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Intermediate dose enoxaparin in hospitalized patients with moderate-severe COVID19: a pilot phase II single-arm study, INHIXACOVID19

Protocol Number: 1

Investigational Compound: enoxaparin

Short Title: enoxaparin in COVID-19

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PROTOCOL AUTHORIZATION PAGE

I have read this study protocol and agree that it contains all the information required to conduct the study. I agree to conduct the study as set out in this protocol. In particular, I agree to adhere to the moral, ethical and scientific principles governing clinical research as set out in the Declaration of Helsinki, the guidelines on Good Clinical Practice and the appropriate national laws.

Local Investigator

_____ _____ **Date**

Trial Promoter Coordinating Centre

_____ _____ **Date**

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1. Background.

Governments and health official worldwide are currently facing the challenge of the severe respiratory syndrome epidemic due to the Corona Virus Disease19 (COVID19) (1). The full spectrum of disease includes four stages depending on clinical severity: mild, moderate, severe and critical (1). The largest epidemiological study done by China CDC showed that among 44672 confirmed cases, 80.9% were considered mild/common pneumonia, 13.8% were severe cases, 4.7% were critical cases. Case fatality rate for critical patients was 49%, with patients with comorbidities (cardiovascular disease, diabetes, chronic respiratory disease, hypertension, cancers) having higher case fatality rates (10.5%, 7.3%, 6.5%, 6.0%, 5.6% respectively) than those without comorbidities (0.9%). (1). The median time from first symptom to dyspnea was 5.0 days, to hospital admission was 7.0 days, and to ARDS was 8.0 days. As a result, the disease can rapidly progress from moderate to severe/critical. Laboratory features of COVID-19 included Lymphopenia with depletion of CD4 and CD8 lymphocytes, prolonged prothrombin time, elevated lactate dehydrogenase (3), elevated D-Dimer, elevated alanine transaminase, C-reactive protein, and creatinine kinase (4). ICU patients had higher plasma levels of IL2, IL7, IL10, GSCF, IP10, MCP1, MIP1A, and TNFα, compared with non-ICU patients. One of the most important mechanism underlying the deterioration of disease is cytokine storm (5).

1.1. Study Rationale

There are currently no treatments of proven efficacy to reduce the progression of the disease from mild/moderate to severe/critical, in particular counteracting the cytokine storm (6). In such patients WHO recommends thrombo-prophylaxis with either unfractionated or low molecular weight heparin (LMWH) as these patients are likely at increased risk of for prevention of venous thromboembolism (VTE) (7, 8) as acute infections are strong prothrombotic stimuli as vascular inflammation, endothelial dysfunction all contribute to an hypercoagulable state.

Although there are no published data, abnormal coagulation has been reported in a multicentre retrospective study in Chinese hospitalized patients with severe disease (9) in whom elevated D-dimer >1 gr/L was associated with in-hospital death, even after multivariable adjustment. In

another study, non survivors had a significant higher levels of D-dimer and 71% met clinical criteria for disseminated intravascular dissemination (DIC) (10). Severe and critically ill COVID-19 patients with prolonged immobilization are inherently at high risk of VTE and in those with clinical deterioration with hypoxia and hemodynamic instability pulmonary embolism (PE) should be also considered.

The optimal thrombo-prophylactic regimen in hospitalized patients with COVID-19 related illness is unknown (8). Given drug-drug interaction with direct oral anticoagulants and some anti-viral regimens, heparins either unfractionated or low molecular weight, with or without mechanical prophylaxis may be preferred in acutely ill patients. Standard LMWH prophylactic may be insufficient in these patients especially in the ICU patients characterized by a dynamic day-to-day variation both of thromboembolic that bleeding risk. Monitoring of anti-Xa activity may be considered when LMWH is used in these patients (11) and yet failure rates with standard pharmacological prophylaxis with LMWH or UFH may not be negligible (5-15%) (12).

In addition, LMWH have been shown to also possess anti-inflammatory properties. The non-anticoagulant fraction of enoxaparin have also been shown in-vitro suppression of IL-6 and IL-8 release from human pulmonary epithelial cells (8). Moreover in vitro and in vivo experimental studies have shown that human coronaviruses utilizes heparin sulfate proteoglycans for attachment to target cells (9). Indeed, interaction between the SARS-CoV-2 Spike S1 protein receptor binding domain (SARS-CoV-2 S1 RBD) and heparin has been recently showed suggesting a role for heparin in the therapeutic armamentarium against COVID-19 (10).

1.2. Benefit/Risk Assessment

Weight adjusted intermediate doses of LMWH enoxaparin could reduce thromboembolic complications with improved outcomes and potentially ameliorate the progression of the disease. Anti-factor-Xa activity determination with dose adjustiments would allow to monitor enoxaparin anticoagulant effects with the aim to minimize bleeding complications.

Detailed information about the known and expected benefits and risks and reasonably expected adverse events of enoxaparin may be found in the Summary of Product Characteristic (SPC) of the drug, even if referred to other usage.

2. Objectives and Endpoints

General objective of the study

To assess the efficacy and safety of enoxaparin in hospitalized patients with moderate-severe COVID-19 infection.

Specific objectives

Primary	**Endpoints**
To investigate the efficacy of enoxaparin in improving the clinical outcome of hospitalized patients with moderate-severe COVID-19.	• All-cause in-hospital, 30-day and 90-day mortality rates. • Evolution of the clinical severity during treatment. • ICU admission and length of ICU stay. • Length of hospital stay.
Secondary	**Endpoints**
To analyse the safety of enoxaparin in hospitalized patients with with moderate-severe COVID-19.	• Rate of adverse events (AEs) during treatment, at the end of treatment (EOT) and at 30 days after EOT. • Severity of AEs classified according to common terminology criteria for adverse events (CTCAE). The worst degree ever suffered will be

	considered.
To describe the rates and the types of thromboembolic events among hospitalized patients with confirmed diagnosis of COVID-19.	• Occurrence of thromboembolic event at 90 days after COVID-19 diagnosis. • Description of the type, distribution and severity of thromboembolic events.

3. Study Design

3.1. Overall Design

The study consists of two parts:

- a phase II single-arm interventional prospective study including all patients treated with the study drug;
- an observational prospective cohort study including all patients screened for receiving the study drug but not included in the phase II study.

Patients will be enrolled from “date of study approval” for 1 month. Each patient will be followed-up for a minimum of 90 days after COVID19 diagnosis.

Setting

The promoting center, Policlinico S. Orsola, is a 1,420-bed tertiary care University Hospital in Bologna with an average of 72,000 admissions per year. Since the beginning of SARS-CoV-2 epidemic in Italy, the Infectious Diseases Unit of the promoting center is dealing with COVID-19 patients coming from all the metropolitan area of Bologna and from other cities of the Region Emilia Romagna.

The promoting centre hosts the regional referral laboratory for emerging infectious diseases (CRREM). To March 18, 2020, 8,176 tests for ruling out SARS-CoV-2 infection have been performed yielding a diagnosis of COVID-19 in 2,261 patients, of which 233 have been hospitalized at the Policlinico Sant’Orsola and managed by the Infectious Diseases Unit.

3.2. End of Study Definition

A participant is considered to have completed the study if he/she has completed the last scheduled procedure shown in the Schedule of assessments. The end of the study is defined as the date of the last scheduled procedure shown in the Schedule of assessments for the last participant in the trial.

4. Study Population

4.1. Inclusion Criteria

For both interventional study and observational cohort, hospitalized patients are eligible to be included if the following criteria apply:

Inclusion criteria:

- Age >=18 y
- Microbiologically confirmed COVID-19 infection
- Patients with moderate-severe disease according to study definitions (see below)
- Informed consent to participate and to use data for interventional study, only to use data for observational cohort

4.2. Exclusion Criteria

Participants are excluded from the interventional study if any of the following criteria apply:

- Thrombocytopenia (platelet count < 50.000 mm3)
- Coagulopathy: INR >1.5, aPTT ratio >1.4
- Impaired renal function (clearance to creatinine less than 15 ml/min)
- Known hypersensitivity to heparin
- History of heparin induced thrombocytopenia
- Presence of an active bleeding or a pathology susceptible of bleeding in presence of anticoagulation (e.g. recent haemorrhagic stroke, peptic ulcer, malignat tumors at hig risk of haemorrahges, recent neurosurgery or ophthalmic surgery, vascular aneurysms, arteriovenous malformations)

- Body weight <45 or > 150 kg
- Concomitant anticoagulant treatment for other indications (eg atrial fibrillation, venous thromboembolism , prosthetic heart valves).
- Dual antiplatelet therapy
- Pregnant or breast-feeding women

Definitions

Clinical severity of COVID-19 will be assessed at the diagnosis of COVID19, during the treatment with the study drug, and at the end of treatment according to the following criteria (1):

- Mild patients: only show mild symptoms without radiographic features
- Moderate patients: have fever, respiratory symptoms, and radiographic signs of pneumonia
- Severe patients: have fever, respiratory symptoms, and radiographic signs of pneumonia *plus* at least one of three criteria: (1) RR>30 times/min, (2) oxygen saturation <93% on ambient air, (3) PaO2/FiO2<300 mmHg.
- Critical patients: meet one of three criteria: (1) respiratory failure needing invasive ventilation, (2) septic shock, (3) multiple organ failure.

Major bleeding will be defined according to the ISTH criteria as one of the following:

- Fatal bleeding
- Symptomatic bleeding in a critical area or organ, such as intracranial, intraspinal, intraocular, retroperitoneal, intra-articular or pericardial, or intramuscular with compartment syndrome
- Bleeding causing a fall in haemoglobin level of 2 g/dL or more, or leading to transfusion of two or more units of whole blood or red cells (11).

5. Treatments

5.1. Administration and monitoring of study drug

All patients screened for being included in the study will receive standard thrombo-prophylaxis with LMWH (e.g. enoxaparin 40 mg/die). Patients included in the observational cohort will continue on standard thrombo-prophylaxis, while patients included in the interventional study will receive subcutaneous enoxaparin in a single daily dose of:

- 60 mg once daily in case of body weight of 45 to 60 kg
- 80 mg per day in case of weight from 61 to 100 kg or
- 100 mg once daily in case of bodyweight >100 kg

Enoxaparin will be started on the first day of COVID19 diagnosis and continued for 14 days, after determination of baseline PT, aPTT, complete blood cell count and creatinine levels.

After reaching the steady state (usually after the third dose), heparin levels will be measured with the determination of anti-Xa activity on a blood sample obtained at 4 hours after the morning injection. LMWH dose may be then increased or reduced on the basis of target anti-Xa activity (0.4 -0.6 antiFXaUI/ml for intermediate doses). The determination of anti-Xa activity will be repeated on the fifth or sixth day to monitor any drug accumulation.

Complete blood cell count will be obtained every second day to monitor for heparin induced thrombocytopenia.

Single low dose antiplatelet agents will be allowed.

In all patients, RT-PCR nasopharyngeal swabs will be performed every 7 days to assess virus clearance and blood samples will be collected at baseline and on day 7 and will be retrospectively analysed to measure viral load.

5.2. Preparation/Handling/Storage/Accountability

1. The investigator, institution, or the head of the medical institution (where applicable) is responsible for study treatment accountability, reconciliation, and record maintenance (ie, receipt, reconciliation, and final disposition records). Specific forms for drug accountability will be provided by the promoter.

2. The vial must be stored below 25 °C. Do not freeze.

5.3. Treatment Compliance

The effective doses of study drugs received by each participant during the study will be recorded.

5.4. Concomitant Therapy

There is no contraindication to concomitant treatment (including antiviral drugs) that can be defined in advance given the severity of the disease and the availability of very few data on pharmacological interactions of the enoxaparin schedule planned in this study.

In case of suspected or demonstrated concomitant infections that can be successfully treated with antimicrobials in order to make the patient eligible, such treatments are allowed.

However, any medication that the participant is receiving at the time of enrolment or receives during the study will be recorded along with:

- Reason for use
- Dates of administration including start and end dates
- Dosage information including dose and frequency

6. Study Assessments and Procedures

Screening evaluations must be completed and reviewed to confirm that potential participants meet eligibility criteria. The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.

Procedures conducted as part of the participant's routine clinical management (eg, blood count) and obtained before informed consent may be used for screening or baseline purposes provided the procedures met the protocol-specified criteria.

6.1 Screening and procedures during hospitalization period

Informed Consent Form

Demography (age, gender, ethnicity)

Medical history (previous and current diseases, all medications started within 14 days prior to screening visit)

Full physical examination including height and weight.

Vital signs (respiratory rate, pulse, blood pressure and temperature)

Laboratory assessments: blood count and PLT, bilirubin, AST, ALT, creatinine, PT, PTT

PaO2/FiO2

Chest XR or Thoracic CT scan (if clinically indicated)

AE review (including SAEs)

Concomitant medication review

All data will be recorded anonymously by an electronic case report form using RedCap platform.

6.2. Follow up procedures

Patients will be followed-up to 90 days after study drug initiation. Follow-up information will be collected via telephone calls, patient medical records and/or clinical visits according to clinical evolution.

6.3. Efficacy Assessments

Endpoints

Primary

- All cause 30-day mortality

Secondary

- Proportion of patients in the severe or critical stage of disease at the end of treatment
- Proportion of patients who develop major and non-major bleeding events
- Time to first negative RT-PCR on nasofaringeal swab
- Reduction of viral load in blood

6.4. Adverse Events

6.4.1 Definitions

An adverse event (AE) is any untoward medical occurrence in a study participant administered the medicinal products and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

An adverse reaction (AR) is an untoward and unintended response to the investigational medicinal products related to any dose administered, judged by either the investigator or the promoter.

An unexpected adverse reaction (UAR) is an adverse reaction, the nature or severity of which is not consistent with the applicable products information (investigator's brochure).

A Serious Adverse Event (SAE) is untoward medical occurrence or effect that at any dose results in death, risk of death, permanent disability/incapacity, hospitalisation or prolongation of existing hospitalization or need for urgent medical treatment, or another medically important serious event as judged by the investigator. Further, any unexpected changes in relation to the toxicity profile of the drugs used of grade 3, as well as adverse event(s) which, although not falling within this definition, are considered unexpected and serious by the Investigator should be reported.

The causality of SAEs (their relationship to all study treatment/procedures) will be assessed by the investigator(s) and communicated to the coordinating centre.

The SAE of special interest for the study drug if major bleeding. This will be defined, according to the ISTH criteria, as one of the following:

- Fatal bleeding
- Symptomatic bleeding in a critical area or organ, such as intracranial, intraspinal, intraocular, retroperitoneal, intra-articular or pericardial, or intramuscular with compartment syndrome
- Bleeding causing a fall in haemoglobin level of 2 g/dL or more, or leading to transfusion of two or more units of whole blood or red cells (11).

A Suspected Unexpected Serious Adverse Reaction (SUSAR) is an unexpected adverse reaction judged serious by the Investigator and/or Promoter, that is not consistent, either in nature or in severity, with the applicable product information.

6.4.2. Collection and reporting of adverse events

All adverse events recorded from time of signature of informed consent, throughout the treatment and observation period up to 30 days following registration, have to be reported in the toxicity case report form, graded according to the corresponding CTCAE term (Version 5.0).

The Investigator must immediately report to the promoter all serious adverse events. The report should be made using the SAE report form online or by sending the paper copy by fax +39 051 343500) to the coordinating office immediately and not exceeding 24 hours following knowledge of the event.

The investigator is responsible for following all SAEs until resolution, until the subject returns to baseline status, or until the condition has stabilized with the expectation that it will remain chronic, even if this extends beyond study participation.

All SAEs related to bleeding will be assessed by an independent adjudication safety committee with and interim safety analysis after enrolling the first 50 patients.

The cause of death will also be evaluated by an independent adjudication safety committee.

7. Statistical Considerations

7.1. Sample Size Determination

This is a pilot study and an initial sample of 100 patients for the phase II single-arm interventional trial is established. As stated above a first safety analysis after enrolling the first 50 patients in the interventional study arm is planned and it will be done by an independent committee. According to safety and efficacy data obtained, using as control the observational cohort, a large study with a more robust design is planned.

7.2. Statistical Analyses

The statistical analysis plan will be developed and finalized before database lock and will describe the participant populations to be included in the analyses, and procedures for accounting for missing, unused, and spurious data.

For descriptive analysis, categorical variables will be presented as absolute number and their relative frequencies, continuous variables will be presented as mean ± standard deviation (SD) or median and interquartile range (IQR) according to their distribution.

For efficacy analysis, the patients treated with the study drug will be compared with the observational cohort. A propensity score to receive enoxaparin will be calculated and two propensity matched groups of patients will be obtained. Differences in 30-day mortality in these two groups will be analysed. The evolution of the clinical severity (progression from moderate to severe, of from severe to critical disease) of COVID19 between the two groups will be further assessed by a competing risk analysis, considering death as competing event.

8. Ethics, Quality Assurance and Monitoring

The procedures set out in this study protocol are designed to ensure that the promoter and the Investigators abide by the principles of the Good Clinical Practice guidelines of the International Conference on Harmonization (ICH) and the Declaration of Helsinki in the conduct, evaluation and documentation of this study. The study will be carried out adhering to local legal requirements and the applicable national law, whichever represents the greater protection for the individual.

Study protocol, patient information and informed consent will be submitted to the appropriate Ethical Committee for approval. The promoter will inform the appropriate Ethical Committee about any changes in the study protocol which could interfere with the patient's safety.

The monitoring activities during pandemia will be primarily or exclusively performed without peripheral visits. Remote monitoring will be performed through periodic, comprehensive connections through the web or the telephone with all participating centres by promoter personnel or representatives.

8.1. Informed Consent Process

The physicians treating the hospitalized patient are responsible for information of the patient and obtaining of the Informed Consent.

The consent can be oral if a written consent cannot be expressed. If the subject is incapable of giving an informed consent and an authorized representative is not available without a delay that would, in the opinion of the Investigator, compromise the potential life-saving effect of the treatment this can be administered without consent. Consent to remain in the research should be sought as soon the conditions of the patient will allow it.

The same procedure apply to the information of the patient and providing of consent to the processing of personal data according to the European Regulation n. 679/2016 on the Protection of Personal Data, the Personal Data Protection Code (Legislative Decree 196/03) and subsequent amendments and additions, and to the provisions, guidelines and general authorizations of the National Guarantor for Personal Data Protection.

9. Administrative aspects

This is a non-profit investigator-initiated trial. In this trial, the experimental drug enoxaparin will be provided at no cost by the manufacturer.

Study protocol, patient information, and informed consent at beginning and at each required amendment will be submitted to the appropriate Ethical Committee for approval. After the first approval the study will be started at each Italian centre requiring to participate and such participation will be notified together with the approved protocol to the local Institutional Ethical Committee.

Coverage for any damage resulting from the participation of the subjects in the clinical trial is included in the general insurance of the individual participating clinical centres.

10. Coordinating centre contacts

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Appendix 1. Laboratory tests

Baseline	PT, aPTT, complete blood cell count, creatinine levels, ALT, AST, triglycerides, ferritine, fibrinogen Blood for determination of viral load
Days 2, 4, 6, 8, 10, 12, 14	Complete blood cell count
Day 3 (after 4 hour from third enoxaparin administration)	Anti-Xa activity
Day 5 (after 4 hour from fifth enoxaparin administration)	Anti-Xa activity
Day 7	PT, aPTT, complete blood cell count, creatinine levels, ALT, AST, triglycerides, ferritine, fibrinogen Blood for determination of viral load Nasofaringeal swab for RT-PCR for SARS-2-CoV
Day 14	PT, aPTT, complete blood cell count, creatinine levels, ALT, AST, triglycerides, ferritine, fibrinogen Nasofaringeal swab for RT-PCR for SARS-2-CoV