Protocol Title: Feasibility Study of Unfractionated Heparin in Acute Chest Syndrome

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Abstract

This is a single-center, randomized controlled, open-label, pilot study to determine the feasibility of performing a larger multicenter phase III trial to assess the effects of unfractionated heparin (UFH) in acute chest syndrome (ACS). Adult patients with sickle cell disease (HbSS, SC, or $S\beta^0$) aged 18 years or older admitted to the University of Pittsburgh Medical Center (Presbyterian, Montefiore, Magee, Shadyside) hospitals with a diagnosis of ACS will be eligible for screening to participate in the trial. Subjects meeting inclusion and exclusion criteria and consenting to study will be randomized within 24 hours of diagnosis to one of two treatment arms, Arm A, anticoagulation and standard of care, or Arm B, no anticoagulation and standard of care. Weight-adjusted UFH will be given at doses of 80 units per kilogram followed by 18 units per kilogram per hour intravenously for 7 days, or until discharge, if discharge is shorter than 7 days. UFH will be monitored by standard protocol to maintain the anti-Xa UFH level in the therapeutic range per institutional guidelines. Standard of care will include intravenous fluids, antibiotics, supplemental oxygen, incentive spirometry, pain medication, red blood cell transfusions, and exchange transfusion. Prespecified feasibility criteria consists of the ability to enroll potential study participants, which includes the timely notification of hospitalized patients with ACS, the capacity to consent eligible individuals, and the ability to appropriately randomize screened patients within 24 hours of diagnosis. Additional feasibility objectives involve ensuring appropriate inclusion and exclusion criteria, proper administration of the study drug, and the ability to completely and accurately collect clinical data of interest. The final aim of our pilot study is to provide preliminary data, with respect to treatment effect and variance, to allow sample size calculation in a larger trial given the lack of data available to help guide this process. All clinical outcomes, except the number of red blood cell transfusions administered, will be defined as beginning with randomization and measured in hours. The primary clinical outcome is duration of hospitalization. Secondary clinical outcomes include the duration of hypoxemia, duration of fever, duration of leukocytosis, duration of moderate to severe pain, total dose of opioids, and the number of red blood cell transfusions administered. Additional clinical outcomes will include the rate of transfer to the intensive care unit, requirement for mechanical ventilation, and development of multiorgan dysfunction syndrome. Further data collected will include demographics and medical information. A sample size of 20 subjects for the pilot study is based on clinical rather than statistical considerations and should provide a preliminary estimate of primary and secondary clinical outcomes and assess prespecified feasibility criteria as detailed above. This is a pilot feasibility study, so comparisons will be descriptive, including frequencies and percentages for categorical variables and mean, median, range, and standard deviation for continuous variables.

1.0 Objective and Specific Aims

The purpose of this single-center, randomized controlled, open-label, pilot study is to determine the feasibility of performing a larger multicenter phase III trial to assess the effects of unfractionated heparin (UFH) in acute chest syndrome (ACS). Specific feasibility criteria include the ability to enroll potential study participants, which entails the timely notification of all hospitalized patients diagnosed with ACS, the capacity to consent eligible individuals, and the ability to appropriately randomize screened patients within 24 hours of diagnosis. Additional feasibility objectives involve ensuring appropriate inclusion and exclusion criteria, proper administration of the study drug, and the ability to completely and accurately collect clinical data of interest. The final aim of our pilot study is to provide preliminary data, with respect to treatment effect and variance, for future sample size calculations given the lack of existing data to help guide this process. The primary clinical outcome is duration of hospitalization. Secondary clinical outcomes include the duration of hypoxemia, duration of fever, duration of leukocytosis, duration of moderate to severe pain, total dose of opioids, and the number of red blood cell transfusions administered. Additional clinical outcomes will include the rate of transfer to the intensive care unit (ICU), requirement for mechanical ventilation, and development of multiorgan dysfunction syndrome (MODS).

2.0 Background and Significance

2.1 Background

Sickle cell disease (SCD) is an autosomal recessive hemoglobinopathy caused by a glutamic acid to valine substitution at the sixth codon of the beta globin gene resulting in the production of mutant hemoglobin S that is prone to intraerythrocytic polymerization and subsequent microvascular occlusion clinically manifested as episodes of severe pain, organ damage, and early death.[1,2] SCD affects approximately 75,000 to 100,000 African Americans in the United States.[3] The second leading cause of admission to the hospital and leading cause of mortality in SCD is ACS, defined as a new pulmonary infiltrate associated with fever, chest pain, and signs and symptoms of pulmonary disease, such as cough, dyspnea, and tachypnea.[4] While a frequent cause of hospitalization in SCD, 48% of ACS episodes are diagnosed following a hospital admission for another reason, typically vasoocclusive crises in nearly 75% of instances, usually occurring within 3 days.[5] The incidence of ACS in adults is 8.8 per 100 patient years.[4] Nearly ½ of all patients with SCD will develop ACS at least once with a smaller subset suffering multiple episodes.[4] Repeated episodes of ACS are associated with the development of chronic lung disease and early death.[4] ACS is responsible for 25% of deaths from SCD.[4] The rate of death in ACS is 9% in adults.[5]

Recently, the role of thrombosis in SCD has been the subject of greater investigation. Nearly every component of hemostasis, including platelet function and the procoagulant, anticoagulant, and fibrinolytic systems, are altered in the direction of the procoagulant phenotype, in SCD.[6] The pathogenesis of the thrombophilic state in SCD is multifactorial and involves every aspect of Virchow's triad, increased coagulability, endothelial dysfunction, and impaired blood flow, resulting in a very thrombogenic environment.[7] Specific mechanisms of hypercoagulability include the loss of normal membrane phospholipid asymmetry from repeated cycles of sickling and unsickling resulting in abnormal phosphatidylserine exposure which is associated with increased levels of markers of thrombin generation and increased endothelial tissue factor expression.[6] Additionally, free plasma hemoglobin from hemolysis in SCD scavenges nitric oxide, which normally inhibits platelet activation and aggregation, resulting in chronic platelet activation.[8] Sickled red blood cells (RBCs) result in microvascular occlusion and subsequent ischemia precipitating endothelial injury as well as impaired blood flow.[4] There is a 50 to 100-fold increase in the incidence of PE in hospitalized SCD patients compared with the general population.[9]

The pathogenesis of ACS is complex and not entirely understood with multiple etiologies likely contributing simultaneously in any one particular episode. The most common etiologies of ACS include infection, infarction due to pulmonary vascular occlusion, and fat embolism.[5] Pulmonary vascular occlusion may be microvascular, due to thrombosis or sickled RBCs, or occur in larger vessels as seen with PE.[4]

Additionally, fat embolism can result in pulmonary vascular occlusion.[4] Infarction is the second leading cause of ACS and one of the leading causes of death in ACS.[5]

Pulmonary vascular occlusion due to thrombosis is becoming increasingly more recognized as an important player in the pathogenesis of ACS. There is a 17% prevalence of pulmonary artery thrombosis in patients admitted to the hospital with ACS.[10] At autopsy, 38.1% of patients had PE and 28.5% of patients had pulmonary microvascular thrombi defined as <1 millimeter in size and undetectable with current computed tomography (CT) techniques.[11] Pulmonary artery thrombosis in ACS may be more of a local rather than embolic phenomenon evidenced by one study that found none of the individuals diagnosed with ACS and pulmonary artery thrombosis had a concurrent lower extremity deep venous thrombosis (DVT).[10] This is in contrast to data that shows approximately 45% of pulmonary emboli in the general population are associated with lower extremity DVTs.[10]

UFH decreases sickle cell adhesion to the endothelium under static conditions as well as P-selectin mediated flow adherence of sickle cells to thrombin treated human vascular endothelial cells.[4] A few studies have evaluated the use of anticoagulation in SCD with varying results. One randomized, double-blind, placebo-controlled study of patients with SCD during vasooclusive (VOC) crises treated with tinzaparin resulted in a significant reduction in duration of hospitalization, duration of VOC, and the number of days with the most severe pain scores.[12] Another randomized, double-blind, placebo-controlled trial is evaluating hypercoagulable markers and pain scores in hospitalized SCD patients treated with dalteparin versus placebo.[13] This trial is open and enrolling patients.

Anticoagulation as an adjunct in the treatment of ACS has not been studied. We propose a single-center, randomized controlled, open-label, pilot study to determine the feasibility of performing a larger multicenter phase III trial to assess the effects of UFH in ACS. Specific feasibility criteria include the ability to enroll potential study participants, which entails the timely notification of all hospitalized patients diagnosed with ACS, the capacity to consent eligible individuals, and the ability to appropriately randomize screened patients within 24 hours of diagnosis. Additional feasibility objectives involve ensuring appropriate inclusion and exclusion criteria, proper administration of the study drug, and the ability to completely and accurately collect clinical data of interest. The final aim of our pilot study is to provide preliminary data, with respect to treatment effect and variance, for future sample size calculations given the lack of existing data to help guide this process. We hypothesize that the use of UFH in ACS will result in a decrease in the duration of hospitalization and improve other clinical outcomes, such as the duration of hypoxemia and duration of moderate to severe pain.

2.2 Significance

ACS is common complication of SCD that will affect a large percentage of individuals at some point in their lifetime.[4] Furthermore, ACS is the leading cause of death in SCD and repeated episodes can lead to early death.[4] Clinically, ACS can be unpredictable, and it is difficult to identify which patients will have an uncomplicated course versus those that will develop severe disease with refractory hypoxemia and respiratory failure. Despite the above, the optimal treatment of ACS remains undefined. Currently, standard of care involves a combination of the following: intravenous fluids, supplemental oxygen, pain medication, antibiotics tailored to treat community acquired pathogens involved in the development of pneumonia, red blood cell and/or exchange transfusions, and respiratory therapies, such as incentive spirometry and nebulizers if a reactive airway disease component is felt to be present. Given the high morbidity and mortality, better therapies are needed to improve the clinical course and outcomes in ACS.

Given the increasingly recognized role of the thrombophilic state associated with SCD, anticoagulation is an attractive therapeutic modality in the treatment of VOC and ACS. Moreover, the use of anticoagulation in SCD, appears to be an interesting option because it has been shown that UFH decreases sickle cell adhesion to the endothelium under static conditions as well as P-selectin mediated flow adherence of sickle cells to thrombin treated human vascular endothelial cells.[4] At this time, there is limited data available regarding the use of anticoagulation in VOC with only one randomized controlled trial in the literature and another randomized controlled trial actively enrolling patients.[12,13] To our knowledge, the use of anticoagulation in the treatment of ACS has not been evaluated.

Given the continued morbidity and mortality associated with ACS in SCD and no optimally defined therapy, it is imperative that other treatments be evaluated in well-designed clinical trials to provide high quality evidence of efficacy. UFH is potential treatment, for reasons outlined above, that we hypothesize will decrease the duration of hospitalization and improve other clinical outcomes, such as the duration of hypoxemia and duration of moderate to severe pain. Other indirect benefits related to decreased length of hospital stay include limiting the risk for potential iatrogenic complications, such as venous thromboembolism (VTE), if not already present, pneumonia, if not already present, central venous catheter related infections, delirium, and adverse affects of medications among others. Additionally, decreased duration of hospitalization will result in healthcare related cost savings.

3.0 Research Design and Methods

3.1 Drug Information

Heparin is an anionic, highly sulfated, mucopolysaccharide that binds to antithrombin III via a unique pentasaccharide sequence.[14] The heparin/antithrombin III complex catalyzes antithrombin III mediated inactivation of factors IIa, IXa, Xa, XIa, and XIIa.[14] Heparin therapy is indicated for the treatment and prevention of arterial and venous thromboses in a wide variety of clinical circumstances, including DVT, PE, atrial fibrillation, myocardial infarction, etc.

Patients receiving UFH will do so intravenously via a peripheral intravenous catheter, or central venous access device if already present, per treating physician preference. Peripheral intravenous catheter, if needed, will be inserted by the inpatient nursing staff. UFH will be obtained from the University of Pittsburgh Medical Center (UPMC) inpatient hospital pharmacy. UFH will be ordered by a member of the research staff via the electronic medical record system. 25,000 units of UFH in 250 milliliters dextrose 5% in water, 100 units per 1 milliliter, will be infused according to institutional guidelines.[15] An initial dose will be given as an 80 unit per kilogram bolus rounded to the nearest 100 units not exceeding 10,000 units (regardless of weight). Subsequently, a continuous infusion will begin at 18 units per kilogram per hour rounded to the nearest tenth but not exceeding 1600 units per hour (regardless of weight). An anti-Xa UFH level will be obtained 6 hours after the start of the heparin infusion with adjustments as follows:

- If the anti-Xa UFH level is less than 0.2 units per milliliter, an 80 unit per kilogram bolus (maximum 10,000 units) will be given followed by an increase in the rate of the infusion by 4 units per kilogram per hour. A repeat anti-Xa UFH level will be checked 6 hours after the change in the infusion rate.
- If the anti-Xa UFH level is 0.21 to 0.29 units per milliliter, a 40 unit per kilogram bolus (maximum 10,000 units) will be given followed by an increase in the rate of the infusion by 2 units per kilogram per hour. A repeat anti-Xa UFH level will be checked 6 hours after the change in the infusion rate.
- If the anti-Xa UFH level is 0.30 to 0.70 units per milliliter, or therapeutic, no changes are necessary. Once a therapeutic anti-Xa UFH level is obtained, an anti-Xa UFH level will be repeated every 6 hours until two consecutive therapeutic results are achieved at which time the anti-Xa UFH level can be checked daily.
- If the anti-Xa UFH level is 0.71 to 0.80 units per milliliter, the rate of the infusion will be decreased by 2 units per kilogram per hour. A repeat anti-Xa UFH level will be checked 6 hours after the change in the infusion rate.
- If the anti-Xa UFH level is 0.81 to 1.00 units per milliliter, the infusion will be held for 1 hour then restarted after decreasing the rate of infusion by 3 units per kilogram per hour followed by a repeat anti-Xa UFH level in 2 hours.
- If the anti-Xa UFH level greater than 1.00 units per milliliter, the infusion will be held for 2 hours then restarted after decreasing the rate of infusion by 4 units per kilogram per hour followed by a repeat anti-Xa UFH level in 2 hours.

In the event of major bleeding, the anticoagulation effects of heparin will be reversed with protamine sulfate, a cationic protein that binds to heparin forming an ionic complex that neutralizes the anticoagulant effects of heparin.[14] Protamine will be administered according to institutional guidelines[15]. The maximum protamine dose is not to exceed 50 mg and no more than 50 mg is to be given over 10 minutes. Protamine will be given by slow intravenous infusion via a peripheral intravenous catheter, or central venous access device if already present, per treating physician preference, over 10 minutes to minimize the risk of hypotension, bradycardia, dyspnea, flushing, feeling of warmth, nausea, vomiting, or rarely anaphylaxis.[14] If a heparin bolus

was given within 2 hours of bleeding, protamine 1 mg per 100 units of heparin bolus will be administered. If no heparin bolus was given in the preceding 2 hours then the following protocol will be followed:

- If the heparin infusion, prior to bleeding, was less than 600 units per hours then 10 mg of protamine will be given.
- If the heparin infusion was 600 to 800 units per hour then 15 mg of protamine will be given.
- If the heparin infusion was 801 to 1100 units per hour then 20 mg of protamine will be given.
- If the heparin infusion was 1101 to 1400 units per hour then 25 mg of protamine will be given.
- If the heparin infusion was 1401 to 1700 units per hour then 30 mg of protamine will be given.
- If the heparin infusion was 1701 to 2000 units per hour then 35 mg of protamine will be given.
- If the heparin infusion was 2001 to 2300 units per hour prior then 40 mg of protamine will be given.
- If the heparin infusion was 2301 to 2600 units per hour then 45 mg of protamine will be given.
- If the heparin infusion was greater than 2600 units per hour then 50 mg of protamine will be given.

Adherence will be measured in hours UFH is not being infused (unless infusion is stopped per institutional guidelines, such as a supratherapeutic anti-Xa UFH level) according to the inpatient electronic medical record.

3.2 Study Design

This is a single-center, randomized controlled, open-label, pilot study to determine the feasibility of performing a larger multicenter phase III trial to assess the effects of UFH in ACS. Adult patients with SCD (HbSS, SC, or $S\beta^0$) aged 18 years or older admitted to UPMC (Presbyterian, Montefiore, Magee, Shadyside) hospitals with a diagnosis of ACS will be eligible for screening to participate in the trial. Subjects meeting inclusion and exclusion criteria and consenting to study will be randomized within 24 hours of diagnosis to one of two treatment arms, Arm A, anticoagulation and standard of care, or Arm B, no anticoagulation and standard of care. Weight-adjusted UFH will be given at doses of 80 units per kilogram followed by 18 units per kilogram per hour intravenously for 7 days, or until discharge, if discharge is shorter than 7 days. UFH will be monitored by standard protocol to maintain the anti-Xa UFH level in the therapeutic range per institutional guidelines.[15] During the study period, if therapeutic anticoagulation is indicated for non-research purposes, it will be given at the discretion of the treating physician.

Standard of care, per the treating physician, will include the following:

- Intravenous fluids, preferably hypotonic saline, will be given to maintain adequate hydration. The specific rate, subsequent changes in rate, and duration will be per the treating physician's discretion.
- Antibiotics will be given to treat pathogens that are responsible for community acquired pneumonia, such as "atypicals" or, if necessary, to provide a broader spectrum of coverage against antimicrobial organisms as the treating physician sees fit.
- Supplemental oxygen will be provided to maintain the arterial oxygen saturation, as recorded by pulse oximetry, greater than or equal to 90%.
- Incentive spirometry will be provided with recommendations to take 10 breaths every 2 hours while awake.
- Pain management will consist of acetaminophen, nonsteroidal antiinflammatory drugs (NSAIDs), or opioids as needed to maintain adequate analgesia. Specifics regarding pain management will be individualized according to each patient's established plan of care as dictated by his or her outpatient hematologist.
- Red blood cell transfusions will be given routinely as standard clinical practice in acute chest syndrome with the goal of preventing progression to acute respiratory failure.
- Exchange transfusions will be performed if extensive bilateral pulmonary disease is present, hypoxemia is not corrected with supplemental oxygen, or rapid clinical deterioration occurs with a goal HgbS less than or equal to 30%.

Following completion of the study, primary care or another accountable physician will assume patient care responsibilities.

3.3 Data Collection and Statistical Considerations

Data collection is summarized in Table 1. Body temperature and pulse oximetry will be checked by the inpatient nursing staff at least three times daily, beginning with randomization and ending with resolution of fever and hypoxemia, respectively, or study completion. Body temperature and pulse oximetry orders will be entered into the electronic medical record by a member of the research staff if not already ordered as part of standard of care. Documentation will be entered into the electronic medical record in the form of a research progress note informing the treating physician of vital signs ordered for research purposes to avoid duplication of tasks. The visual analog scale (VAS) for pain will be assessed once daily beginning with randomization and ending with resolution of moderate to severe pain or study completion. Laboratory evaluation will include a complete blood count (white blood cell count, hemoglobin and hematocrit, and platelet count). Complete blood count (white blood cell count) will be obtained daily beginning with randomization and ending with resolution of leukocytosis or study completion. Complete blood count (hemoglobin, hematocrit, and platelet count) will be obtained daily beginning with randomization and ending with study completion in subjects receiving UFH. Anti-Xa UFH level will be ordered according to the heparin infusion protocol as detailed earlier in subjects receiving UFH. Complete blood count will be entered into the electronic medical record by a member of the research staff if not already ordered as part of standard of care. Documentation will be entered into the electronic medical record in the form of a research progress note informing the treating physician of laboratory studies ordered for research purposes to avoid repeat blood draws. All blood samples will be collected by the inpatient phlebotomy team or nursing staff and sent to the inpatient laboratory for analysis.

Further data collected will include information regarding standard of care therapy (collected daily during duration of study), demographics (collected following study enrollment and screening), and medical information (collected following study enrollment and screening). Regarding standard of care therapy, data collected will consist of whether or not intravenous fluids were given; whether or not antibiotics were administered, number of antibiotics received, and class of antibiotics given (penicillin, cephalosporin, macrolide, fluoroquinolone, vancomycin, other); whether or not supplemental oxygen was received with documentation of need for noninvasive positive pressure ventilation or mechanical ventilation; whether or not pain medication was provided, including type of pain medication (opioid, NSAIDs, acetaminophen, other); number of red blood cell transfusions given; and whether or not exchange transfusion was necessary. Demographics include age, race, and sex. SCD related medical information includes number of previous episodes of ACS, current administration of outpatient hydroxyurea, and prior DVT or PE. General medical information includes history of myocardial infarction; chronic lung disease, defined as a documented baseline arterial oxygen saturation less than 90% and/or home supplemental oxygen requirement; previous stroke; and current or former smoker.

Prespecified feasibility criteria are as follows: the ability to enroll potential study participants, which includes the timely notification of hospitalized patients with ACS, the capacity to consent eligible individuals, and the ability to appropriately randomize screened patients within 24 hours of diagnosis, all of which should occur at a frequency greater than or equal to 80%; inclusion and exclusion criteria that does not exclude potential study participants at a frequency greater than or equal to 20%; the proper administration of the study drug at a frequency greater than or equal to 90%; and the ability to completely and accurately collect clinical data of interest at frequency greater than or equal to 90%. The final aim of our pilot study is to provide preliminary data, with respect to treatment effect and variance, for future sample size calculations given the lack of existing data to help guide this process.

The primary clinical outcome is duration of hospitalization. Secondary clinical outcomes include the duration of hypoxemia, duration of fever, duration of leukocytosis, duration of moderate to severe pain, total dose of opioids, and the number of red blood cell transfusions administered. Additional clinical outcomes will include the rate of transfer to the ICU, requirement for mechanical ventilation, and development of MODS.

Duration of hospitalization will be defined as beginning with randomization and measured in hours. Conclusion of hospitalization will be defined as the time of order entry into the inpatient electronic medical record system stating the patient is appropriate for medical discharge, per the discretion of the treating physician. Duration of hypoxemia will be defined as beginning with randomization and measured in hours. Hypoxemia will be defined as arterial oxygen saturation less than 90% according to pulse oximetry and/or the continued need for supplemental oxygen. If the patient's baseline arterial oxygen saturation is less than 90%, hypoxemia will be defined as a decrease in arterial oxygen saturation below the patient's baseline. Similarly, if

the patient has a baseline supplemental oxygen requirement, hypoxemia will be defined as an increase in supplemental oxygen requirement above the patient's baseline. If the patient does not meet criteria for hypoxemia following the initial 3 nursing vital signs assessments after randomization, hypoxemia will be considered absent and no further assessments will occur. Resolution of hypoxemia will be defined as failure to meet the criteria for hypoxemia on 3 consecutive nursing vital signs assessments. Duration of fever will be defined as beginning with randomization and measured in hours. Fever will be defined as a body temperature greater than or equal to 38.0 degrees Celsius measured by a thermometer orally. If the patient does not meet criteria for fever following the initial 3 nursing vital signs assessments after randomization, fever will be considered absent and no further assessments will occur. Resolution of fever will be defined as failure to meet the criteria for fever on 3 consecutive nursing vital signs assessments. Duration of leukocytosis will be defined as beginning with randomization and measured in hours. Leukocytosis will be defined as a white blood cell count greater than 10,000 per liter. If the patient does not meet criteria for leukocytosis following the initial 2 complete blood counts after randomization, leukocytosis will be considered absent and no further assessments will occur. Resolution of leukocytosis will be defined as failure to meet the criteria for leukocytosis on 2 consecutive complete blood counts. Duration of moderate to severe pain will be defined as beginning with randomization and measured in hours. If the patient does not report a score of 4 or greater on the VAS for pain following the initial 2 assessments after randomization, moderate to severe pain will be considered absent and no further assessments will occur. The end of the duration of moderate to severe pain will be defined as 2 consecutive scores of less than 4 on the VAS for pain. Total dose of opioids will be defined as beginning with randomization, ending with study completion, and measured in milligrams of oral morphine equivalents. Red blood cell transfusions will be given routinely as standard clinical practice in acute chest syndrome with the goal of preventing progression to acute respiratory failure. Number of red blood cells transfused will be defined as beginning with randomization, ending with study completion, and measured in units of packed red blood cells received. Red blood cell transfusions given for suspected or proven hemorrhage secondary to UFH will not be included in the outcome as defined above. Rate of transfer to the ICU, requirement for mechanical ventilation, and development of MODS will be defined as beginning with randomization, ending with study completion, and categorized as present or absent. Further, MODS will be defined as the acute development of 2 or more organs or organ systems unable to maintain homeostasis in a critically ill individual.

Potential adverse events include heparin-associated risks, such as allergic reaction, major bleeding, minor bleeding, thrombocytopenia, and heparin induced thrombocytopenia (HIT), or any other adverse reaction suspected secondary to drug administration. All subjects enrolled in the study and received any UFH will be included in the safety evaluation.

A member of the research staff will collect the study data. All data required by the study protocol will be documented in the inpatient electronic medical record system. De-identified subject information will be written on Case Report Forms (CRFs) and all data collected will be stored in locked files at the Hemophilia Center of Western Pennsylvania (HCWP). Additionally, data will be entered into research electronic capture (REDCap) and compared to ensure consistency. No personal identifiers will be associated with the data. The Institutional Review Board (IRB) may inspect records at any time.

A sample size of 20 subjects for the pilot study is based on clinical rather than statistical considerations and should provide a preliminary estimate of primary and secondary clinical outcomes and assess prespecified feasibility criteria as detailed above. Based on 75,000 to 100,000 African Americans with SCD in the United States who develop ACS at a frequency of 8.8 per 100 patient years, with 50% developing ACS at some point during his or her lifetime, and given the fact that approximately 30 patients are admitted annually at UPMC hospitals (Presbyterian, Montefiore, Magee, Shadyside) with ACS, we estimate that at least 80%, or 24 patients per year, will meet inclusion and exclusion criteria, and expect to enroll 10 patients, or slightly less than 50% per year, which should meet the n=20 subjects required for the pilot study.[3,4] This is a pilot feasibility study, so comparisons will be descriptive, including frequencies and percentages for categorical variables and mean, median, range, and standard deviation for continuous variables.

Demographics and medical information will be summarized with descriptive statistics as well. Adverse events will be summarized by frequency, relatedness, and severity.

4.0 Human Subjects

4.1 General Characteristics – Minority Inclusion and Non-Discriminatory Statements

Adult patients with SCD (HbSS, SC, or $S\beta^0$) aged 18 years or older admitted to UPMC (Presbyterian, Montefiore, Magee, Shadyside) hospitals with a diagnosis of ACS will be eligible for screening to participate in the trial. Children and pregnant females will not be enrolled in the study. Any patient with a predisposition towards thrombosis, other than SCD, and bleeding, as detailed in the inclusion and exclusion criteria, will excluded from study participation. Specifically, moyamoya disease has a 20% incidence of intracranial hemorrhage in adults in the United States.[16] The incidence of the disease is 1 in 1 million in the United States; therefore, even with an increased association between moyamoya disease and SCD, it is unlikely to affect patient recruitment.[16] Additionally, proliferative retinopathy predisposes towards vitreous hemorrhages with visual loss; however, the incidence of the disease is 0.5 per 100 patients, which, again, will be unlikely to affect patient recruitment.[17]

As SCD is an autosomal recessive disorder that predominantly affects individuals of African descent, it is anticipated that the study population will be comprised largely of African Americans, and the percentage of male and female participants will be similar in number. There will be no discrimination based on gender, race, or ethnicity. The gender, racial, and ethnic characteristics of the proposed study population will reflect the demographics of the disease, the city of Pittsburgh and surrounding areas, and UPMC. Written informed consent will be obtained from all subjects prior to enrollment in the study.

4.2 Recruitment Procedures

Patients with SCD will be given a flyer presenting the study during SCD clinic visits to produce study awareness and increase the liklihood of future study participation. All subjects will be enrolled at UPMC (Presbyterian, Montefiore, Magee, Shadyside) hospitals. The SCD inpatient service, including Drs. Novelli and Kato, two of the study co-investigators, is consulted to assist in the management of all hospitalized patients with SCD. Individuals approached for participation in this study will be subjects age 18 years or older admitted to one of the aforementioned hospitals with a diagnosis of ACS. A member of the research staff will meet with prospective subjects during inpatient admission and determine the patient's interest in study participation, and if interested, discuss the study in further detail. Informed consent will be obtained by Dr. Seaman, and include the purpose of the study, protocol specifics as they relate to the patient, potential adverse effects, and risks and benefit of the study. Each potential subject will be encouraged to ask all questions that he or she feels are necessary to enable him or her to make an informed decision regarding participation in the study. Each potential subject will be encouraged to take the time needed for thoughtful consideration whether or not to participate in the study; however, due to the design of the trial, which requires randomization within 24 hours of the diagnosis of ACS, there will be some time constraints with respect to the decision regarding study participation. Subjects who read the consent form are free to refuse enrollment. No experimental interventions will occur until after informed consent is obtained.

Subjects will be considered enrolled in the study after informed consent is obtained. Each study participant will receive a unique subject identification number. Afterwards, screening assessment will be completed by a member of the research staff. All individuals will have any reason for study exclusion documented. Following enrollment and screening, patients will undergo randomization. Prior to beginning the study, an independent statistician will perform a 1:1 randomization using randomization software ensuring 10 patients are placed in each arm of the study. A card will be labeled heparin or standard of care, placed in a sealed envelope numbered 1 to 20, and stored in locked files at HCWP. Following enrollment and screening, a member of the research staff will access the appropriate numbered envelope to ascertain patient assignment.

Study participants will be free to withdraw at any time by notifying a member of the research staff. Any data collected prior to the time of withdrawal will continue to be used, but no additional information will be collected. Processed blood sample results will continue to be used; however, remaining samples will be destroyed or used as indicated by the subject. The reason and date of study withdrawal for all subjects will be recorded.

4.3 Inclusion/Exclusion Criteria

Inclusion criteria:

1. Diagnosis of ACS defined as a new pulmonary infiltrate involving at least one segment of the lung on a chest x-ray or chest CT scan with 2 or more of the following: chest pain, tachypnea (defined as a respiratory rate greater than 20 breaths per minute), dyspnea, cough, hypoxemia (see 3.3 Data Collection and Statistical Considerations for definition of hypoxemia), or body temperature greater than or equal to 38.0 degrees Celsius.

2. Hemoglobin electrophoresis confirming (HbSS, SC, or $S\beta^0$) (historical records sufficient).

3. Age greater than or equal to 18.

Exclusion Criteria:

1. Any absolute contraindication to heparin, including known bleeding diathesis, history of HIT, or history of an allergic reaction.

2. Platelet count less than 50 per microliter (current admission).

3. Historical diagnosis of moyamoya disease as documented in medical records.

4. Historical diagnosis of proliferative retinopathy as documented in medical records.

5. Current participation in a chronic exchange transfusion program.

6. Underlying hypercoagulable disorder other than SCD.

7. Currently receiving therapeutic anticoagulation.

8. Currently receiving antiplatelet agents.

9. Currently receiving estrogen containing oral contraceptives.

10. Chest CT scan documented PE performed as standard of care prior to study enrollment (current admission).

4.4 Risk/Benefit Ratio

Risk of blood drawing
There may be discomfort with drawing blood, which may include pain, lightheadedness, dizziness, syncope, ecchymosis, bleeding, or infection in the tissue surrounding the vein. Appropriate medical intervention may consist of applying pressure to resolve bleeding or antibiotics in the setting of infection.

Risk of peripheral intravenous catheter placement
There may be discomfort with peripheral intravenous catheter placement, which may include pain, ecchymosis, bleeding, thrombophlebitis, or infection in the tissue surrounding the vein. Appropriate medical intervention may consist of applying pressure to resolve bleeding or antibiotics in the setting of infection.

Heparin associated risks
1. Allergic reaction
Allergic reactions are rarely reported with the use of intravenous UFH.[18] Because of this, the exact incidence is unknown, but expected to be rare.[18] Adverse events will be classified using the Common Terminology Criteria for Adverse Events (CTCAE) system, volume 4.03, 2010.[19] Allergic type symptoms may include fever, chills, pruritis, rash, urticaria, paresthesias, chest pain, dyspnea, wheezing, nausea, vomiting, edema, tachycardia, hypotension, anaphylaxis, or death. Subjects will be monitored for the development of these symptoms. If these symptoms occur, and are felt to be the result of UFH, the infusion will be discontinued, and diphenhydramine, methylprednisolone, or other appropriate medical interventions will be administered. Anaphylaxis will result in the permanent discontinuation of UFH. The decision to permanently discontinue UFH

in less severe, or minor, allergic reactions will be at the discretion of the investigator. A known history of an allergic reaction to heparin will result in exclusion from study participation.

2. Bleeding
Heparin therapy, like any other form of anticoagulation, increases the risk of bleeding. The risk of bleeding with UFH varies among clinical settings. The risk of bleeding with intravenous UFH in acute VTE is less than 3%.[20] Bleeding may be major, defined as a decrease in hemoglobin 2 grams per deciliter or more; transfusion of 2 or more units of red blood cells; or bleeding in a critical organ, such as intracranial, intraspinal, intraocular, pericardial, or retroperitoneal.[21] The presence of major bleeding will result in the permanent discontinuation of UFH. If major bleeding occurs, protamine will be administered to reverse the anticoagulant effects of heparin. Protamine may result in hypotension, bradycardia, dyspnea, flushing, feeling of warmth, nausea, vomiting, or rarely anaphylaxis.[14] Other medical interventions, such as intravenous fluids and red blood cell transfusions, will be administered as deemed appropriate. Minor bleeding is defined as all bleeding episodes that do not meet criteria for major bleeding. The treatment of minor bleeding may vary and will be determined by the specific clinical scenario. The decision to discontinue UFH in the setting of minor bleeding, permanently or only for a brief period of time, will be at the investigator's discretion. A known history of a bleeding diathesis will result in exclusion from study participation.

3. Thrombocytopenia
The risk of non-immune related thrombocytopenia with heparin varies anywhere between 5 and 30%.[22] Thrombocytopenia will be defined as a platelet count less than 100,000 per microliter. UFH will be discontinued if the platelet count is less than 50,000 per microliter and may be resumed if the platelet count increases to greater than 50,000 per microliter during the study period. A baseline platelet count less than 50,000 per microliter will result in exclusion from study participation.

4. HIT
HIT is an immunologically mediated thrombotic state that occurs in 1-2% of patients receiving heparin therapy.[23] Antibodies of the IgG class recognize large macromolecular complexes of platelet factor 4 bound to heparin.[23] Subsequently, these antibodies produce strong activation of platelets by binding to platelet FcγIIa receptors.[23] The ensuing thrombotic state may lead to arterial and venous thrombosis, skin necrosis, venous limb gangrene, stroke, and myocardial infarction. The diagnosis of HIT is a clinical-pathologic syndrome, which means the diagnosis requires a compatible clinical picture and laboratory evidence of platelet activating antibodies.[23] The laboratory diagnosis of HIT will be made with the serotonin release assay (SRA) that has a sensitivity of greater than 95% and a specificity of approximately 90% for the detection of platelet activating antibodies.[23] If HIT is suspected and/or confirmed, UFH will be discontinued for the duration of the study period, and an alternate non-heparin-based anticoagulant will be instituted at the discretion of the investigator. Subsequently, the SRA will be performed to confirm or exclude the diagnosis. The SRA will be collected by the inpatient phlebotomy team or nursing staff and sent to the inpatient laboratory for analysis. A known history of HIT will result in exclusion from study participation.

Risk of inadvertent disclosure
Study participation and related data will be protected to maintain confidentiality. There is a possibility that the subject's personal information could become generally known. This information could impact future insurability, employability, have a negative impact on family relationships, or result in stigmatization. In order to reduce risks of disclosure or breach of confidentiality, the research related documents, blood samples, and clinical information stored in the subject's research file will be assigned an alphanumeric identifier, i.e. ACS001, which does not contain any personal identifying information. A linkage key for linking the assigned alphanumeric identifier with the subject's name will be stored in locked files at HCWP. Any publication arising from this study will not contain names or other identifying information.

Potential benefits
UFH may result in a decrease in the duration of hospitalization, hypoxemia, fever, leukocytosis, moderate to severe pain, and the number of red blood cell transfusions administered; however, as this is a pilot study, specifically designed to evaluate predefined feasibility criteria for conducting a large scale clinical trial, there may be no benefit from participation in the study.

4.5 Data Safety Monitoring Plan (DSMP)

The Data Safety Monitoring Committee (DSMC) will meet at least weekly to review all aspects of the study. The DSMC is comprised of the research team at HCWP consisting of three physicians (Dr. Seaman, Dr. Ragni, and another hematologist), two research nurses, and one regulatory coordinator. Subjects will be closely monitored to ensure subject safety and to ensure that procedures are in place to maintain privacy and confidentiality, progress of study, integrity of the data, procedure reviews, and for discussion of pertinent scientific literature or events which could effect the benefit to risk ratio. All serious and unexpected adverse events and/or major breaches of confidentiality will be reported to the IRB according to regulations outlined in the IRB *Reference Manual for the Use of Human Subjects*. The DSMC will determine if the risk benefit ratio is sufficiently favorable that it is appropriate to continue the trial. The events are:

- A subject develops major bleeding in association with UFH.
- A subject develops HIT in association with UFH.
- A subject develops a Grade 2 or greater allergic reaction in association with UFH, defined as follows using the CTCAE grading.
    - Grade 2 - Intervention or interruption of infusion necessary. Responds promptly to symptomatic treatment. Prophylactic medications indicated for less than or equal to 24 hours.
    - Grade 3 - Prolonged, or not rapidly responsive to symptomatic treatment and/or interruption of infusion. Recurrence of symptoms following initial improvement.
    - Grade 4 - Life-threatening consequences. Urgent intervention indicated.
- A subject develops anaphylaxis in association with UFH, defined as follows using the CTCAE grading (all anaphylactic events are Grade 3 or higher).
    - Grade 3 - Symptomatic bronchospasm with or without urticaria. Allergy-related edema and/or angioedema. Hypotension. Parenteral intervention needed.
    - Grade 4 - Life-threatening consequences. Urgent intervention indicated.

Such events will be handled as serious adverse events (SAEs) and reported in an expedited time frame to the IRB. The data concerning the event will be reviewed by the DSMC along with all other available data to determine appropriate follow-up, with a decision to continue enrollment and treatment of subjects at that time. Additionally, the following may also stop further subject enrollment and treatment.

- The DSMC warrants temporary suspension of enrollment for further review of data generated to date.
- The investigator determines that a medically important event warrants further evaluation by the DSMC.

A report summarizing the above DSMP activities will be submitted to the IRB at the time of annual renewal.

4.6 Adverse Event Reporting

All adverse events (AE) experienced by study subjects are to be recorded on the CRF, regardless of the severity of the event or its relationship to study treatment. The AE reporting procedures are based on the CTCAE system, volume 4.03, 2010. Subjects will report any symptoms to the investigator, research staff, inpatient nursing staff, or treating physician. Dr. Seaman will determine if symptoms qualify as an AE or SAE with confirmation by Dr. Ragni. AEs will be classified as mild (does not interfere with routine activities), moderate (interferes somewhat with routine activities), or severe (impossible to perform routine activities). The following algorithm will be used to assess the causality of all AEs:

- **Not related:** The event can readily be explained by factors not involving UFH, and a temporal relationship with UFH does not exist.
- **Possibly related:** A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of UFH but which could also be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal may be lacking or unclear.
- **Probably related:** The temporal relationship between the administration of UFH is compelling, and the event cannot be explained by the subject's medical condition or other therapies.
- **Related:** The event follows a reasonable temporal sequence from the administration of UFH, follows a known or suspected response pattern to UFH, is confirmed by improvement upon stopping UFH, and reappears upon repeated exposure to UFH.

The investigator will follow up all AEs, regardless of severity, until satisfactory resolution. All subjects experiencing AEs will be monitored until symptoms subside and any abnormal laboratory values have returned to baseline, or until there is a satisfactory explanation for the changes observed, or until death, in which case a full pathologist's report will be supplied, if possible. Withdrawal from the clinical study and therapeutic measures shall be at the discretion of the investigator. As required by the IRB, if there is a serious, unexpected or adverse event that is determined to be associated with UFH, it will be reported to the IRB within 24 hours. If the event is not serious, unexpected or related to UFH, it will be reported within 5 days. All events must be assessed to determine the following:

- If the event meets the criteria for an SAE.
- The relationship of the event to study treatment.
- The severity of the event.

An AE is any untoward medical occurrence in a subject in whom a pharmaceutical product is administered but does not necessarily have a causal relationship with the pharmaceutical product. 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 pharmaceutical product, whether or not related to the pharmaceutical product.[27] A SAE is any untoward medical occurrence that at any dose:

- Results in death.
- In the view of the investigator, places the subject at immediate risk of death.
- Requires inpatient hospitalization or prolongation of existing hospitalization.
- Results in persistent or significant disability/incapacity.
- Results in a congenital anomaly/birth defect.

An SAE may also be any other medically important event that, in the opinion of the investigator, may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above.

5.0 Costs and Payments

5.1 Research Study Costs

The subject will incur no costs for study participation. The Vascular Medicine Institute (VMI) of UPMC will provide compensation for research related costs as outlined in the budget proposal.

5.2 Research Study Payments

Subjects will receive compensation in the amount of $100.00 following randomization and at the completion of the study, using the WePay system, for a total compensation of $200.00.

6.0 Appendices

6.1 References

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Table 1. Schedule of Events

Schedule of Events[1]	Study Day						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Demographic information[2]	X						
Medical information[3]	X						
Body temperature and pulse oximetry	X	X	X	X	X	X	X
Visual analog scale for pain score	X	X	X	X	X	X	X
Complete blood count	X	X	X	X	X	X	X
Anti-Xa UFH level	X	X	X	X	X	X	X
Clinical data (standard of care[4])	X	X	X	X	X	X	X
Primary and secondary outcomes[5]	X	X	X	X	X	X	X
Adverse events	X	X	X	X	X	X	X
Adherence	X	X	X	X	X	X	X

[1]All data will be obtained by a member of the research staff.

[2]Demographics include age, race, and sex.

[3]Medical information includes number of previous episodes of ACS, current administration of outpatient hydroxyurea, prior DVT or PE, history of myocardial infarction, chronic lung disease, previous stroke, and current or former smoker.

[4]SOC data includes whether or not intravenous fluids were given; whether or not antibiotics were administered, number of antibiotics received, and class of antibiotics given (penicillin, cephalosporin, macrolide, fluoroquinolone, vancomycin, other); whether or not supplemental oxygen was received with documentation of need for noninvasive positive pressure ventilation or mechanical ventilation; whether or not pain medication was provided, including type of pain medication (opioid, NSAIDs, acetaminophen, other); number of red blood cell transfusions given; and whether or not exchange transfusion was necessary.

[5]Primary clinical outcome is duration of hospitalization. Secondary clinical outcomes include duration of hypoxemia, fever, leukocytosis, and moderate to severe pain; total dose of opioids; number of red blood cell transfusions administered; rate of transfer to the ICU; requirement for mechanical ventilation; and development of MODS.