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Nebulized Enriched Heparin to Treat no Critical Patients With Sars-Cov-2 - Triple Blind Clinical Trial

Brief Title:

Enriched Heparin Anti COVID-19 Trial (EnHanCed)

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Background

The first cases of coronavirus in humans were initially identified in 1937 and their particular crown-like form was demonstrated in 1965.[1] A new coronavirus was discovered in December 2019, after the first cases were diagnosed in Wuhan, China, being called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causing coronavirus disease (Covid-19).[2] The disease spread rapidly throughout the country of origin and internationally, reaching pandemic status. A portion of these more severe cases evolved with severe respiratory failure related to COVID-19 and can quickly progress to the need for assisted mechanical ventilation.[3] The severity of the cases and the need for highly complex medical and hospital resources, associated with a large number of cases due to the high infectivity of the virus, has led numerous health systems to their maximum support capacity, which caused the collapse of countless health services, in addition to a strong socioeconomic impact related to health policies and social isolation imposed in order to decrease the speed of spread of the disease.

Rationale

Heparins are natural polydisperse and heterogeneous acidic polysaccharides belonging to the group of glycosaminoglycans.[4] It is being used as an anticoagulant for over 80 years, with characteristics of well-established safety, stability, bioavailability, and pharmacokinetics. Heparins have other clinical applications that are not completely explored, including antiviral activity against flavivirus,[5, 6] herpes,[7] influenza[8] and HIV,[9, 10] and, more recently, evidenced action antiviral against the current SARS-CoV-2.[11]

The interactions that occur between viral surface proteins and host cell receptors are the way for viral transmissibility[12]. SARS-COV-2 expresses the surface glycoprotein S1, which contains a receptor binding domain (RBD) that interacts with the angiotensin-converting enzyme 2 (ACE2), enabling the onset of infection.[13] Previous studies demonstrated that heparin and its analogues are able to bind to the SARS-CoV-2 spike protein and block SARS-CoV-2 infection using therapeutic relevant concentrations. Such inhibition is likely to be caused by overlapping heparin / HS binding sites on S1 RBD restricting binding with the angiotensin-converting enzyme 2.[14]

We have developed a method to enrich commercially available unfractionated heparin through simple filtration. Unfractionated heparin, containing chains of molecular weight between ~5,000 Da and ~30,000 Da,[15] is filtered (Amicon-10 kDa® centrifuge - Millipore™) to obtain an enriched heparin preparation that possesses higher amounts of chains of molecular weight greater than 10.000 Da.

This enriched heparin showed antiviral action *in vitro* (data not shown), using standardized cell-based assays in the VERO cells infected with a clinical isolate SARS-CoV-2/SP02/human/2020/BRA (GenBank access n° MT126808.1). Upon heparin treatment, there was a variable decrease in viral load up to 100% with no observed cytotoxicity in the tested doses (62.5µg/mL to 250µg/mL). Together with widespread evidence that heparin molecular weight affects its anti-SARS-CoV-2 infectivity, we envisage that this enriched heparin preparation can be more effective in viral inhibition.

Inhaled Heparin

The mechanism of nebulized heparin has been tested in several clinical studies in the last two decades, mainly for the treatment of acute airway inflammation caused by smoke inhalation and severe acute respiratory diseases.[16] As it is a systemic anticoagulant, the administration of heparin by inhalation presents an initial concern regarding the risk of bleeding during treatment. However, it is known that the systemic absorption of heparin by this route is minimal.

A study carried out by Bendstrup, et al (2002),[17] evaluated the effects of the inhibition of systemic coagulation through nebulized unfractionated heparin at doses up to 64 mg and identified that there is a dose-dependent effect for factor Xa inactivation, inducing minimal changes in aPTT, and it is safe for clinical or research purposes, without increasing the risk of bleeding.[18]

Phelps, et al (2020),[19] carried out a systematic review on the use of UFH inhalation for the treatment of inhalation injury caused by heat in burn patients and concluded that nebulized heparin did not demonstrate an increase in the risk of bleeding rates and decreased the duration of mechanical ventilation.

For COVID-19, the heparin anti-viral effect is expected to be related to the fact that heparin blocks viral interaction with host receptors thereby inhibiting virus interaction with the cell and, consequently, viral invasion.[20, 21] To this moment, some studies indicate that treatment with systemic heparin reduced hospital mortality in patients with COVID-19[22] and a previous study that strongly supported the clinical investigation of heparin as a potential treatment for patients with COVID-19,[23, 24] justifies further investigation to prove these clinical effects.

Objectives

Primary objectives assessment

Safety: related to the use of nebulized enriched heparin by patients with SARS-CoV-2 through the assessment of hemorrhagic events of any nature, alteration of the coagulation testing, like prolonged activated partial thromboplastin time (aPTT), indicated by an increase in aPTT>1.5, and heparin-induced thrombocytopenia;

Efficacy: related to the proposed treatment, through analysis of the SARS-CoV-2 viral load of the participants treated by the sequential assessment of reverse transcription-polymerase chain reaction (RT-PCR) in the nasal swab.

Secondary objectives assessment

Safety measured by respiratory, clinical, laboratorial and tomographic findings as follows, in number of patients, and hospitalization days:

Supplemental oxygen therapy needed;

Mechanical pulmonary ventilation needed;

Renal failure development;

Development of major cardiovascular events such as pulmonary embolism and acute myocardial infarction;

Demand for intensive care unit (ICU) treatment;

Secondary pulmonary bacterial infections (bacterial pneumonia);

Deep vein thrombosis (DVT) through Doppler ultrasonography assessment;

Pancreatitis characterized by increase in amylase >200 U/L;

Demand for corticosteroid therapy such as hydrocortisone, dexamethasone and other corticosteroids duo to inflammatory pulmonary disease;

Death;

Increase in white blood cell count (>10.000 cells/mm^3);

Increase in C reactive protein test (CRP) (>3.00mg/L);

Deterioration of arterial blood gas paO2/pFiO2 ratio (<200);

Altered sodium (< 135mEq/L or > 145mEq/L);

Altered potassium (< 3,5mEq/L or > 5,5mEq/L);

Worsening of pulmonary area compromised (%) by inflammation or infection through tomographic assessment.

Method

EnHanCed study design

The present protocol refers to a phase I / II, prospective, randomized, parallel, triple blind clinical trial. This design is justified by the lack of a drug-specific cost-efficient treatment for SARS-CoV-2 patients. The use of a therapeutic drug justifies the need for a placebo control group. A minimum number of participants will be allocated to this study, as the main intention will be to evaluate the safety of the treatment. Triple blind clinical studies have the lowest levels of bias and, therefore, the highest level of evidence for a clinical study. It is understood that there is no need for a purely phase I study based on the literature data previously presented.

The study will be carried out at the São Paulo State University (UNESP) Clinical Hospital of the Botucatu Medical School, Botucatu, São Paulo, Brazil.

Dosage and administration

Considering the pulmonary water loss of 8 to 10mL during the gas exchange in 30 minutes (of inhalation) and compensating the dilution of the solution in the pulmonary fluids, we opted for the dose of 2.5 mg heparin in 10mL of 0.9% saline solution, aiming the effective mean tested doses (62.5µg/mL to 250µg/mL), nebulized 4/4 hours, except the nebulization that would be administered between 12 pm and 6 am. The treatment will be administered for 7 days because it will be assumed that the reduction in viral load promoted by enriched heparin will have a significant impact during the first 14 days of the infectious process. Considering that the patient has already had the disease for at least seven days when admitted and that the viral load tends to reduce spontaneously after the referred period, it is known that complications persist due to other factors such as inflammatory response.

Individual vials containing 10 mL of enriched heparin (250ug/mL, that will be adjusted after the filtration process based on the result of biochemical analysis) or placebo (adding to the same total volume of 0.9% saline solution) will be prepared by the pharmacist, stored in a freezer at -30º Celsius, and delivered to the research team in a blind character. After receiving the preparation (enriched heparin or placebo), it must be inserted into the nebulizer cup and administered in a source of oxygen or compressed air (5L/min or according to the patient's need) in isolated rooms, a process that spend 30 minutes. Nebulization therapy will be performed after isolation of the patient and with support from the hospital infection control committee, so that there will be no greater risk of spreading the virus.

Eligibility criteria

Sign and agree to the free and informed consent form;

Both sexes, of any ethnic origin, aged between 18 and 90 years;

COVID-19 infected, diagnosed by reverse-transcriptase polymerase chain reaction (RT-PCR) or with a strong suspicion of COVID-19 through compatible clinical and radiological findings;

Time of disease evolution less than 10 days;

Radiological diagnosis of grade IIA pneumonia, with gas exchange ratio>200 in blood gas analysis (paO2 / pFiO2), characterizing mild hypoxemia;

Indication of hospital treatment regime, provided that the period of hospitalization before inclusion is not more than 24 hours;

Demand for supplemental oxygen therapy (O2) less than 5 L/min.

Exclusion criteria

Disagree with the terms of the study;

Moderate or severe respiratory failure requiring ICU admission requiring invasive mechanical ventilation or positive pressure non-invasive ventilation (NIV);

Pregnancy or puerperium;

Hematological diseases;

Coagulation disorders;

Previous use of anticoagulants in therapeutic dosages for more than 72 hours;

Previous heparin-induced allergy or thrombocytopenia or current thrombocytopenia with a count of less than 50,000 platelets/mm3.

Discontinuation criteria

Participants can withdraw from the study, if they wish, at any time and regardless of the reason, without consent of the research team. Any type of severe adverse events, according to the rules of the Common Terminology Criteria for Adverse Events (CTC-AE 5.0)[25] will be considered criteria for discontinuation.

The reasons for discontinuing treatment will be properly documented by the research team and, in case the study is closed, the researchers will ensure the continuity of the medical and hospital assistance to the participant.

If COVID-19 is not confirmed by RT-PCR within 72 hours of the inclusion, the patient will be withdrawn from the study.

Randomization and study arms

Participants will be divided in two groups and randomized 1:1. In this way, they will receive a sequential inclusion number from an electronically drawn number table and will receive the treatment according to the allocation in one of the groups:

Group 1 - Control: Nebulization with 10mL of 0.9% saline solution, administered every 4 hours for 7 days, except the nebulization that would be administered between 12pm and 6 am (5 inhalations per day).

Group 2 - Experimental: Nebulization with enriched heparin (250µg/mL diluted in 10mL of 0.9% saline solution), administered every 4 hours, for 7 days, except the nebulization that would be administered between 12 pm and 6 am (5 inhalations per day).

The randomization list will be prepared by an independent statistician, using Stat Trek, a program to construct random number tables, available at http://stattrek.com/Tables/Random.aspx. Fifty positions will be drawn in a random and consecutive manner. The envelope containing the randomization will be kept in possession of an independent professional (pharmacist), so that the members of the study team will not know the order of allocation.

Blinding

The clinical trial will be triple-blind. Participants, researchers, the data and statistical analysis team will not have access to the allocation numbers. A pharmacist will produce the heparin and the placebo and will assign a researcher of the team to distribute the product to each participant, according to the randomization list. In addition, presentation of the preparation to the control and experimental group will be identical, since enriched heparin is colorless and odorless to the human eye and nose, as well as the saline solution. The blinding code will not be broken until all research participants have completed the study and the database is closed, that is, it will not undergo further changes. In an emergency, and if necessary, members of the data and security monitoring committee can request a blinding break, only for the participant at risk.

Sample size, recruiting and statistical analysis

The total recruitment size will be 50 participants, aiming at 40 patients followed during the study period. The sample was estimated considering the number of patients diagnosed as COVID-19 at the São Paulo State University (UNESP) Clinical Hospital that met the study's eligibility criteria.

The sample calculation of 40 patients took into account two independent study arms, with an estimated proportion in the viral load reduction rate of 30% for group 1, that will receive placebo, and 75% for group 2 that will be treated with enriched nebulized heparin as proposed in this study, considering 80% test power and a 5% significance level for a bilateral test.

Categorical variables will be evaluated with Fisher's exact test, continuous variables will be compared with non-parametric methods such as Mann-Whitney test or U test, paired comparisons will be evaluated with the Wilcoxon test, multivariate analyzes will be evaluated with regression Cox analysis and long-term clinical analyzes will be compiled on Kaplan-Meir curves.

Interventions and inpatient schedule

Intervention	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Informed consent form	X								
Medical visit, patient story and physical examination	X	X	X	X	X	X	X	X	X
Vital signs assessment	X	X	X	X	X	X	X	X	X
Medications in use	X	X	X	X	X	X	X	X	X
Inclusion and exclusion criteria	X								
COVID-19 viral load in RT-qPCR	X		X			X		X	X
Complete blood count, D-dimer, aPTT, urea, creatinine, glucose, reactive protein C, blood gas and amylase	X		X			X		X	X
Computed thoracic tomography	X								X
HMWH or placebo administration		X	X	X	X	X	X	X	
Adverse events	X	X	X	X	X	X	X	X	X
Discontinuation criteria	X	X	X	X	X	X	X	X	

Table 1. Schedule for assessments and intervention. The participants will be followed up and evaluated on days 0, 1, 2, 3, 4, 5, 6, 7 and 8 days after randomization.

The closure assessment will be carried out on day 8. Extra data can be collected if the patient's clinical condition changes. Randomization will be performed in up to 24 hours after admission. After the end of the study, the participant will be referred and accompanied at the COVID-19 outpatient clinic São Paulo State University (UNESP) Clinical Hospital.

Adverse events

Adverse effects related to the nebulized use of heparins will be tracked and analyzed according to the Common Terminology Criteria for Adverse Events (CTC-AE 5.0),[25] and will be graded from 1 to 5, accordingly to the severity of the event.

Grade	Description
Grade 1: Mild	Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2: Moderate	Minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily life.
Grade 3: Severe or medically significant but not immediately life-threatening	Medical hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily life.

Grade 4: Life-threatening consequences	Urgent intervention indicated.
Grade 5: Death	Death related to adverse effect.

Table 2. Common Terminology Criteria for Adverse Events (CTC-AE) 5.0 classification.[24]

Adverse effects secondary to the use of heparins will cause the discontinuation of the study if graded 3 or more. All severe adverse effects noted by the researchers will be reported to the independent data and security monitoring committee.

The following data will be analyzed in case of an adverse event: the type of event or reaction, including predefined events (major bleeding, pulmonary bleeding and heparin-induced thrombocytopenia); start date and time; date and time of the most recent administration of heparin; the extent of any causal link to the enriched heparin; the severity of the event.

The following adverse events and reactions will be considered severe:

Any event that is related to the use of enriched heparin and causes prolongation of hospital stay, disabling, limiting self-care activities, urgent interventions needed and death; bleeding that results in death, that occurs in a critical area or organ (intracranial, spinal, intraocular, retroperitoneal, intra-articular or intramuscular), that results in a drop in hemoglobin of 20g/L or more or demands transfusion of two or more units of whole blood or red blood cells; lungbleeding, which is the notable bleeding from the lungs, trachea or bronchi with repeated hemoptysis or which requires repeated aspiration and is associated with acute deterioration of the respiratory state; heparin-induced thrombocytopenia, which is an unexplained drop in platelet count and a positive heparin antibody test; any other adverse event and reaction that, after evaluated by the independent data and security monitoring committee, are considered not part of the expected clinical course and may be related to the study.

Assessment forms

Epidemiological and personal antecedents form					
Form Number:	**Date:**				
Sex	F ◯	M ◯			
Age (in years)					
Color (self-declared) (Brazilian Institute of Geography and Statistics terminology)[34]	White ◯	Black ◯	Indigenous ◯	Brown ◯	Asian ◯
Residency	Urban ◯	Rural ◯			
Height (m)					
Weight (kg)					
Abdominal circumference (cm)					
Diabetes	Yes ◯	No ◯			
Hypertension	Yes ◯	No ◯			
Heart disease	Yes ◯	No ◯			
Dyslipidemia	Yes ◯	No ◯			
Obesity	Yes ◯	No ◯			
Sedentary lifestyle	Yes ◯	No ◯			
Smoking	Yes ◯	No ◯			
If smoker, smoking load (pack-year)					
Alcoholism	Yes ◯	No ◯			
Previous cardiovascular ischemic events	Yes ◯	No ◯			
Previous cerebrovascular ischemic events	Yes ◯	No ◯			
Previous venous thrombosis or pulmonary thromboembolism	Yes ◯	No ◯			
Neoplasia under treatment (<5 years)	Yes ◯	No ◯			
Acquired immunodeficiency syndrome	Yes ◯	No ◯			
Autoimmune disease	Yes ◯	No ◯			
Other diseases (report)					

Figure 1. Epidemiological and personal antecedents form based o Brazilian epidemiology.

Clinical assessment and physical examination form

Form Number:	**Date:**		
Diagnosis day			
Time elapsed since diagnosis (days)			
Time elapsed since start of treatment (days)			
Time elapsed since star of symptoms (days)			
Correct use of inhalation treatment	Yes ◯	No ◯	
Symptomatic medications use	Yes ◯	No ◯	
Supplementary oxygen use	Yes ◯	No ◯	
Non-invasive ventilation use	Yes ◯	No ◯	
Mechanical ventilation use	Yes ◯	No ◯	
Bleeding	Yes ◯	No ◯	
Type of bleeding (epistaxis, hematuria, upper / lower digestive hemorrhage, others)			
Bleeding start date (blank if not present)			
Bleeding end date (blank if still present)			
Fever	Yes ◯	No ◯	
Fever start date (blank if not present)			
Fever end date (blank if still present)			
Dry cough	Yes ◯	No ◯	
Dry cough start date (blank if not present)			
Dry cough end date (blank if still present)			
Tiredness	Yes ◯	No ◯	
Tiredness start date (blank if not present)			
Tiredness end date (blank if still present)			
Osteomuscular aching and pain	Yes ◯	No ◯	
Osteomuscular aching and pain start date (blank if not present)			
Osteomuscular aching and pain end date (blank if still present)			
Sore throat	Yes ◯	No ◯	
Sore throat start date (blank if not present)			
Sore throat end date (blank if still present)			
Diarrhea	Yes ◯	No ◯	
Diarrhea start date (blank if not present)			

Diarrhea end date (blank if still present)			
Conjunctivitis	Yes ◯	No ◯	
Conjunctivitis start date (blank if not present)			
Conjunctivitis end date (blank if still present)			
Headache	Yes ◯	No ◯	
Headache start date (blank if not present)			
Headache end date (blank if still present)			
Loss of smell	Yes ◯	No ◯	
Loss of smell start date (blank if not present)			
Loss of smell end date (blank if still present)			
Loss of taste	Yes ◯	No ◯	
Loss of taste start date (blank if not present)			
Loss of taste end date (blank if still present)			
Cutaneous rash	Yes ◯	No ◯	
Cutaneous rash start date (blank if not present)			
Cutaneous rash end date (blank if still present)			
Cutaneous pallor	Yes ◯	No ◯	
Cutaneous pallor start date (blank if not present)			
Cutaneous pallor end date (blank if still present)			
Cutaneous cyanosis	Yes ◯	No ◯	
Cutaneous cyanosis start date (blank if not present)			
Cutaneous cyanosis end date (blank if still present)			
Dyspnea	Yes ◯	No ◯	
Dyspnea start date (blank if not present)			
Dyspnea end date (blank if still present)			
Chest pain	Yes ◯	No ◯	
Chest pain start date (blank if not present)			
Chest pain end date (blank if still present)			
Aphasia	Yes ◯	No ◯	
Aphasia start date (blank if not present)			
Aphasia end date (blank if still present)			
Hyporexia	Yes ◯	No ◯	
Hyporexia start date (blank if not present)			
Hyporexia end date (blank if still present)			
Heart rate (BPM)			

Brachial systolic pressure (mmHg)					
Brachial diastolic pressure (mmHg)					
Peripheral oxygen saturation (%)					
Axillary temperature (Celsius)					
Paleness	Normal ○	1+/4+ ○	2+/4+ ○	3+/4+ ○	4+/4+ ○
Cyanosis	Normal ○	1+/4+ ○	2+/4+ ○	3+/4+ ○	4+/4+ ○
Oral cavity	Normal ○	Altered ○			
Description					
Pulmonary auscultation	Normal ○	Altered ○			
Description					
Cardiac auscultation	Normal ○	Altered ○			
Description					
Abdominal examination	Normal ○	Altered ○			
Description					
Limbs (pulses, edema, skin conditions)	Normal ○	Altered ○			
Description					

Figure 2. Clinical assessment and physical examination form based on SARS-CoV-2 most prevalent symptoms.

Laboratory results and tomographic findings form			
Form Number:	**Date:**		
RT-qPCR	Positive ○	Negative ○	
RT-qPCR quantitative viral load			
Blood count	Normal ○	Altered ○	
Hemoglobin (g / dL)			
Hematocrit (%)			
Mean corpuscular volume (fL)			
Red cell distribution width (%)			
Platelets count (10^3 / µL)			
White cell count (10^3 / µL)			
Neutrophils (%)			
Lymphocytes (%)			
Bands (%)			
Arterial gas	Normal ○	Altered ○	
pH			
pCO2 (mmHg)			
PaO2 / FiO2			
HCO3 (mEq / L)			
SO2 (%)			
Base Excess			
D-Dimer (µg / mL)			
aPTT	Normal ○	Altered ○	
Prothrombin time (PT)			
International normalized ratio (INR)			
Activated partial thromboplastin time (aPTT)			
Normalized aPTT Ratio			
Renal function	Normal ○	Altered ○	
Urea (mg / dL)			
Creatinine (mg / dL)			

Fasted glucose (mg / dL)	
C reactive protein (CRP) (mg / L)	
Amylase (U / L)	
Thoracic tomography description	

Figure 3. Laboratory and tomographic findings form based on SARS-CoV-2 inpatient laboratorial routine.

Adverse events form					
Form Number:	**Date:**				
Thrombotic thrombocytopenic purpura	Grade 1 ○	Grade 2 ○	Grade 3 ○	Grade 4 ○	Grade 5 ○
Time between event and last dose administered (hours)					
Disseminated intravascular coagulation	Grade 1 ○	Grade 2 ○	Grade 3 ○	Grade 4 ○	Grade 5 ○
Time between event and last dose administered (hours)					
Hemorrhage	Grade 1 ○	Grade 2 ○	Grade 3 ○	Grade 4 ○	Grade 5 ○
Site of hemorrhage					
Time between event and last dose administered (hours)					
Anaphylaxis	Grade 1 ○	Grade 2 ○	Grade 3 ○	Grade 4 ○	Grade 5 ○
Time between event and last dose administered (hours)					
Hypophysitis	Grade 1 ○	Grade 2 ○	Grade 3 ○	Grade 4 ○	Grade 5 ○
Time between event and last dose administered (hours)					
Ischemia	Grade 1 ○	Grade 2 ○	Grade 3 ○	Grade 4 ○	Grade 5 ○
Site of Ischemia					
Time between event and last dose administered (hours)					
Other adverse events (description)					
Grading	Grade 1 ○	Grade 2 ○	Grade 3 ○	Grade 4 ○	Grade 5 ○
Time between event and last dose administered (hours)					
Other adverse events (description)					
Grading	Grade 1 ○	Grade 2 ○	Grade 3 ○	Grade 4 ○	Grade 5 ○
Time between event and last dose administered (hours)					
Other adverse events (description)					
Grading	Grade 1 ○	Grade 2 ○	Grade 3 ○	Grade 4 ○	Grade 5 ○
Time between event and last dose administered (hours)					

Figure 4. Adverse events form based on frequency of observation of heparin induced adverse events and categorized according to the Common Terminology Criteria for Adverse Events (CTC-AE) 5.0.[25]

Ethics and dissemination

The EnHanCed clinical trial will be conducted in accordance with all applicable Brazilian national laws and international guidelines; in accordance with the ethical principles defined in the 18th World Medical Assembly, Helsinki, 1964, and all applicable amendments; following the International Council for Harmonization (ICH) guidelines for Good Clinical Practice (GCP).

EnHanCed is registered on several Brazilian and international platforms under the following registration IDs:

World Health Organization (WHO) International Clinical Trials Registry Platform (ICTRP) Universal Trial Number (UTN): U1111-1264-8253, under the Brazilian Clinical Trials Registry (ReBEC) approval.

National Institute of Health (NIH) United States National Library of Medicine ClinicalTrials.gov platform ID: NCT04743011.

Ethics approval

Brazilian National Health Council's "Plataforma Brasil": CAEE 39872920.0.0000.5411, under the São Paulo State University (UNESP) Medical School research ethics committee approval number 4.436.447.

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