1) Protocol Title

Antithrombin III (AT3) in Infectious Disease Caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)

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ClinicalTrials.gov Identifier
NCT04899232

1) Objectives*

This will be a prospective, randomized, open label, interventional pilot trial to test the hypothesis that deficient endogenous AT3 is associated with a prothrombotic hyperinflammatory state associated with COVID-19.

2) Background*

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). As of 6/24/20, as social distancing was easing, the U.S. recorded >2.3M COVID-19 infections and >121K deaths, with new cases rising in at least 27 states [https://coronavirus.jhu.edu/map.html]. Worldwide, cases are rising at an even more alarming pace; there were 1M on 4/1/20, and >9.2 M cases with 478K deaths by 6/24/20. [https://coronavirus.jhu.edu/map.html].

A unique feature of the acute respiratory distress syndrome (ARDS) associated with COVID is a relatively preserved lung compliance and acute inflammation associated with a high alveolar-arterial oxygen gradient [PMID: 32267998]. The pathogenesis involves a rapid increase in airway cytokines which promotes pulmonary leukocyte sequestration, further propagating the local inflammatory response. Fibrin is deposited in the air spaces and lung parenchyma with elevated D-dimers and fibrinogen. Tissue factor (TF) is exposed on damaged alveolar endothelial cells and on the surface of leukocytes promoting more fibrin deposition, while increasing plasminogen activator inhibitor 1 (PAI-1) from lung epithelium and endothelial cells which creates a hypofibrinolytic state [PMID: 32329246]. The signature finding on autopsy is diffuse pulmonary microthrombi associated with an ARDS caused by vascular occlusion rather than the more classic findings of low-compliance [PMID: 32267998].

The most severely ill patients often present with mild symptoms that rapidly progress within 12-24h to multi-system involvement including ARDS, myocarditis, arrhythmias, cardiac arrest, gastrointestinal symptoms, hypoxemic brain injury, acute liver, renal function impairment, and cutaneous lesions. The common link seems to be microvascular occlusion with thrombi or viral laden antigen-antibody immune complexes [PMID: 32559324].

Thus, COVID might be a dysregulated inflammatory state superimposed on disseminated intravascular coagulation (DIC) with consumptive coagulopathy [PMID: 32302438]. These patients are at high risk for both thrombosis and bleeding, as well as an overwhelming cytokine storm. The pathogenic mechanism is not fully elucidated, but the dysregulated coagulation is probably orchestrated by inflammatory cytokines, lymphocyte cell-death, hypoxia, and endothelial damage [PMID: 32558075]. This is a project to develop an entirely novel evidence-based treatment that targets both the coagulopathy and the inflammation.

In 183 consecutive patients with COVID, the overall mortality was 11.5%. On admission, the non-survivors had higher D-dimer and fibrin degradation product (FDP) levels, longer prothrombin time and activated partial thromboplastin time compared to survivors; 71.4% of the deaths and only 0.6% survivors met the criteria of DIC [PMID: 32073213]

In 40 patients with COVID, whole blood thromboelastometry (TEM) was consistent with hypercoagulability characterized by an acceleration of blood clot formation and higher clot strength. This hypercoagulable state persists for at least 5-10 days and was associated with deep vein thrombosis in six patients (15%), thromboembolism in 2 patients (5%), and catheter-related thrombosis in 12 patients (30%). The ICU and the hospital mortality were 10% and 12.5%, respectively. [PMID: 32394236]

In 16 COVID patients, the pro-coagulant TEM profile was characterized by an increased clot strength (CS), platelet contribution to CS, fibrinogen contribution to CS elevated D-dimer levels, and hyperfibrinogenemia. Fibrinogen levels were associated with interleukin-6 values. [PMID: 32302448].

In 24 COVID patients, the R, K, Alpha, and MA TEM parameters were all consistent with hypercoagulability. Platelet count was normal or increased, prothrombin time and activated partial thromboplastin time were near normal. Fibrinogen, D-dimer, C-reactive protein, Factor VIII and von Willebrand factor, protein C were all increased whereas AT3 was decreased [PMID: 32302438].
In 94 patients with COVID vs 40 healthy controls, whereas D-dimer, fibrin/fibrinogen degradation products (FDP), and fibrinogen (FIB) in all SARS-CoV-2 cases were all higher, but AT3 was lower [PMID: 32172226].

The most logical prophylaxis or treatment for this hypercoagulable state is either unfractionated heparin (UFH) or low molecular weight heparin (LMWH), but this often fails. One possible explanation is that both UFH and LMWH require AT3 to exert their anticoagulant effect. [PMID: 32516429]. Therefore, endogenous AT3 deficiency can result in failure to achieve adequate anticoagulation with standard chemoprophylaxis with either UFH or LMWH.

AT3 is currently FDA approved for prophylaxis and treatment of thrombotic and thromboembolic disorders in patients with hereditary AT3 deficiency (i.e. AT3 below 70% of normal). In this project, we test the intriguing idea that AT3 can be repurposed, to treat the procoagulant state and resulting cytokine storm associated with COVID. There are virtually no studies on whether low endogenous AT3 explain the COVID-associated coagulopathy and/or cytokine storm.

AT3 is a nonvitamin K-dependent serine protease inhibitor that anticoagulates by neutralizing the enzymatic activity of thrombin. It is a glycoprotein produced by the liver and consists of 432 amino acids. Its activity is potentiated by UFH or by LMWH such as dalteparin or enoxaparin. TEG-guided adjusted enoxaparin dosing increased anti-Xa activity, but this did not decrease the rate of venous thromboembolism (VTE) in trauma patients. Failure to reduce the VTE rate despite high supplemental LMWH and increased anti-Xa activity was consistent with AT3 deficiency (PMID 24662855). Thus, the effectiveness of thromboprophylaxis may depend in part endogenous AT3. Furthermore, some evidence suggests that AT3 has anti-inflammatory properties.

AT3 is protective against renal, myocardial and cerebral ischemic injury by suppressing macrophage activity and reducing production of pro-inflammatory cytokines In contrast, AT3 deficiency augments coagulation and inflammation in the brain in a mouse model of acute cerebral ischemia-reperfusion [PMID: 32305521]..

The fact is that several factors have major modulatory role in both coagulation and inflammation [PMID: 26676884]. AT3, tissue factor pathway inhibitor, and protein C, all suppress proinflammatory mediators. Conversely, inflammation blunts anticoagulant activity and, when uncontrolled, promotes DIC. AT3 has broad anticoagulant activity and potent anti-inflammatory properties in hereditary or acquired AT deficiency [PMID: 26676884]. AT3 modulates inflammation by inhibiting thrombin and other clotting factors that induce cytokine activity and leukocyte-endothelial cell interaction, and also by coagulation-independent effects, including direct interaction with cellular mediators of inflammation [PMID: 26676884]. An increasing body of evidence suggests that AT3 may be a potential therapeutic agent in certain clinical settings associated with inflammation [PMID: 26676884], but there is no data on whether AT3 modulates the hypercoagulation/ inflammation associated with COVID.

3) Inclusion and Exclusion Criteria*

INCLUSION CRITERIA:

a. >18y of age,
b. Subject or proxy who can provide informed consent
c. Positive SARS COV2 by PCR or as determined by clinical team

EXCLUSION CRITERIA:

a. Adults or Proxy unable to consent
b. Individuals who are not yet adults (infants, children, teenagers)
c. Pregnant women
d. Prisoners
e. Patients expected to die within 24 hours or with a "do not resuscitate" order,
f. Multi-organ failure,
g. History of hypersensitivity or allergy to any component of the study drug,
h. Ongoing massive surgical or unexplained bleeding,
i. History of bleeding or clotting disorder,
j. Severe traumatic brain injury (Glasgow Coma Scale <6),
k. Spinal or multiple-trauma,
l. Cancer (incurable/terminal phase) and/or patients receiving palliative care,
m. Enrollment in another concurrent clinical interventional study
   - if considered interfering with this study objectives
n. Per study team discretion, any condition(s) that may prevent safe treatment, preclude adequate evaluation or adding further risk to their underlying illness.

4) Number of Subjects*

The study population will be comprised of 75 patients assigned to one of three groups of 25 COVID (+) patients each.

The proportion of subjects with AT3 levels < 100% will be 50/50 randomization of which 25 subjects with AT3 <100% will be standard of care and 25 subjects with AT3<100% will be standard of care with exogenous AT3 supplementation. The third group will be subjects with AT3 levels ≥100%.

a. 25 patients with endogenous AT3 >100% who receive standard of care only or
b. 25 patients with endogenous AT3 <100% who receive standard of care only or
c. 25 patients with endogenous AT3 <100% who receive standard of care plus supplemental AT3.

5) Study-Wide Recruitment Methods*

Potential subjects will be recruited as inpatients under the care of an attending which may include the PI or Sub-Is on this study. Eligibility will be determined by any member of the study team under direction of the PI and in collaboration with the attending physician through review of inclusion and exclusion criteria as compared to the patients' presentation, diagnoses and medical history. Jackson Health System's medical records within Cerner will be accessed under a waiver of HIPAA authorization (see section 21). After collaboration with the clinical team and initial screening for entry criteria, a member of the study team will approach potential subjects for consent and enrollment.

6) Study Timelines*

a. Subject participation will be approximately 11 days from the time of consent to the last blood draw. After the last blood draw, only routine clinical data will be followed and captured from the medical record to outcomes.
b. Enrollment of 75 subject will be over the course of 12 to 18 months.
c. The primary analyses should be completed within 24 months from the time of study initiation.

7) Study Endpoints*

The Primary endpoint is change from baseline in the ISTH DIC Score to Day 9.

The Secondary endpoints include change from baseline D-dimers, Fibrinogen & Prothrombin time, length of hospital stay (days), mortality (%), and Pulmonary function (including Time (days) to mechanical ventilation and/or ECMO Duration (days) of new ventilator or extracorporeal membrane oxygenation (ECMO) use, Change from baseline SOFA and SOFA Respiratory sub-score, Number of venous thromboembolisms, Number of major bleeding events

Subjects discharged prior to completion of intervention would be classified as having a successful outcome. We anticipate that all cause morbidity will occur in 10-20% of the COVID study population, despite standard of care. We anticipate that these patients will be deficient in endogenous AT3.

8) Procedures Involved*

Any member of the study team will discuss potential subjects with the clinical team for appropriate pursuit of informed consent and screening for eligibility.

75 eligible subjects, after consent, will have laboratory blood tests for further screening.

25 of those subjects with endogenous **AT3 ≥ 100% will continue with SOC**.

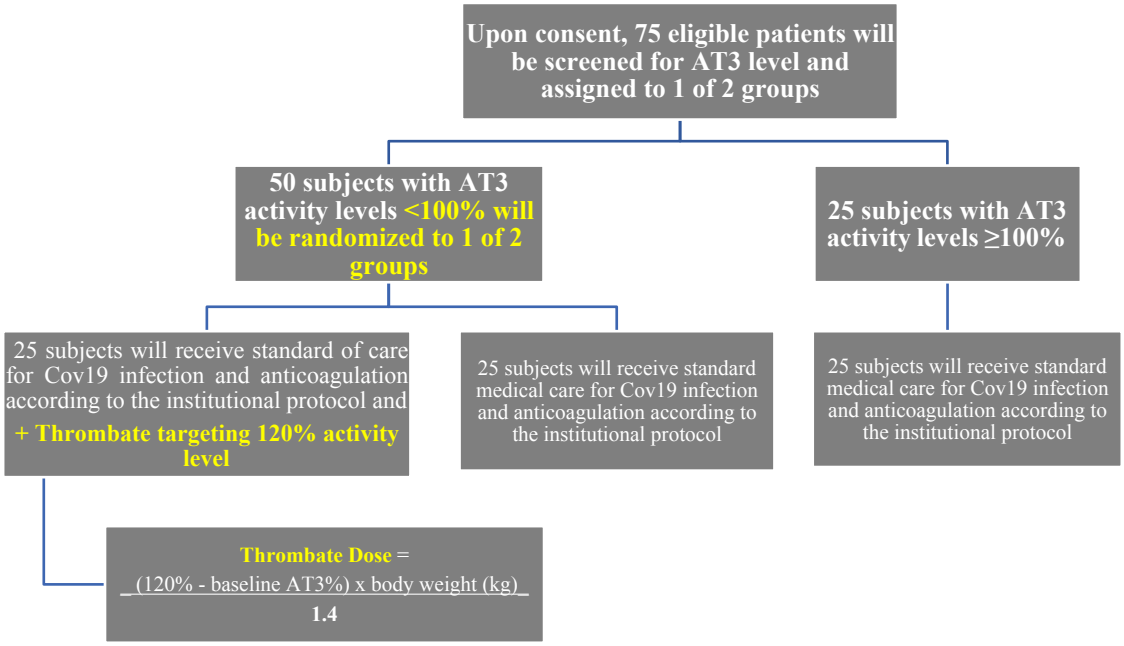
50 of those subjects with endogenous **AT3 < 100%** will be will be randomly assigned to begin **SOC plus treatment with exogenous AT3 supplementation or only SOC** treatment. The randomization schedule will be established by a member of the study team that is not involved in the informed consent process.

They will all be followed up for laboratory blood tests, study endpoints and adverse events.
If they do not meet entry criteria they will be excluded from the study.

Consented subjects will be scored according to The International Society of Thrombosis and Haemostasis Disseminated Intravascular Coagulation scale (ISTH DIC) on study visit days 1, 3, 5, 7, 9 and 11.

The SOFA score will be performed only when the parameter is obtained clinically and monitored for changes in patient status on study visit days 1, 3, 5, 7, 9 and 11. Each organ system is assigned a point value from 0 (normal) to 4 (high degree of dysfunction/failure)). It will be calculated on admission and Day 11 using the worst parameters measured during the study visit day. If a parameter is not obtained clinically it will be voided for research purposes.

After enrollment and each 48h period (+/- 12 hours) thereafter, blood samples are collected and assayed for routine clinical coagulation and inflammation markers, including AT3 activity, D-Dimer, anti-Xa, fibrinogen, and prothrombin time until Day 11.



Dosing Rationale-

In those COVID(+) patients with an endogenous AT3 activity level <100% and at the discretion of the attending physician, Day 1 infusion of AT3 will be at a dose to be adjusted based on initial body weight up to 100 kg maximum and to a set target plasma activity level of 120% according to the following formula:

$$= \frac{(120\% - \text{baseline AT3\%}) \times \text{body weight (kg)}}{1.4}$$

Based on an example of an 85kg patient with a baseline AT activity level of 90%,

- The dose would be 1,821 units on a dosing schedule of Day 1, 3, 5, 7 & 9
- For a total of approximately 9100 units
- Each Thrombate III vial contains 500 units each reconstituted with 10 mL of Sterile Water for Injection, USP, the final concentration is approximately 50 units per mL.
- Requiring 4 vials per dose or 20 vials per patient

Each 85kg patient will receive ~9100 IUs over 5 doses requiring 20 vials

- (10% overage to account for any dose adjustments needed)
- therefore each patient would require 10,000 IUs
- for a total of 250,000 IUs or 500 vials for the study.

AT3 will be drawn every other day, pre-dose, for monitoring, and verification of 120% target plasma activity level. Dosing schedule will be as follows: Day 1, 3, 5, 7 & 9 a dose will be given if AT3 activity level remains less than 100% on those days, otherwise no dose will be given. Days refer to a 24 hour period.

Data to be collected from the medical record according to the Study Flowsheet (Appendix B) will include:

a. Demographics: age, comorbidities, medical history, concomitant meds, vital signs, height and weight,
b. ISTH DIC score and a Sofa Score,
c. Laboratory values:
    a. Platelets, PT, INR, PTT, D-Dimer, Fibrinogen, AT3 level, Anti-Xa,
d. Outcomes:
    a. Length of Stay, Mortality, change in PF ratio, Vent/ECMO days, # and severity VTE's, # and severity of bleeding events

9) Statistics*

Statistical analyses will be performed using SPSS version 21 (IBM Corporation, Armonk, NY). Parametric data are reported as M±SD and nonparametric data are reported as median (interquartile range). Missing values for continuous variables are handled by applying imputation methods before calculating their statistics. The study population will be stratified into two halves based on the primary outcome of change from baseline SOFA and/or ICTH DC. Those with the fastest rate of recovery are compared to those with the slowest rate of recovery. We expect that AT3 will correlate with rate of recovery and/or with one or more of the secondary outcomes. The demographics and physiologic variables in the fastest and slowest groups are compared using a t test or Mann-Whitney U-test, as appropriate. Categorical variables are compared using a $\chi 2$ or Fisher's exact test, as appropriate. Statistical significance is determined at $p<0.05$. Then multivariate models will be evaluated for predicting the primary or secondary outcomes, using various performance metrics, such as area under the receiver operating characteristic curve, sensitivity and specificity, positive predictive value, and negative predictive value, as we have described in multiple previous studies. In addition, propensity scores will be assigned for each patient based on a 1:1 fixed ratio nearest neighbor matching was performed to compare those with fastest or slowest recovery. A 1:1 fixed ratio nearest neighbor matching is performed to minimize bias without sacrificing power, as we have described in multiple previous studies.

10) Provisions to Monitor the Data to Ensure the Safety of Subjects*

Study Staff will maintain the data on paper Case Report Forms and in an electronic database stored in a secure location and on a password- protected computer system. The consent forms will be kept in files which will be stored in a secure location within Jackson Health System. De-identified data will be uploaded to the electronic database. The PI and/or a member of the study team will confirm the completeness of data being entered in the electronic database

In order to protect all subjects' confidentiality, we will store data on a password-protected network. Subjects will receive a study-specific unique identifier and the medical record number (MRN) will be uncoupled from the data. The decoder key for study identifier and MRN will be stored in a separate, password-protected file.

Trial Monitoring, Auditing, and Inspecting

The investigator will permit trial-related monitoring, quality audits, and inspections by, government regulatory authorities, of all trial-related documents (e.g., source documents, regulatory documents, data collection instruments, case report forms). The investigator will ensure the capability for inspections of applicable trial-related facilities. The investigator will ensure that the trial monitor or any other compliance or QA reviewer is given access to all trial-related documents and trial-related facilities.

Participation as an investigator in this trial implies the acceptance of potential inspection by government regulatory authorities.

In addition to the Clinical Monitoring component of this protocol, Quality Assurance (QA) to assess compliance with GCP and applicable regulatory requirements. Data or documentation audited shall be assessed for compliance to the protocol, accuracy in relation to source documents and compliance to applicable regulations.

Adverse Event (AE) Definition

An AE is defined as any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical/medicinal product. An AE does not necessarily have to have a causal relationship with this treatment.
An AE can therefore be any unfavorable and unintended sign (including a clinically significant abnormal laboratory finding, for example), symptom, or disease temporarily associated with the use of a medicinal product (investigational or marketed), whether or not considered related to treatment with the medicinal product.

An AE includes:

- An exacerbation of a pre-existing illness, sign, symptom, or clinically significant (as determined by the investigator) laboratory test abnormality
- An illness, sign, symptom, or clinically significant laboratory abnormality that is detected or diagnosed after study drug administration
- Pretreatment or post-treatment events that occur as a result of protocol-mandated procedures

An AE does not include:

- The disease or disorder being studied or signs and symptoms associated with the disease or disorder, unless there is worsening of the condition of the disease or disorder
- A pre-existing disease or condition, present at the start of the study, that does not worsen.

Procedures for Reporting Adverse Events

Adverse events may be spontaneously reported, obtained through non-leading questioning, or noted during examination of a subject. Adverse events will be recorded from the signing of informed consent through the last dose of study drug. Serious adverse events will be recorded from the signing of informed consent through hospital discharge.

At each visit, new AEs are recorded sequentially on the **Adverse Event log**. The AE term will note the diagnosis whenever possible, not the individual signs or symptoms (e.g., myocardial infarction will be recorded rather than chest pain, elevated cardiac enzymes, and abnormal ECT). Also recorded are:

- Start and stop date and time
- Whether or not the event is continuing
- Severity (mild, moderate, severe)
  - Mild: usually transient, requiring no special treatment and generally not interfering with usual daily activities
  - Moderate: usually ameliorated by simple therapeutic maneuvers and impairs usual activities
  - Severe: requires vigorous therapeutic intervention and interrupts usual activities. Hospitalization may or may not be required.

Relationship to Study Medication
The relationship of an AE to the administration of study medication (unrelated, probable or definite) is a clinical decision based on all available information at the time of review. The relationship will be evaluated according to the following definitions:

Unrelated: includes the existence of a clear alternative explanation (e.g., mechanical bleeding at surgical site) or non-plausibility (e.g., the patient is struck by an automobile, at least where there is no indication that the study medication caused disorientation that may have led to the event; cancer developing a few days after study medication administration).

Probable: The AE, including clinical laboratory abnormality, follows a reasonable sequence from the time of study medication administration, follows a known response pattern of the study medication class, is unlikely to be attributed to the patient's clinical state, and is confirmed by improvement on stopping the study medication (de-challenge).

Definite: The AE, including clinical laboratory abnormality, follows a plausible sequence from the time of study medication administration; follows a known or expected response pattern to the study medication class; cannot be explained by disease or it disappears or decreases on cessation or reduction in study medication dose; and/or it reappears or worsens when the study medication is re-administered.

Actions taken (none, study drug dose interrupted, study drug discontinued, change in treatment).

Outcome (resolved, severity or frequency increased, ongoing, fatal). An individual AE receives only one outcome.

Adverse events not resolved at the end of treatment will be followed until discharge or until the AE is judged by the investigator to have stabilized.

Serious Adverse Events
An event that is serious will be recorded on the Serious Adverse Event log. SAEs will be recorded from the signing of informed consent hospital discharge.

The most common adverse events are mild and related to underlying disease such as respiratory distress. If these occur, they will be noted in the adverse event tracking log and reported to the IRB during Continuing Review.

More serious adverse events and any unexpected adverse events will be recorded as per UM HSRO policies and procedures.

Disease Related Adverse Events
It is recognized that this patient population who require critical care support will experience a number of common aberrations in laboratory values, signs, and symptoms due to the severity of the underlying disease and the impact of standard therapies. These will not necessarily constitute an AE unless they require significant intervention, lead to discontinuation of study medication, felt to be related to study medication or are considered to be of concern, in the Investigator's clinical judgment. Examples of the type of AEs that may be associated with the patient population are listed below.

List of Expected Adverse Events
Acute respiratory failure; respiratory distress; acute lung injury; acute respiratory distress syndrome (ARDS); acute renal failure; coagulation dysfunction; decreased platelets count; neutropenia; hypothermia; fever, bacteremia; sepsis, septic shock; hypotension; metabolic acidosis, pleural effusion; abdominal compartment syndrome; repeated surgeries/debridement; amputation.

For purposes of this study, deaths will be captured as clinical outcomes and do not need to be reported as SAEs unless felt related to study drug administration.

11) Withdrawal of Subjects*
The occurrence of any of the following events may necessitate premature withdrawal of a subject from the study:

- Development of any of the following Inclusion/Exclusion criteria:
  - Subjects with a "do not resuscitate" order,
  - Multi-organ failure,
  - History of hypersensitivity or allergy to any component of the study drug,
  - Ongoing massive surgical or unexplained bleeding,
  - History of bleeding or clotting disorder,
- Occurrence of a serious adverse event (SAE) thought to be related to study drug and not alleviated by symptomatic treatment
- Unwillingness to continue in the clinical study
- Death of the subject
- Principal Investigator decision (i.e. subject non-compliance with study procedures)
- Significant AE or medical decision that precludes the subject from adhering to study requirements

12) Stopping rules.
The following events will require the study to pause and be evaluated prior to resuming the study. These events will be reported to the FDA in a timely manner as required per 21 CFR 312.64(b)

a. Any major bleeding event
b. Any anaphylaxis event
c. 2 infusion reactions

13) Risks to Subjects*

Hypersensitivity reactions, including anaphylaxis, are possible. Should symptoms occur, THROMBATE III infusion will be discontinued and appropriate treatment begun as per clinical judgement.

• Because THROMBATE III is made from human blood, it may carry a risk of transmitting infectious agents, e.g., viruses, the variant Creutzfeldt-Jakob disease (vCJD) agent and, theoretically, the Creutzfeldt-Jakob disease (CJD) agent.

• We will perform coagulation tests to avoid excessive or insufficient anticoagulation and monitor for bleeding or thrombosis. Assessment will be based on clinical findings at the discretion of the treating physician along with laboratory values of PT, PTT, Hemoglobin and Hematocrit analysis. Treatment may include heparin for excessive coagulation or blood transfusion for insufficient coagulation ultimately left to the discretion of the treating physician.

Two clinical trials were conducted in 33 subjects with congenital AT deficiency. Nine subjects (27%) experienced 29 adverse reactions which occurred during 17 of 389 infusions. There were no serious adverse reactions reported. The severity of adverse reactions was reported as mild or moderate, except for wound secretion and hematoma, which was severe.
Dizziness (12%), Chest discomfort (9%), Nausea (9%), Dysgeusia (6%), Pain (cramps) (6%), and each of the following were reported in 3% of the subjects: Chills, Wound secretion and hematoma, Vision blurred, Chest pain, Dyspnea, Intestinal dilatation, Pyrexia and Urticaria,

DRUG INTERACTIONS

The anticoagulant effect of heparin is enhanced by concurrent treatment with THROMBATE III in patients with hereditary AT deficiency. Thus, in order to avoid bleeding, the dosage of heparin (or low molecular weight heparin) may need to be reduced during treatment with THROMBATE III.
The effect of drugs that use antithrombin to exert their anticoagulation may be altered when THROMBATE III is added or withdrawn. We will regularly perform coagulation tests suitable for the anticoagulant used (e.g., aPTT and anti-Factor Xa activity) and at close intervals to avoid excessive or insufficient anticoagulation. Adjust dosage of anticoagulant as necessary. Additionally, we will monitor the patients for the occurrence of bleeding or thrombosis.

14) Potential Benefits to Subjects*

We expect subjects with deficient endogenous AT3 can lessen the risk associated with a prothrombotic hyperinflammatory state with infusion of AT3.

15) Setting: Jackson South Medical Center , Jackson Memorial Hospital

16) Resources Available

The PI has experience conducting and being trained for clinical research. All study personnel will be trained on the protocol by the investigator or designee and complete CITI training. Those obtaining consent will also have prior experience communicating with patients.

17) Prior Approvals: We have preliminary approval of funding from Grifols.

18) Confidentiality

The protected health information collected for the purpose of this research study will be assigned a research code number and any obvious patient identifiers (name, social security number, hospital record number) will be removed from this information. Both the confidential health information and the information linking the research code numbers to the patients' identities will be stored in a secure manner (e.g., locked file cabinet, password protected database) accessible only to the study team. The information linking the research code numbers to the patients' identities will be stored separate from the confidential health information.

Data will be de-identified and stored on a password-protected network accessible only to research staff. Data will be retained for at least six years following conclusion of the study on this network.

Please note: Any data, including Personally Identifiable Information (PII) and/or Protected Health Information (PHI), acquired from JHS with a waiver of HIPAA Authorization (without a signed HIPAA authorization) may only be stored in the secure JHS SharePoint environment provided by JHS. The Study Team is not permitted to copy or store JHS data in any other system. We will contact the JHS Office of Research for additional information or clarification.

Protected Health Information (PHI) is defined under HIPAA in 45 CFR § 160.103. The following list of identifiers of an individual, or of relatives, employers, or household members of the individual, are defined under HIPAA in 45 CFR § 164.541:
(A) Names;
(B) All geographic subdivisions smaller than a State, including street address, city, county, precinct, zip code, and their equivalent geocodes, except for the initial three digits of a zip code if, according to the current publicly available data from the Bureau of the Census:
(1) The geographic unit formed by combining all zip codes with the same three initial digits contains more than 20,000 people; and
(2) The initial three digits of a zip code for all such geographic units containing 20,000 or fewer people is changed to 000.
(C) All elements of dates (except year) for dates directly related to an individual, including birth date, admission date, discharge date, date of death; and all ages over 89 and all elements of dates (including year) indicative of such age, except that such ages and elements may be aggregated into a single category of age 90 or older;
(D) Telephone numbers;

(E) Fax numbers;
(F) Electronic mail addresses;
(G) Social security numbers;
(H) Medical record numbers;
(I) Health plan beneficiary numbers;
(J) Account numbers;
(K) Certificate/license numbers;
(L) Vehicle identifiers and serial numbers, including license plate numbers;
(M) Device identifiers and serial numbers;
(N) Web Universal Resource Locators (URLs);
(O) Internet Protocol (IP) address numbers;
(P) Biometric identifiers, including finger and voice prints;
(Q) Full face photographic images and any comparable images; and
(R) Any other unique identifying number, characteristic, or code

The name, date of birth and mrn are required to conduct this study.

19) Choose the statements below that are applicable to this research:

20(a). Will the research collect protected health information or personally identifiable information from the EMR or from subjects at UHealth and/or JHS?

☒ Yes (If checked go to 21(b))
☐ No (If checked, go to Section 22)

20(b). Check the box next to the correct statement below
☒ Research Subjects will sign a HIPAA Authorization before the research will collect this data.
□ Research Subjects will not sign a HIPAA Authorization for this data collection and the
□ Research is requesting a waiver of HIPAA authorization from the IRB. (If checked, complete Section 17 below

20(c). How will the research store the data? (See Section 21(e) below)
□ On a University of Miami electronic device (e.g. encrypted, password-protected computer)
☒ On a cloud-based storage system that is approved by the University of Miami
☒ On a secured JHS SharePoint environment (required for protected health information or identifiable information collected from JHS records without a waiver of authorization from IRB.)
☐ Other, specify: Click here to enter text.

20(d) Select one of the following:

☐ The Principal Investigator (and/or Study Team members) will record (e.g. write down, abstract) data acquired in a manner that does not include any indirect or direct identifiers (listed in the instructions for Section 19 of this protocol), and the recorded data will not be linked to the individual's' identity.
OR
☒ The Principal investigator (and/or Study Team members) will record (e.g. write down, abstract) the data collected in a manner that does not include any direct identifiers (see list in the instructions for

Section 19 (Confidentiality) of this protocol) of any subject. Instead, the Principal Investigator and/or Study Team members shall will assign a code (that is not derived in whole or in part from any direct or indirect identifiers of the individual) to each study subject and link the code to the study subject's identity. The link to each subject's identity and/ or other identifiable information will be maintained on a document separate from the research data.

20(e) Additional requirement for Jackson Health System Data:
This section applies to data that will be collected from JHS under a waiver of HIPAA authorization (without a signed HIPAA Authorization from the participant).

☐ Not-applicable, no data will be acquired from JHS under a waiver of authorization.

☒ JHS data, including Protected Health Information (PHI) and/or Personally Identifiable Information (PII), acquired from JHS for this research under a waiver of authorization shall only be stored on the secured JHS SharePoint environment made available by JHS. I and the Study Team members shall not copy or store the JHS sourced personally identifiable information (PII), including protected health information (PHI) data to any other system, including any systems maintained or provided by the University of Miami. I and the Study Team shall only copy or transfer JHS-sourced data that has been properly de-identified in accordance with all requirements contained in the HIPAA Rules by removing all of the identifiers listed in the instructions for Section 19 of this protocol.

20) Provisions to Protect the Privacy Interests of Subjects

Access to patients and their protected health information along with the collection and analysis is necessary in order to conduct this research study. Consistent with the "minimum necessary standard" of the HIPAA privacy rule, we will only access and collect the specific health information necessary to complete this research study.

Some of the research investigators who will access and use the protected health information may also be involved directly in the care of the respective patients, thus obviating the privacy and confidentiality concerns.

Authorization to obtain protected health information will be obtained from the patient as soon as possible.

The protected health information collected for the purpose of this research study will be assigned a research code number and any obvious patient identifiers (name, social security number, etc) will be removed from this information. Both the confidential health information and the information linking the research code numbers to the patients' identities will be stored in a secure manner (e.g., locked file cabinet, password protected database) accessible only to the study team.

21) Consent Process

22a. Remote Consenting for subject proxies unavailable for inperson consenting will includethe following:

- Send a copy of the consent document via secure email or U.S. Mail.
- Arrange for an impartial witness not part of the study team to attend and witness the consent discussion.

- Let the participant know that a witness will join the consent meeting.
- Set up a video meeting or 3-way call and send an invitation to the attendees.

We will ask the participant to scan or take a picture of each page of the documents and email the signed/dated documents to the study team. As an alternative, we may establish a secure network location for uploading the consent document.

During meeting:
- Identify everyone on the call.
- Review the informed consent with the participant, answer the participant's questions and ask questions of the participant to confirm comprehension.
- Ask the participant if s/he consents to participate/continue participation.
- If the participant agrees, ask him/her to sign and date the consent document.
- If using 3-way call, ask the participant to confirm s/he signed & dated the document.

We will ask the participant to scan or take a picture of each page of the documents and email the signed/dated documents to the study team. As an alternative, the research could establish a "UM Box" location for uploading the consent document.

If the person is unable to take a picture, we will document the circumstances.

The person conducting the consent process will sign and date a copy of the consent document.

The impartial witness will sign & date on the witness line of a copy of the consent document.

The person conducting the consent process will document the purpose for the remote consent (COVID-19), and each step of the process. The note will explain why the research team doesn't have the signed and dated document.

<u>Documenting the Remote Consent process</u>

The originals of the informed consent document signed by the investigator (and possibly the witness) will be placed in the participant's research record.

The person obtaining consent will document how s/he confirmed that the patient consented and signed the consent form. The participant may retain the signed original due to contamination. The note will include a statement indicating why the informed consent document signed by the participant was not retained, (e.g., due to contamination of the document by infectious material.)

If the participant cannot send a picture of the signed document, the person obtaining consent will document why a copy of the signed document is not available. See example below.

Example:
Informed consent was obtained on Date at Time. The participant could not come to the site for the consent process due to COVID-19 social distancing requirements. A copy of the consent document was emailed to the prospective participant before the consent discussion

The consent process was performed by phone/ZOOM. The individuals attending the discussion were: (list the names of the individuals). The person obtaining consent explained the research to the participant and answered the participant's questions. The person obtaining consent asked the participant questions to ascertain whether the s/he understood the study, and the participant was able to answer the questions. The participant voluntarily agreed to participate. The subject/LAR signed and dated the consent document. The research was not able to obtain a copy of the signed original consent document because consent was obtained remotely, and the document may transmit the COVID-19 infection.

After signing the consent document, the participant took a picture and sent it to the research team/ OR The participant was unable to send a picture of the document. An impartial witness observed the entire process.

The consenter will then add similar documentation about the HIPAA authorization.

22b. In person consenting:

The subject will be approached in the clinic area by a member of the research staff. Any of the staff members listed on the protocol will discuss the risks and benefits of participation. If the staff member is not fluent in the patient's preferred language, they will use a translator to obtain informed consent in a document written in the patient's preferred language. Initiating this process may also occur over the phone and during scheduling and may include mailing of the consent form to allow for more time to read and understand prior to coming into clinic.

Subject will be given an ICF in their preferred language (English/Spanish only). They will be given ample time to review the ICF with family, clergy, friends or primary physicians. Once they have agreed they will be required to sign ICF before any study procedures can be done on the subject. A copy of the ICF will be given to the subject and one will be placed in the medical records. The original will stay in the subject's source documentation folder located in the Trauma Research Office.

22) Process to Document Consent in Writing

We will document consent with a translated written informed consent document in a language understandable to the participant. If subjects who do not speak English are eligible, we ensure that the oral and written information provided to those subjects will be in that language either by research personnel fluent in that language or by an institutional translator. We will pursue approval of the consent documents in Spanish as a significant portion of the subject population may speak Spanish. We also request permission to utilize the short forms for documentation of consent and translation into other than the English or Spanish for the rare circumstance that the subject cannot communicate in either of those languages.

23) Authorization for Use and Disclosure of Protected Health Information (HIPAA)

Type of Request:

☒ Waiver of Authorization for access to medical record for subject identification/recruitment.

☐ Waiver of Authorization for access to medical record to obtain data for the research.

Confirm that you will destroy the Protected Health Information (PHI) you and/or your Study Team acquire receive from JHS and/or UHealth at the earliest opportunity.

☒ **I confirm**

Confirm that the Protected Health Inform (PHI) you acquire from JHS and/or UHealth will not be re-used or disclosed to any other person or entity, except as required by law or for authorized oversight of the research study or for other research for which the use or disclosure of PHI is permissible.

☒ **I confirm**

If you are collecting health information from JHS under a waiver of authorization, you must read the paragraph below and sign the signature block to indicate your agreement:

☐ Not applicable. This research will not collect data from JHS record under a waiver of authorization

Notwithstanding the preceding "I confirm" statements above, I agree that neither I nor any member of the study team listed on the IRB submission for this Protocol shall ever re-use or re-disclose any of the information acquired from Jackson Health System in any format, whether identifiable or de-identified, to any individual or entity without first obtaining written permission from Jackson Health System, even if such re-use or re-disclosure is permissible by law (e.g., HIPAA).

_____ _____

Signature Date

24) Drugs or Devices

Drug Administration, Dispensation and Disposition

Once consent is obtained and necessary baseline data collected, and if the subject is enrolled and falls into the Thrombate III infusion arm, they will begin receiving THROMBATE III (Antithrombin III [Human]) at 120% target dose every other day for 9 days.

Study drug will be packaged, labeled, and delivered to the clinical center by the drug manufacturer. All medication supplied to be used in this study will be manufactured, tested, labeled, and released according to current legal requirements and Good Manufacturing Practice (GMP).

The pharmacy will conduct an inventory upon receipt of the clinical supplies and will acknowledge receipt of the supplies to the sponsor. A copy of the shipping documents will be maintained for records. Study drug will be stored in accordance with the package insert in a secure location until dispensed to the subject.

Study drug kits will be dispensed according to the dosing schedule. The pharmacy will keep a current record of the inventory and dispensing of all clinical supplies. This record will be made available to the sponsor's monitor for the purpose of accounting for all clinical supplies. Any discrepancy or deficiency will be recorded, with an explanation. All supplies sent to the investigator will be accounted for and in no case will clinical supplies be used in an unauthorized situation.

25) REFERENCES

PMID: 32267998
Wang J, Hajizadeh N, Moore EE, et al. Tissue plasminogen activator (tPA) treatment for COVID-19 associated acute respiratory distress syndrome (ARDS): A case series. J Thromb Haemost. 2020;18(7):1752-1755. doi:10.1111/jth.14828

PMID: 32329246
Whyte CS, Morrow GB, Mitchell JL, Chowdary P, Mutch NJ. Fibrinolytic abnormalities in acute respiratory distress syndrome (ARDS) and versatility of thrombolytic drugs to treat COVID-19. J Thromb Haemost. 2020;18(7):1548-1555. doi:10.1111/jth.14872

PMID: 32559324
Garg S, Garg M, Prabhakar N, Malhotra P, Agarwal R. Unraveling the mystery of Covid-19 cytokine storm: From skin to organ systems [published online ahead of print, 2020 Jun 19]. Dermatol Ther. 2020;e13859. doi:10.1111/dth.13859

PMID: 32302438
Panigada M, Bottino N, Tagliabue P, et al. Hypercoagulability of COVID-19 patients in intensive care unit: A report of thromboelastography findings and other parameters of hemostasis. J Thromb Haemost. 2020;18(7):1738-1742. doi:10.1111/jth.14850

PMID: 32558075
Iba T, Levy JH, Levi M, Thachil J. Coagulopathy in COVID-19 [published online ahead of print, 2020 Jun 18]. J Thromb Haemost. 2020;10.1111/jth.14975. doi:10.1111/jth.14975

PMID: 32073213
Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. J Thromb Haemost. 2020;18(4):844-847. doi:10.1111/jth.14768

PMID: 32394236
Pavoni V, Gianesello L, Pazzi M, Stera C, Meconi T, Frigieri FC. Evaluation of coagulation function by rotation thromboelastometry in critically ill patients with severe COVID-19 pneumonia. J Thromb Thrombolysis. 2020;50(2):281-286. doi:10.1007/s11239-020-02130-7

PMID: 32302448
Ranucci M, Ballotta A, Di Dedda U, et al. The procoagulant pattern of patients with COVID-19 acute respiratory distress syndrome. J Thromb Haemost. 2020;18(7):1747-1751. doi:10.1111/jth.14854

PMID: 32172226

Han H, Yang L, Liu R, et al. Prominent changes in blood coagulation of patients with SARS-CoV-2 infection. Clin Chem Lab Med. 2020;58(7):1116-1120. doi:10.1515/cclm-2020-0188

PMID: 32516429
Arachchillage DJ, Remmington C, Rosenberg A, et al. Anticoagulation with argatroban in patients with acute antithrombin deficiency in severe COVID-19 [published online ahead of print, 2020 Jun 9]. Br J Haematol. 2020;10.1111/bjh.16927. doi:10.1111/bjh.16927

PMID: 24662855
Louis SG, Van PY, Riha GM, et al. Thromboelastogram-guided enoxaparin dosing does not confer protection from deep venous thrombosis: a randomized controlled pilot trial. J Trauma Acute Care Surg. 2014;76(4):937-943. doi:10.1097/TA.0000000000000165

PMID: 32305521
Nakano T, Nakamura Y, Irie K, et al. Antithrombin gamma attenuates macrophage/microglial activation and brain damage after transient focal cerebral ischemia in mice. Life Sci. 2020;252:117665. doi:10.1016/j.lfs.2020.117665

PMID: 26676884
Levy JH, Sniecinski RM, Welsby IJ, Levi M. Antithrombin: anti-inflammatory properties and clinical applications. Thromb Haemost. 2016;115(4):712-728. doi:10.1160/TH15-08-0687

Appendix A

ISTH DIC, the score is increased by 2 points for abnormal baseline D-dimers (fibrin-related marker) and/or abnormal fibrinogen <1.0 gram/L level (1 point). This scoring algorithm was developed by the ISTH Scientific and Standardization Committee (SSC) committee on DIC and published in 2001. The ISTH DIC is a set of objective criteria for the diagnosis and severity of DIC with a score of ≥5 correlating with overt DIC, whereas < 5 does not rule out DIC but may indicate DIC that is not overt. The score is only appropriate for use in patients with an underlying disorder that is known to be associated with DIC; e.g., sepsis, severe immunological reactions, etc.

The ISTH DIC score is calculated using

- platelet count (≥100,000 = 0; 50,000-99,999 = 1; <50,000 = 2),
- fibrinogen level (≥100 mg/dL = 0; <100 mg/dL = 1),
- prothrombin time prolongation above upper limit of normal (ULN)
  - (<3 seconds = 0, 3-6 seconds = 1, >6 seconds = 2),
- and D-dimer (<2 times ULN = 0, 2-4 times ULN = 2, >4 times ULN = 3).

SOFA SCORE

SOFA SCORE (Sequential Organ Failure Assessment)					
ORGAN SYSTEM	**0**	**1**	**2**	**3**	**4**
Respiratory* PaO2 / FIO2 Ratio	> 400	≤ 400	≤ 300	≤ 200 w/support	≤ 100 w/support
Hematologic Platelets	> 150	≤150	≤ 100	≤ 50	≤ 20
Hepatic Serum Bilirubin	< 1.2	1.2 – 1.9	2.0 – 5.9	6.0 – 11.9	> 12.0
CV MAP	No hypotension	< 70	Dopamine ≤ 5 **or** Any dobutamine	Dobutamine > 5 **or** Epi ≤ 0.1 **or** Norepi ≤ 0.1	Dopamine > 15 **or** Epi > 0.1 **or** Norepi > 0.1
Neurologic* Glasgow Coma Score	15	13-14	10-12	6-9	< 6
Renal Serum Creatinine	< 1.2	1.2 – 1.9	2.0 – 3.4	3.5 – 4.9 **or** <500 u/o	>5 **or** < 200 u/o
*See Appendix D. A Modified GCS is provided for intubated patients. Also provided for cases when it is not possible to obtain arterial blood gases to determine the SOFA respiratory parameter, SpO2/FiO2 ratio can be imputed for PaO2/FiO2 ratio. For patients on supplemental oxygen via nasal cannula, nasopharyngeal catheter or mask and for converting SpO2 values to PaO2 see Appendix D for oxygen conversion tables.					

Appendix B

Study Subject Flowsheet	Enrollment - Day 1*	Days 3, 5, 7 & 9	Day 11	On Discharge
Informed Consent	x			
Entry Criteria	x			
Demographics	x			
Medical and Surgical History	x			
Prior & Concomitant Medications	x	x	x	x
Vital Sign Measurements	x	x	x	
ISTH DIC Score	x	x	x	
SOFA Score	x		x	
Blood Draw	x	x	x	
Urine or Serum Pregnancy Test if applicable	x			
Platelet	x	x	x	
PT,INR	x	x	x	
PTT	x	x	x	
Hemoglobin and Hematocrit	x	x	x	
Fibrinogen	x	x	x	
D-Dimer	x	x	x	
AT3 Level	x	x	x	
Anti-Xa	x	x	x	
Dosing if randomized to group with AT3 Infusion[1]	x[1]	x[1]		
Adverse Events[2]	x	x	x	x[2]
Hospital LOS				x
Mortality				x
PF ratio change				x
Days on Ventilator				x
Days on ECMO				x
VTE's				x
Bleeding Events				x

*: Day refers to a 24 hour period.
x: To be completed
1: Only if randomized to AT3 Infusion and AT3 level is at specified level
2: Only Serious Adverse Events will be captured from last dose to hospital discharge.

Appendix C

The Secondary endpoint measurements:

a. D-dimer level change from baseline to Day 9 in mcg/mL FEU or DDU,
b. Fibrinogen level change from baseline to Day 9 in mg/dL
c. Prothrombin time change from baseline to Day 9 in seconds
d. Length of hospital stay in days,
e. Mortality (%) of overall and between groups,
f. Pulmonary function
    a. Time (days) to mechanical ventilation and/or
    b. ECMO Duration (days) or extracorporeal membrane oxygenation (ECMO) use,
g. SOFA score change from baseline to Day 9 and
h. SOFA Respiratory sub-score change from baseline to Day 9,
i. Number of venous thromboembolisms from admission to hospital discharge,
j. Number of major bleeding events from admission to hospital discharge

Appendix D

Estimating Fi02		
Method	**O2 flow (l/min)**	**~FiO2 (%)**
Nasal cannula	1	24
	2	27
	3	30
	4	33
	5	36
	6	40
Nasopharyngeal catheter	4	40
	5	50
	6	60
Face mask	5	40
	6-7	50
	7-8	60
Face mask with reservoir	6	60
	7	70
	8	80
	9	90
	10	95

Estimated Pa02 from a given Sp02	
SpO2 (%)	PaO2 (mmHg)
80	44
81	45
82	46
83	47
84	49
85	50
86	52
87	53
88	55
89	57
90	60
91	62
92	65
93	69
94	73
95	79
96	86
97	96
98	112
99	145

Modified GCS	
Eyes	4 = spontaneously 3 = to verbal 2 = to painful stimuli 1 = no response
Motor	6 = to verbal command 5 = localized to pain 4 = withdraws to pain 3 = decorticate, arms flex 2 = decerebrate, arms ext 1 = no response
Verbal No ETT	5 = oriented, converses 4 = disoriented & talks 3 = inappropriate words 2 = incomprehensible words 1 = no response
Verbal w/ETT	5 = seems able to talk 3 = questionable ability to talk 1 = generally unresponsive