigator to the Sponsor within 24 hours from when it becomes aware by filling the Sponsor's SAE form. In addition, the Investigator must record it in eCRF.

Any SAE observed after the 28th day, must be reported to the Sponsor only if the Investigator suspects a causal relationship to the investigational product.

Recurrent episodes, complications, or progression of the initial SAE must be reported as follow-up to the original episode within 24 hours of the Investigator receiving the follow-up information.

Any SAE must be followed up until resolution or, in case it has caused permanent impairment, until considered stabilised.

2.5.5 Abnormal laboratory findings and non-SAE reporting

Abnormal laboratory values or test results occurring after informed consent constitute AE only if they induce clinical signs or symptoms, are considered clinically significant, require therapy or require changes in investigational product.

Disease-related events such as disease progression (including fatal outcomes) should not be reported as AEs.

All non-SAE must be recorded and described on the eCRF.

2.5.6 SUSAR reporting

SUSARs are SAEs that are unexpected and judged by the Investigator or Sponsor to be related to the investigational product. All SUSAR must be notified by the Sponsor to the Competent Authorities through the EudraVigilance Clinical Trial Module (EVCTM) and to the Ethics Committee that issued the Single Opinion: all fatal or life-threatening SUSARs within 7 days, all other SUSARs within 15 days.

SUSARs require follow-up by the Sponsor, who must actively collect information on their evolution and provide updates, in the same way as SUSARs are reported. The follow-up must be notified within 8 days for fatal or life-threatening SUSARs, while no time is defined for other SUSARs, but it must be notified in the shortest time possible.

2.6 Data collection

eCRF will be used for retrieval of information using a validated system that addresses traceability. Thus, the data will be collected through REDCap, being a secure web application for building and managing online surveys and databases. REDCap provides automated export procedures for data downloads to Excel and the R software which will be used to analyse the data. Also, a built-in project calendar, a scheduling module, ad hoc reporting tools, and advanced features, such as branching logic, file uploading, and calculated fields will be used. Furthermore, it provides audit trails for tracking data manipulation and

user activity.

3. STUDY OBJECTIVES

3.1 Primary objective

Assess the effect of 25,000 IU Q4 of nebulised UFH in COVID-19 patients undergoing non-invasive cPAP helmet ventilation in terms of reducing cPAP use.

3.1 Primary endpoint

The primary endpoint is the number of hours of cPAP use in COVID-19 patients undergoing non-invasive cPAP helmet ventilation who neither die nor undergo mechanical ventilation.

3.2 Secondary objectives

Assess the effect of 25,000 IU Q4 nebulised UFH in terms of:

- need for cPAP helmet ventilation;
- need for mechanical ventilation;
- duration of mechanical ventilation (when needed);
- mortality during the 28 days following the beginning of cPAP;
- safety

in patients undergoing non-invasive cPAP helmet ventilation for COVID-19 pneumonia.

3.2 Secondary endpoints

The secondary endpoints are:

- removal of cPAP helmet ventilation (point 4, 3, 2, 1 on the WHO ordinal scale) within 10 days from randomisation;
- proportion of patients requiring invasive mechanical ventilation (point 6-7 on the WHO ordinal scale) within 10 days from randomisation;
- number of days of mechanical ventilation;
- proportion of patients who die within 28 days following the beginning of cPAP (point 8 on the WHO ordinal scale);
- daily ratio of partial pressure of oxygen to FiO_2 ($PaO2/FiO_2$);
- number and type of adverse reactions

in patients undergoing non-invasive cPAP helmet ventilation for COVID-19 pneumonia.

3.3 Process of care assessments

The following data will be collected to assess the process of care:

- time from hospital admission to randomisation;
- total cumulative dose of nebulised heparin;
- days of treatment with nebulised heparin;
- mean daily APTT among all patients;
- days of treatment with each of the following therapies while in the study: lopinavir-ritonavir, remdesevir, hydroxychloroquine, interferon-ß, interleukin antagonists, oseltamivir, laninamivir, zaninamivir or peramivir, macrolide, non-macrolide antibacterials, antifungal, corticosteroid, vasopressor infusion and renal replacement.

4. INCLUSION CRITERIA

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

- Age 18 years or older
- Positive molecular COVID-19 test on nasopharyngeal swab
- Interstitial pneumonia confirmed on lung CT scan
- Indication to helmet cPAP according to the COVID-19 Treatment Guideline criteria (21), i.e. the presence of at least one of the following signs during O_2 therapy with O_2 mask reservoir-bag 15 L/min:
    - $SpO_2 < 92\%$,
    - respiratory rate > 26/min and/or evident respiratory distress

  (point 5 on the WHO ordinal scale)

5. EXCLUSION CRITERIA

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

- Age 80 years or older
- Ongoing anticoagulant therapy
- Clinically not compatible with helmet cPAP interruption for 10-25 minutes 4 times a day
- Home cPAP treatment
- Home O_2 therapy
- Insulin-dependent diabetes mellitus
- Acute myocardial infarction during the previous 6 months
- Ongoing oncological disease
- Chronic renal failure

- CT scan diagnosis of pulmonary thromboembolism
- Body Mass Index (BMI) over 40
- Uncontrolled bleeding
- Not able to understand or sign the informed consent

6. STATISTICS

Unpublished data of the ASST Fatebenefratelli Sacco Hospital from both the March-April and the October-December 2020 time spans indicate that the duration of cPAP in COVID-19 patients, who neither died nor were admitted to the ICU, was 210±144 h (mean±SD).

Aiming at observing a difference between two equally sized groups of at least 3 days (144 h) with alpha = 0.05 and power = 80%, a parametric evaluation of the sample size requires 64 patients per group. Taking into account dropouts and deviations from normality, we will enrol 80 patients per group (total 160 patients).

Data analysis will be performed according to the "intention-to-treat" principle.

The primary endpoint (hours on helmet cPAP in patients who neither die nor are admitted to the ICU) will be analysed with the unpaired two-samples Wilcoxon rank sum (Mann-Whitney) test.

Among the secondary endpoints, the proportion of died patients and of patients admitted to the ICU will be analysed with the Chi-square test, and the number of days on mechanical ventilation (when needed) will be analysed with the unpaired two-samples Wilcoxon rank sum (Mann-Whitney) test.

Continuous variables will be reported as mean±SD [median (IQR)] and categorical variables as number(percentage).

Data will be analysed with the R software (R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/).

7. ETHICAL CONSIDERATIONS

This study will be performed in accordance with the ethical principles of the Declaration of Helsinki and ICH Good Clinical Practice. Approval of the protocol and related documents will be obtained by the Ethical Committee prior to the beginning of the study at each centre. The Investigators will ensure that all conditions for the conduct of the study are met and that all information is submitted to the Ethical Committee.

Figure 1. The vibrating mesh nebuliser (Aerogen Solo), the valved collection chamber (Aerogen Ultra) and the valved facemask

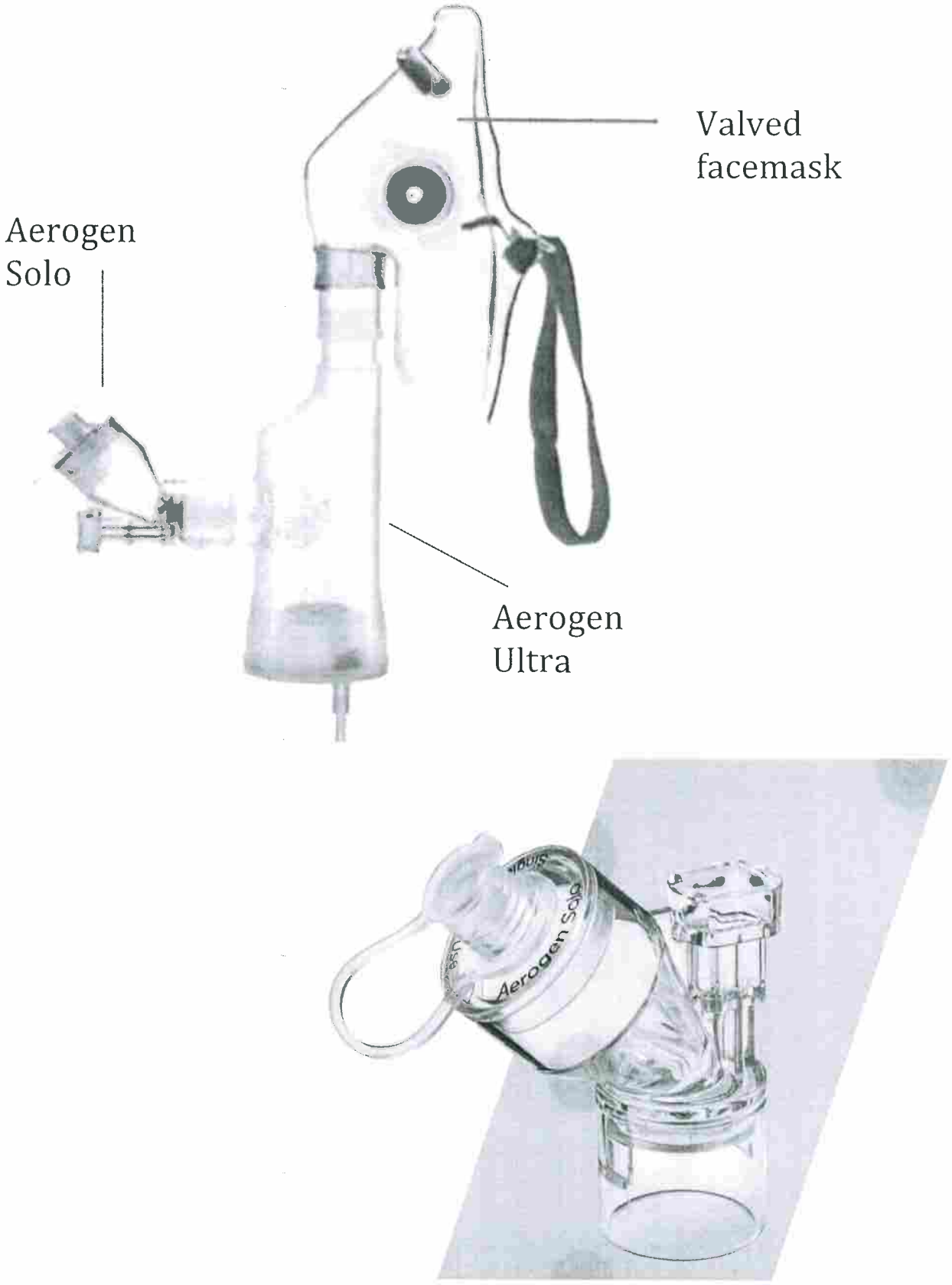

Table 1 Studies registered in clinicaltrials.gov, Eudract and AIFA on nebulised UFH for COVID-19 pneumonia

Study title	Institution	Status	intervention	Identifier Number	Protocol name
Nebulized Heparin in Severe Acute Respiratory Syndrome COVID-19	Clinica San Camilo Ciudad Autonoma de Buenos Aire, Buenos Aires, Argentina	On-going	Nebulised heparin + LMWH in pneumonia	NCT04530578	NEBUHEPA
Protocol of the Can Nebulised Heparin Reduce Time to Extubation in SARS-CoV- 2 Study	St Vincent's Hospital (Melbourne), Victoria, Australia	On-going	Nebulised heparin 25000 UI each 6h in ICU intubated patients	ACTRN: 12620000517976	CHARTER
A Multicentre, Seamless, Phase 2 Adaptive Randomisation Platform Study to Assess the Efficacy and Safety of Multiple Candidate Agents for the Treatment of COVID 19 in Hospitalised Patients	University Hospital Southampton NHS Foundation Trust	On-going	Nebulised heparin 25000 UI each 6h up to 21 days	EudraCT: 2020-001736-95	ACCORD 2
Inhaled Heparin for Hospitalised COVID -19 Patients	Helwan University Clinica San Camilo, Argentina	On-going	inhaled nebulised unfractionated heparin in addition to standard care Dose 25,000 IU every 6 hours for up to 21 days	NCT04635241	INHALE-HEP
Nebulised Heparin in Patients With Severe COVID-19	Frederick Health Hospital Frederick, Maryland, United States	On-going	Mechanical ventilated covid-19 randomised to receive nebulised heparin	NCT04545541	CHARTER-MT
Nebulised Heparin to	Galway University	Not-yet	Nebulised	NCT04511923	CHARTER-

Reduce COVID-19 Induced Acute Lung Injury	Hospital Galway, Ireland	recuitin g	heparin mechanical ventilation		Irl

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