



Aneurysmal Subarachnoid Hemorrhage Trial RandOmizing Heparin:

Continuous Low-dose Intravenous Heparin Therapy in Coiled Low-grade Aneurysmal
Subarachnoid Hemorrhage Patients with Significant Hemorrhage Burden

Study Protocol

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STATEMENT OF COMPLIANCE

The study will be conducted in accordance with the International Conference on Harmonisation guidelines for Good Clinical Practice (ICH E6), the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46). All personnel involved in the conduct of this study have completed human subjects protection training.

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PROTOCOL SIGNATURE PAGE

I have read this protocol and agree to adhere to the requirements. I will provide copies of this protocol and all pertinent information to the study personnel under my supervision. I will discuss this material with them and ensure they are fully informed regarding the investigational plan and the conduct of the study according to 21 CFR parts 50, 54, 56 and 812, ICH Good Clinical Practices Guidelines and Institutional Review Board (IRB) requirements. The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Clinical Site

Site Principal Investigator Signature

Date

Site Principal Investigator Printed Name

1. STUDY SYNOPSIS AND OBJECTIVES

1.1 STUDY SYNOPSIS

STUDY TITLE	<u>A</u>neurysmal <u>S</u>ubarachnoid hemorrhage <u>T</u>rial <u>R</u>and<u>O</u>mizing <u>H</u>eparin (ASTROH)
PRIMARY OBJECTIVES	The primary objective of this study is to investigate the safety and clinical effect of a continuous low-dose intravenous unfractionated heparin (LDIVH) infusion for the prevention of aneurysmal subarachnoid hemorrhage (aSAH) induced neurocognitive dysfunction. The rate of “Major Bleeding” or “Clinically Relevant Non-Major Bleeding” as defined by the International Society of Thrombosis and Haemostasis (ISTH) between the LDIVH and the control group will be compared. The mean Montreal Cognitive Assessment (MoCA) scores obtained at a 90-day follow-up visit will be compared between LDIVH treated patients and controls. We expect the results of this study to inform on whether a properly powered Phase III study evaluating a LDIVH infusion for aSAH is warranted.
SECONDARY OBJECTIVES	Increased blood and CSF levels of certain inflammatory biomarkers have been correlated to aSAH patients with poor clinical outcomes. Unfractionated heparin (UFH) has known anti-inflammatory actions. As a result, a secondary objective of this study will be to evaluate whether LDIVH can reduce blood and CSF inflammatory biomarker levels compared to controls and whether there is any association between inflammatory biomarker levels and cognitive outcomes in aSAH.
STUDY DESIGN	Investigator initiated and sponsored, multicenter, prospective, 1:1 randomized, open-label, blinded adjudication phase II study. Study patients randomized to the LDIVH arm will receive normal standard of care (SOC) plus a continuous low-dose intravenous infusion of UFH for up to 14 days. During the 14-day infusion, the LDIVH arm study subjects will not receive any chemical deep venous thrombosis

	<p>(DVT) prophylaxis but may resume chemical DVT prophylaxis after the 14-day infusion is complete. Study patients randomized to the control arm will receive the normal standard of care (SOC), which will be UFH 5,000 units, twice daily.</p>
STUDY POPULATION	<p>Patients with a supratentorial aSAH secondary to a ruptured cerebral aneurysm that has been secured by endovascular coil embolization. Study subjects must score as a WFNS SAH grade ≤ 2 post-coiling with no significant aphasia at the time of enrollment and have a significant hemorrhage burden on the initial head computed tomographic (CT) scan that demonstrates a modified-Fisher grade 3, or grade 4 (with minimal intraparenchymal blood or IVH less than 25%.)</p>
CLINICAL SITES	<p>Eight to ten designated clinical sites in the USA.</p>
PLANNED ENROLLMENT	<p>88 patients (44 in each treatment allocation group; Approximately 5-6 patients per site per year average)</p>
STUDY DURATION	<p>3 years [2 years enrollment; 3 months primary end-point follow-up visit; 3 months data analysis and reporting of results; 1-year follow-up visit secondary endpoint].</p>
PRIMARY SAFETY ENDPOINT	<p>Rate of “Major Bleeding” or “Clinically Relevant Non-Major Bleeding” as defined by the International Society of Thrombosis and Haemostasis (ISTH) between the groups from enrollment until discharge from the acute inpatient setting.</p>
SECONDARY SAFETY ENDPOINT	<p>Rate of Symptomatic Intracranial Hemorrhage between the two groups from enrollment until discharge from the acute inpatient setting. A symptomatic hemorrhage is defined as a 4-point or greater increase in the NIHSS or a decrease in the GCS by 2 or more points not explained by other causes.</p>
ADDITIONAL PRESPECIFIED SAFETY OUTCOMES TO BE ANALYZED	<p>Rate of any new intracranial hemorrhage between the two groups from enrollment until discharge from the acute inpatient setting.</p> <p>Rate of Serious Adverse Events (SAEs) between the two groups from enrollment until</p>

the 90-day follow-up visit as reported by each site and adjudicated by an independent Clinical Events Adjudication Committee (CEC).

Rate of Type II Heparin Induced Thrombocytopenia (HIT) between the two groups from enrollment until the 90-day follow-up visit

Rate of DVT or PE between the two groups from enrollment until the 90-day follow-up visit

All cause and neurological mortality rates between the two groups from enrollment until the 90-day follow-up visit

PRIMARY CLINICAL EFFECT ENDPOINT

Mean Montreal Cognitive Assessment score (MoCA) at 90 (± 10) days post enrollment.

SECONDARY ENDPOINTS:

-PRIMARY BIOMARKER ENDPOINT

Peak and Overall Plasma and CSF levels of hsCRP at enrollment and, infusion days #2, 4, 6, and 10 compared between groups.

-SECONDARY CLINICAL EFFECT ENDPOINTS

Trail Making Test Parts A&B at 90 (± 10) days post enrollment.

MoCA at 1-year (± 30 days)

Center for Epidemiologic Studies Depression Scale (CES-D) at 90 (± 10) days post enrollment

Return to work status between groups at 90 (± 10) days

SECONDARY EXPLORATORY PRESPECIFIED BIOMARKER OUTCOMES

Plasma and CSF levels of IL-6, TNF- α , P-Selectin, sICAM-1, HMGB-1, MRP8/14 plasma levels at enrollment and infusion day #2, 4, 6, and 10

ADDITIONAL PRE-SPECIFIED

Rate of MoCA ≤ 20 between groups at the 90-day follow-up visit.

**EXPLORATORY
OUTCOME ANALYSES**

In-hospital incidence of any fever ($> 38.3^{\circ} \text{C}$; $\geq 101.0^{\circ} \text{F}$), Incidence of multiple fever episodes (≥ 3), and Mean Daily Fever Burden.

GCS and NIHSS at enrollment and infusion days # 2,4,6,10, discharge and 90 (± 10) days

MoCA at post-enrollment day #1, 6 and 10

Correlation analysis between MoCA scores and inflammatory biomarker levels with and without adjusting for hemorrhage burden (Hijdra Sum Score).

Incidence of moderate and severe Radiographic Cerebral Vasospasm based on a CTA, MRA, or Catheter DSA obtained between PBD # 6-12 as determined by an independent and blinded core imaging lab.

Incidence of TCD criteria for significant vasospasm (velocities $>200 \text{ cm/s}$ and / or MCA / ICA (Lindgaard) ratio >6)

Incidence of TCD criteria for absence of vasospasm (velocities $<120 \text{ cm/s}$)

Incidence of clinical vasospasm requiring rescue therapy (eg, pressors, endovascular treatment)

Incidence of "Delayed Cerebral Ischemia" defined as:

- Symptoms of neurological deterioration (e.g. hemiparesis, aphasia, decreased level of consciousness)
- Symptoms last for more than an hour
- Symptoms are not explained by other physiological processes (e.g., hydrocephalus, hypotension)

CT or MRI evidence of Vasospasm Related Cerebral Infarction on post-infusion (discharge) imaging as scored by an independent and blinded core imaging lab

Global outcome as measured between group ordinal regression analysis of the modified Rankin Scale score at 90-days and 1-year.
Return to work status between groups at 1-year.
Lawton IADLs at 90 (± 10) days
QOLIBRI-OS at 90 (± 10) days and 1-year.
Checklist Individual Strength-subscale Fatigue (CIS-f) at 90 (± 10) days
Barthel Index at 90 (± 10) days and 1-year

**INVESTIGATIONAL
STUDY DRUG NAME
AND FORMULATION**

Unfractionated Heparin (UFH) intravenous solution at a concentration of 100 units per ml (25,000 to 50,000 units of heparin in 250 to 500 ml of 0.9% or 0.45% NaCl solution)

INTENDED USE

A prophylactic infusion of LDIVH for up to 14 days with prescribed escalated dosing until the goal aPTT value is achieved. The LDIVH infusion is intended to prevent cognitive deficits in modified-Fisher grade 3 and 4 aneurysmal subarachnoid hemorrhage survivors following endovascular coil embolization of the ruptured cerebral aneurysm. The LDIVH infusion is to be initiated 12 ± 4 hours after coiling of the aneurysm. The infusion will be started at 8 units/kg/hr (0.08 cc/kg/hr) and increased by 2 units/kg/hr every 12 hours until the goal aPTT is reached (typically 12 u/kg/hr) *Note: The goal aPTT is an institution specific aPTT value that correlates with an anti-Xa assay value of 0.1 – 0.3 IU/ml of heparin.*

STUDY SUCCESS

For the purposes of this trial, study success is defined as demonstrating a statistically significant improvement in the mean MoCA score for the LDIVH arm compared to the control arm of the study at the 90-day follow-up visit or a significant reduction in inflammatory biomarker levels in the LDIVH arm compared to the control arm. In addition, there can be no significant differences between groups in the rate of Major Bleeding or Clinically Relevant Non-Major Bleeding attributable to the LDIVH treatment.

PATIENT COMPLETION

Patient completion in the study occurs when the patient has completed his/her 1-year follow-up

visit and all case report forms (CRFs) and Imaging required by the Study Protocol have been completed OR the patient has withdrawn from the study or has been determined to be “Lost to Follow-up” and all available CRFs and Imaging have been collected and submitted to the University of Louisville. The primary endpoint is the 90-day follow-up visit and therefore the primary data-analysis will occur after this date.

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DATABASE

REDCap
<https://neurosurgery-research.redcap.louisville.edu>

<https://astroh.redcap.louisville.edu>

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1.2 STUDY OBJECTIVES

1.2.1 Primary Objective

1. Demonstrate the safety of a low-dose intravenous infusion of unfractionated heparin (LDIVH) in aSAH patients treated with coil embolization.
2. Demonstrate a positive clinical effect of LDIVH in aSAH patients through measurement of significantly better mean Montreal Cognitive Assessment (MoCA) scores in the LDIVH treated subjects at a 90-day follow-up visit compared to control patients.

1.2.2 Secondary Objectives

1. Measure the correlation between blood / CSF levels of inflammatory biomarkers (e.g. hsCRP, IL-6) and cognitive function (MoCA).
2. Demonstrate a reduction in blood / CSF levels of inflammatory biomarkers (e.g. hsCRP, IL-6) in the LDIVH treated subjects compared to controls.
3. Demonstrate a reduction in cerebral vasospasm in the LDIVH treated subjects compared to controls by measuring the proportion of patients in each group who:
 - i. Demonstrate moderately severe or severe angiographic vasospasm by CTA or DSA obtained within the prime vasospasm window
 - ii. Demonstrate moderately severe or severe vasospasm on transcranial Dopplers (TCDs)
 - iii. Demonstrate vasospasm related CT infarctions
4. Demonstrate a reduction in “Delayed Cerebral Ischemia” as defined by the Neurocritical Care Society:
 - i. Symptoms of neurological deterioration (e.g., hemiparesis, aphasia, altered consciousness) presumed to be related to ischemia
 - ii. Symptoms persist for more than one hour
 - iii. Symptoms cannot be explained by other physiological abnormalities
5. Demonstrate a reduction in the need for “rescue therapy” for delayed cerebral ischemia and / or symptomatic cerebral vasospasm
6. Demonstrate a difference between the LDIVH treated subjects and controls in the proportion of patients in each group who:
 - i. Have any fever (Temperature $\geq 38.3^{\circ}\text{C}$ / 101.0°F)
 - ii. Have multiple fevers (≥ 3 fever episodes)
7. Demonstrate a significant difference in “fever burden” between LDIVH treated subjects and control subjects
8. Demonstrate a difference between LDIVH treated subjects and controls in mean Daily Fever Burden during the LDIVH infusion.

9. Demonstrate better clinical outcomes in the LDIVH treated subjects compared to controls with one of the following measures:
 - i. mRS at 90 days and 1-year (Ordinal Regression Analysis)
 - ii. Barthel Index at 90 days
 - iii. Center for Epidemiologic Studies Depression Scale (CES-D) at 90 days and 1-year
 - iv. QOLIBRI-OS at 90 days and 1-year
 - v. Lawton IADLs at 90 days
 - vi. Trail Making Test Parts A&B at 90 days
 - vii. Return to Work Status at 90 days and 1-year
 - viii. MoCA at 1-year
10. Perform Pre-specified Subgroup Analysis of Outcome measures for LDVIH and Control groups using the following subgroup designations:
 - i. Sex (Male versus Female)
 - ii. Admission CT Hijdra Sum Score (<23 versus ≥ 23)
 - iii. Aneurysm location (ACoA Aneurysm location vs. all others; any location; anterior circulation versus posterior circulation)
 - iv. Infection requiring Antibiotic Treatment versus no infection
 - v. Elevated hsCRP at enrollment vs. normal levels at enrollment
 - vi. WFNS 1 vs. 2
 - vii. Modified Fisher grade 3 and 4 while excluding accidental enrollments with Modified Fisher not equal to 3 or 4
 - viii. Chemical DVT prophylaxis: UFH 5000 units twice daily, or increased dose in obese patients according to SOC
 - ix. LDVIH arm: LDIVH infusion >10 days vs. ≤ 10 days
 - x. LDIVH arm: Initiation of LDIVH within 48 hours of hemorrhage ictus vs. > 48 hours
 - xi. LDIVH arm: Anti-Xa (Infusion Day 6) value < 0.20 IU/mL vs. ≥ 0.20 IU/mL
 - xii. Vasospasm related CT infarction vs. No vasospasm related CT infarction
 - xiii. Tobacco smoking versus no tobacco smoking

2. BACKGROUND

2.1 Rationale

Aneurysmal subarachnoid hemorrhage (aSAH) comprises 5–7% of all strokes of which 30% will die from the initial hemorrhage or re-hemorrhage. Unfortunately, aSAH survivors are at high risk for subsequent severe neurological and neurocognitive complications, collectively referred to as “delayed neurological deficits” (DNDs). There is growing recognition that inflammation, both local (neuroinflammation) and systemic, plays an important role in the pathogenesis of cerebral vasospasm (CV) and DNDs after aSAH. Cognitive dysfunction and memory deficits are observed following many inflammatory states, including infection, traumatic brain injury, normal aging, and Alzheimer’s disease. Similarly, cognitive dysfunction and memory deficits are very

common following aSAH, leading to life-altering psychosocial deficits in patients with otherwise “favorable” outcomes.

The vascular inflammatory response in the central nervous system following aSAH induced vascular injury is multifocal and complex and is implicated in the development of cerebral vasospasm, delayed cerebral ischemia, and cognitive dysfunction. Numerous inflammatory and non-inflammatory biomarkers have been proposed as outcome predictors in aSAH. Some of the strongest evidence relates to elevation of inflammatory biomarkers IL-6 and high sensitivity C-reactive protein (hs-CRP) predicting poor outcome following aSAH. Other biomarkers that may predict poor outcome in aSAH include (but not limited to): HMGB-1, S100 family proteins including MRP-8/14 complex, P-Selectin, ICAM-1, TNF- α , MMP-9, and ICAM-1. Therapies designed to mute the severity of neuroinflammation following aSAH are prime candidates for investigation as they may allow an opportunity to prevent the development of DNDs including commonly occurring and often disabling cognitive dysfunction. In addition to the potential for direct neural injury from inflammation, inflammation can cause secondary effects such as fever. Fever has been associated with worse outcomes in many diseases of cerebral injury including aSAH exclusive of whether there is a concomitant infection. Modulation of the inflammatory response that has the secondary effect to reduce fever burden may also prove to be beneficial to aSAH patients.

Unfractionated heparin (UFH) is clinically utilized almost exclusively for its anticoagulant properties, yet heparin binding can interrupt numerous other biological pathways. Among its wide-ranging effects, heparin complexes with free hemoglobin itself, including oxyhemoglobin, blocks the activity of free radicals (FR) including reactive oxygen species (ROS), antagonizes endothelin-mediated vasoconstriction, binds several cytokines and all chemokines, thereby imparting potent anti-inflammatory effects. It is hypothesized that heparin may exert a therapeutic benefit in SAH patients by acting via one or more of these well-established anti-inflammatory mechanisms.

Recently, experiments utilizing a rat model of SAH, found that continuous intravenous administration of unfractionated heparin was able to downregulate neuroinflammation and prevent neurodegeneration, demyelination, and transynaptic apoptosis.

2.2 Supporting Data in Humans

Recent retrospective human aSAH studies evaluating the Maryland low-dose IV heparin (LDIVH) protocol provide evidence that heparin may reduce the incidence of symptomatic vasospasm requiring rescue therapy, vasospasm related CT infarction, and cognitive dysfunction. In Simard JM et al. (J Neurosurg 119:1611-1619) an analysis of 86 Fisher grade 3 aSAH patients treated with craniotomy and clipping was performed. The patients were enrolled concurrently and 43 LDIVH patients were compared to 43 control patients. The incidence of clinical vasospasm in the control group was 47%, and 9% in the LDIVH group ($p=0.0002$). When evaluating for CT evidence of vasospasm related infarction, there was a 21% incidence of CT infarct in the control group, and 0% incidence of CT infarct in the LDIVH treated group ($p=0.003$).

There were no hemorrhagic complications. Unpublished study data (James RF) presented during a symposium at the 2015 AHA/ASA International Stroke Conference demonstrated a significantly better ($p=0.013$) mean MoCA score of 26.4 in the LDIVH treated group ($n=25$) compared to 22.7 in the control group ($n=22$). [MoCA score range 0-30, normal 26-30]. A 3-point difference in mean MoCA scores is considered clinically relevant. The influence of heparin on MoCA remained significant after controlling for potential confounding factors with multivariate regression analysis. Additional preliminary data demonstrate that the Maryland LDIVH protocol can also mute elevations of the inflammatory biomarker myeloperoxidase (MPO) at 48 hours (Simard JM, unpublished data). MPO has been previously identified to be elevated in serum after aSAH and at even higher levels in those patients with cerebral vasospasm. The Maryland LDIVH protocol has been administered to over 200 patients without any serious adverse safety concerns.

3. STUDY DESIGN AND OVERVIEW

This study is a Phase II, multicenter, prospective, stratified randomization, open-label, blinded adjudication, clinical trial evaluating the feasibility of LDIVH treatment in aSAH. The study personnel at the Imaging Core Lab, Biomarker Lab, and all personnel involved in evaluation of neurological and cognitive outcomes during the 90-day follow-up visit will be blinded to treatment allocation.

Each site will designate a separate clinical research team member(s) who will perform the the 90-day and 1-year follow-up visit clinical research assessments with subjects. These team members must remain blinded and therefore are not allowed to be involved in the day-to-day research procedures of the study prior to the 90-day follow-up visit (cannot have been involved with study subjects during the acute hospitalization period). This will usually require most study sites to have two or more clinical research nurses / coordinators available.

In this trial, 88 modified-Fisher grade 3 and 4 (subset of mFisher 4) aSAH patients with clinical WFNS grade ≤ 2 who have had their ruptured anterior circulation or basilar apex cerebral aneurysm secured by endovascular coil embolization and who remain without a new focal neurological deficit, aphasia or other major complication from their procedure will be eligible for enrollment (See formal inclusion and exclusion criteria Sections 4.1 and 4.2). Enrolled subjects will undergo a stratified randomization procedure (1:1) to one of two treatment arms (LDIVH vs Control). The stratified aspect of the randomization will attempt to evenly distribute sex, and the presence of ruptured anterior communicating artery (ACoA) aneurysms among the treatment arms. This will increase the likelihood of an even distribution of patients with these baseline characteristics within both treatment groups. ACoA aneurysms are implicated in causing greater cognitive dysfunction than other aneurysm location types, likely due to their classic frontal bleed pattern. Only subjects aged 70 years or less are allowed for enrollment, as advanced age can confound cognitive testing.

All subjects will receive mechanical DVT prophylaxis with sequential compression devices and / or TED Stockings per the usual SOC at each site. Compliance with this prophylaxis will be documented. Control subjects should additionally receive chemical DVT prophylaxis (5000 units of heparin subcutaneously twice daily) which will be considered within the range of SOC for each clinical site. LDIVH subjects will not receive chemical DVT prophylaxis while actively receiving the study drug. All LDIVH subjects will receive chemical DVT prophylaxis following cessation of the study drug infusion until discharge or ambulatory per the usual SOC at each clinical site. Subjects will undergo DVT / PE screening / assessment per the usual SOC at their facility, although we recommend screening by weekly lower extremity venous duplex as aSAH patients have been reported to have deep venous thrombosis in up to 20% and this may serve as justification for modifying the local SOC. Other heparin drug(s) and heparin coated catheters and devices will not be utilized in this study except for treatment of an urgent or emergent medical condition requiring heparin (eg. DVT, PE, dissection) or as mentioned above, for chemical DVT prophylaxis.

All patients should be monitored for the development of heparin induced thrombocytopenia with daily CBCs (platelet counts) and per the guidelines of the American Society of Hematology:

https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=4&ved=0CD0QFjADahUKEwjuqvWcil3HhAhWEJR4KHWNbCp4&url=http%3A%2F%2Fwww.hematology.org%2FClinicians%2FGuidelines-Quality%2FQuick-Ref%2F529.aspx&ei=rXK_Va6qNITLeOO2qfAJ&usq=AFQjCNF64VDAzo5VXjZEKiWau t2Xmep7iA&bvm=bv.99261572,d.dmo

Daily CBCs will be considered SOC for this study. Potential HIT cases will be evaluated per recommended protocols and SOC at each site based on the guidelines above. Any case (or suspected case) of type II heparin induced thrombocytopenia (Heparin Induced Thrombocytopenia and Thrombosis - HIT) will be treated by stopping the study drug infusion in LDIVH subjects and otherwise treated per the usual SOC at each clinical site. These events will be documented in the adverse event reporting system.

Routine clinical blood work will be drawn per each site's SOC.

hs-CRP (Blood and CSF), Anti-Xa levels, and study related aPTT values are to be analyzed by the clinical lab at each site but will be paid for by the study. The dose adjustments of the LDIVH patients will not be based on the Anti-Xa levels, these are for research data analysis purposes only. The LDIVH dose adjustments will be based solely on aPTT values.

Blood drawn for additional biomarker analysis will be processed to plasma at each site. Plasma and CSF obtained by study personnel will then be stored in properly marked tubes at -70° Celsius until at least 6 patients have all samples collected. This will be the first batch designated for shipment to the Biomarker Core Lab. The second batch shipped to the Biomarker Core Lab will include all remaining subjects for that clinical site. Specimen batches from each site will be shipped on dry ice to the Core Biomarker

Lab at the University of Louisville where it will again be stored at -70° Celsius until batch analysis is performed after all samples have been received.

Neurological and cognitive evaluations (example: MoCA) will be performed during the acute hospitalization at various time points by trained study personnel. These clinical assessments will commonly occur on the same day as the biomarker blood draw (See Schedule of Events). This correlation will allow an enhanced analysis of a potential clinical treatment effect for LDIVH in relation to inflammatory biomarker level changes over time providing both clinical and laboratory biomarkers of disease.

Cognitive testing with MoCA and other clinical outcome measures are to be performed at the 90-day follow-up visit by separate trained study personnel. These personnel will remain blinded to treatment allocation and will not have been previously directly involved with the study subjects. A final MoCA will be performed at a 1-year follow-up.

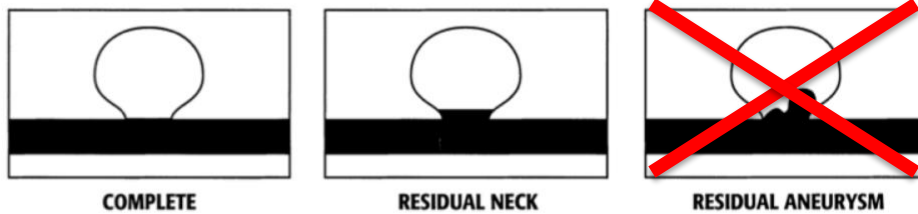
Study enrollment will occur over 24 months or until enrollment goals are met. Data for the clinical trial will be collected via an online, web accessible, HIPAA compliant, secure database (<https://neurosurgery-research.redcap.louisville.edu>; OR <https://astroh.redcap.louisville.edu>) through electronic case report forms (CRFs) [REDCap] during the patient's acute hospitalization for their aSAH as well as during a single 90-day (+/- 10 days) and 1-year study follow-up period following the date of enrollment. Should the REDCap system be non-operational then paper CRFs will be utilized by each site. In this event, a separate binder will be created for each study subject and will be stored in a locked office or cabinet to prevent any HIPAA violations. The study will be complete after all study subjects (i.e., those that have not expired, withdrawn consent to participate in the trial, or been lost to follow-up) have completed their 1-year follow-up visit.

4. SELECTION AND ENROLLMENT OF SUBJECTS

4.1 INCLUSION CRITERIA

1. Patient age is between 18 to 70, inclusive
2. Historical modified Rankin Scale Score 0-1
3. Aneurysmal subarachnoid hemorrhage caused by a ruptured saccular aneurysm confirmed by catheter angiography that is repaired by endovascular coil embolization. Initiation of the coil embolization procedure should occur within 48 hours from the time of the aneurysm rupture (ictus). In patients where the exact time of the ictus is uncertain, a reasonable estimate of the time of ictus may be assigned. This reasonable time estimate should be considered likely accurate to within hours of the true unknown time.

4. Quality of aneurysm embolization is interpreted to be Raymond-Roy Score of 1 (Complete) or 2 (Residual Neck) indicating that the aneurysm is adequately secured. A tiny amount of contrast in the body of the aneurysm is acceptable as long as the physician considers the aneurysm secured and to NOT represent a Raymond-Roy Score of 3 (Residual Aneurysm). Please see diagram below:



5. WFNS grade 1 or 2 as assessed after repair of the aneurysm during screening but prior to randomization. A patient who presents with a WFNS greater than 2 who then improves with resuscitation, ventriculostomy, or time is acceptable.

- The modified GCS (mGCS) is the sum of the best eye-opening response, the best verbal response and the best motor response (best extremity). It is used to determine the WFNS according to the tables below:

Adult modified Glasgow Coma Scale (mGCS)

Test	Responses	Value
Eyes opening Response (1-4)	No Response	1
	to pain	2
	to voice	3
	opens eyes spontaneously	4
Verbal Response (1-5)	No Response	1
	Incomprehensive words	2
	Inappropriate words	3
	Disoriented	4
	Oriented	5
Motor Response (1-6)	No Response	1
	Abnormal extension (decerebrate posturing)	2
	Abnormal flexion (decorticate posturing)	3
	Withdrawal	4
	Localizes	5
	Follows Commands	6

World Federation of Neurological Surgery (WFNS) SAH Grading Scale

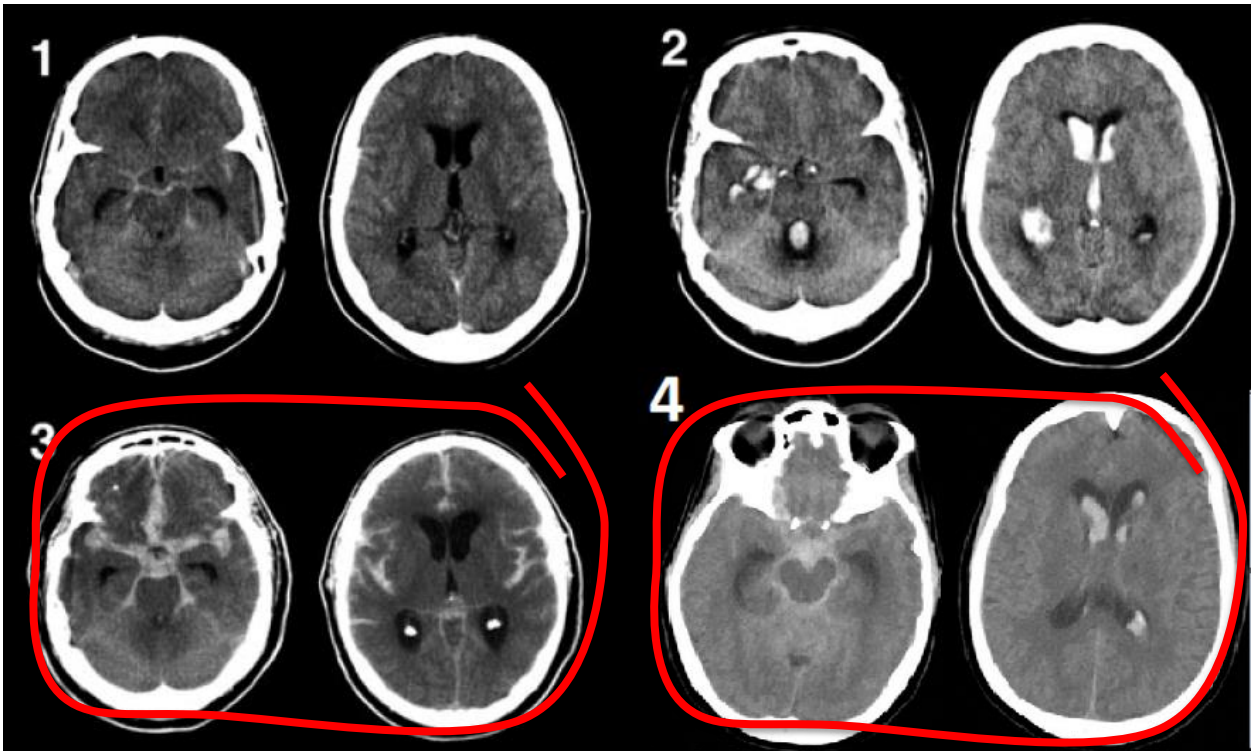
WFNS Grade	mGCS Score	Motor Deficit
1	15	Absent
2	14 - 13	Absent
3	14 - 13	Present
4	12 - 7	Present or Absent
5	6 - 3	Present or Absent

- For patients with endotracheal intubation, we calculate (impute) a mGCS verbal score based on a table comparing the mGCS eye opening response and best motor response scores. In the table below, the green shaded areas represent patients that would continue to meet enrollment criteria based on mGCS score contribution to the WFNS grade (total mGCS of 13-15). This alleviates the challenge of not being able to directly assess the mGCS verbal score due to the patient being intubated during the assessment.

mGCS Verbal Score for Patients that have Endotracheal Intubation

Motor Score (1-6)	Eye Score (1-4)			
	1	2	3	4
1	1	1	1	2
2	1	2	2	2
3	2	2	3	3
4	2	3	3	4
5	3	3	4	4
6	3	4	4	5

- The pre-repair, admission head CT demonstrates an aSAH bleed pattern of “thick and diffuse” or “thick and focal” hemorrhage within the subarachnoid basal cisterns measuring ≥ 4 mm in the short axis and ≥ 20 mm in the long axis which is consistent with a modified Fisher grade 3 or 4. Intraventricular hemorrhage is acceptable. Enrollable patients must **NOT** have a parenchymal hemorrhage greater than 10 cc. Please refer to diagram below for examples. The hemorrhage location should be substantially within the supratentorial space and not isolated to the infratentorial space.



The Modified Fisher CT rating scale: Grade 1 (minimal or diffuse thin SAH without IVH); Grade 2 (minimal or thin SAH with IVH), Grade 3 (thick cisternal clot without IVH), Grade 4 (thick cisternal clot with IVH) [From: Claassen J et al. Effect of cisternal and ventricular blood on risk of delayed cerebral ischemia after subarachnoid hemorrhage: the Fisher scale revisited. *Stroke* 2001;32:2012-2020.]

7. The location of the aneurysm should be the anterior circulation, posterior communicating, **OR** a basilar terminus (apex). Angiographic location of the aneurysm should be confirmed by catheter digital subtraction angiography (DSA) usually obtained during the coil embolization procedure. Patients with PICA or other posterior circulation aneurysms as the cause of the SAH should not be included because they typically cause primarily infratentorial bleed patterns.
8. Ability to screen the patient and obtain a head CT 2-12 hours after the completion of the coiling procedure and the ability to initiate the study drug 12 ± 4 hours after the completion of aneurysm coiling procedure.
9. After recovering from anesthesia following the aneurysm coiling procedure, the patient must remain a WFNS SAH grade ≤ 2 without evidence of a significant new focal neurological deficit including monoparesis / monoplegia, hemiparesis / hemiplegia, or receptive, expressive or global aphasia. New minor cranial nerve defect without any other new findings is permissible. If an NIHSS score was obtained prior to the aneurysm coiling procedure, a post-coiling (pre-enrollment) NIHSS score must not have increased by ≥ 4 points and GCS score must not be decreased by ≤ 2 points. The clinician at the local site should use their best clinical judgment as to whether a significant neurological decline has occurred due to the procedure.

10. Patient is willing and able to return for study follow-up visits.
11. Patient or their Legally Authorized Representative (LAR) has provided written informed consent.

4.2 EXCLUSION CRITERIA

1. Angio-negative SAH.
2. History or imaging suggesting that the current hemorrhage presentation is a recent re-rupture of the aneurysm. Prior sentinel headache with negative CT or prior sentinel headache where the patient did not seek medical attention does not exclude the patient.
3. Surgical Clipping (or plan for clipping) of the ruptured aneurysm or any non-ruptured aneurysm on the same admission.
4. Aneurysm is identified to be traumatic, mycotic, blister or fusiform type by catheter DSA.
5. Any intracranial stent placement or non-coil intra-aneurysmal device where dual-antiplatelet therapy is needed during admission.
6. Patient has additional aneurysm(s) that are untreated and could reasonably be considered a possible alternate cause of the aSAH based on the observed bleeding pattern. Adequate treatment of these aneurysms by coiling embolization would result in the aneurysms no longer causing an exclusion. MRI may be used in some situations to determine that the associated aneurysms did not rupture based on lack of blood seen adjacent to the additional aneurysms.
7. Patient received heparin in any form within the last 100 days prior to current presentation / admission.
8. Thrombocytopenia (platelet count less than 100,000 - assuming clumping has been ruled out as a cause).
9. New intraparenchymal hemorrhage or new infarction larger the 15cc in volume, or significant increased mass effect as seen on the post-coiling, pre-enrollment head CT when compared to baseline admission head CT. New hyperdensity on CT scan related to contrast staining is not an exclusion.

10. Patient has a documented history of heparin induced thrombocytopenia (HIT).
11. Patient developed SAH-induced cardiac stunning prior to enrollment, with an ejection fraction <30%, or requiring IV medications for blood pressure maintenance.
12. Thrombolytic therapy within 24 hours prior to enrollment (rtPA, urokinase, etc.)
13. Plan for antiplatelet or oral anticoagulation therapy from the time of the coil embolization procedure until 14 full days after enrollment. Antiplatelet therapy may be resumed after the 14-day window. A single 325 mg Aspirin (or lower dose) given during the coil embolization peri-procedural period is acceptable if this is the local standard of care but should be documented.
14. Concomitant serious or uncontrolled disease such as severe infection, active (non-remission) cancer, severe organ dysfunction (severe heart failure, severe chronic kidney impairment requiring dialysis or severe chronic liver disease) or any coagulopathy (including DIC or bleeding diathesis).
15. Uncontrollable hypertension (>180 systolic and/or >110 diastolic) that is not correctable prior to enrollment.
16. Prior neurological disease/deficit or psychiatric disease that may continue to alter the results of neuropsychological evaluation, such as dementia, Multiple sclerosis, seizure disorder, severe traumatic brain injury, previous ruptured cerebral aneurysm or active major depression. Childhood seizures that have resolved and no longer require treatment are not part of this exclusion criteria.
17. Active Immunosuppression therapy including chronic corticosteroid usage.
18. History of gastrointestinal hemorrhage or major systemic hemorrhage within 30 days (including large flank or large retroperitoneal hematoma due to current admission coiling procedure requiring treatment), hemoglobin less than 6 g/dL, INR \geq 1.5 after reversal of anticoagulants.
19. Major surgery (including open femoral, aortic, or carotid surgery) within previous 30 days.
20. Currently pregnant.
21. Enrollment in another research study that prescribes a therapeutic treatment that differs from the local standard of care, or that would conflict with this study in some other significant fashion. Registries or coil comparison studies are appropriate.

4.3 Rationale For Selecting The Target Patient Population

Modified-Fisher grade 3 or 4 aSAH patients with good baseline neurological function (WFNS \leq 2) are the ideal patient population for several reasons:

- Focusing on a patient subgroup with homogenous baseline characteristics will favor uncovering a treatment effect with a small sample size
- This specific subgroup is similar to study patients where a similar LDIVH protocol was previously shown to be effective in a retrospective human study.
- These patients are most likely to experience, neuroinflammation, vasospasm and other causes of DNDs due to the high hemorrhage burden and therefore most likely to have the opportunity to show benefit from UFH administration.
- These patients are least likely to have a confounding significant pre-existing neurological injury due to the initial rupturing of the aneurysm prior to initiation of the LDIVH study drug.

4.4 Study Enrollment Procedures

Due to the acute nature of the disease process being studied, there will not be any media advertising for enrollment within this study. Potential participants will be identified by members of the study team as they are a part of the typical patient population for these healthcare providers and clinical sites. Identification and recruitment of study subjects will be done at participating institutions under the supervision of the local site PI and study team. All patients admitted for acute aSAH under the care of study personnel may be identified as potential candidates for screening, including minorities, young adults aged 18-21 years old, women (excluding those who are pregnant), and other underrepresented research populations. The screening, informed consent, randomization and enrollment procedures are detailed below in Section 6.1-6.5. A patient is considered enrolled in this study after informed consent has been obtained **AND** the patient has met all enrollment criteria **AND** has been randomized to a treatment allocation arm.

5. STUDY INTERVENTIONS

5.1 Study Drug

The study drug will be unfractionated heparin intravenous solution with a concentration of 100 units per ml. UFH will be provided and prepared by the investigational / research pharmacy at each clinical site. UFH is a glycosaminoglycan (GAG). GAGs are a physiologically reactive class of acidic, negatively charged, structurally and functionally similar polysaccharides. They are long-chain compounds composed of repeating disaccharide units having a carboxyl group and one or more sulfates, in which one

sugar is N-acetylgalactosamine or N-acetylglucosamine. The endogenous GAGs are heparin, heparan sulfate, keratin sulfate, dermatan sulfate, chondroitin-4-sulfate, chondroitin-6-sulfate and hyaluronic acid.

Metabolism

For UFH, the liver and the reticuloendothelial systems are the sites of biotransformation. Elimination of UFH is thought to be primarily via the reticuloendothelial system, with uptake by the same Hyaluronic acid receptor for endocytosis (HARE receptor) on the sinusoidal epithelium of reticuloendothelial cells that eliminates heparin from the circulation. Renal excretion possibly plays a minor role.

UFH Safety in Humans

UFH has been used as a medical therapy for anticoagulation purposes for decades and its risk profile is well established. LMWHs and heparinoids often cause a temporary class effect related to transient elevation of liver enzymes. This occurs on approximately day 5 - 7 of exposure, with return to baseline values by day 14 after treatment discontinuation. These elevations should be evaluated cautiously as they could confuse diagnosis of myocardial infarction, liver disease, and other disorders.

Osteoporosis has been reported as a class effect for chronic usage of UFH and LMWH. The pathophysiology behind this remains poorly defined.

Bleeding and Heparin Induced Thrombocytopenia are potentially serious side effects that can be related to heparin usage in patients. [Please see sections on HIT and definitions of serious bleeding that can be found later in this protocol].

5.2 Dose Selections And Treatment Duration

This study will plan to infuse the study drug UFH in an escalating fashion with a minimum target rate of 12 u/kg/hr and until a goal aPTT is achieved. The aPTT should be maintained at that level for up to 14 days of study drug infusion. The study drug infusion will begin 12 hours +/- 4 hours after the aneurysm treatment procedure is completed. **The initial infusion rate will be 8 units/kg/hr.** An aPTT will be checked 6 hours later and if a therapeutic level is not achieved the rate will be increased to 10 u/kg/hr 12 hours after study drug initiation. An aPTT level will again be checked 6 hours after the rate increase and the rate will be increased again by 2 u/kg/hr to 12 u/kg/hr, 12 hours after last adjustment until the aPTT goal is reached and then the study drug infusion will be maintained at that level with periodic aPTT levels being checked to confirm that the target aPTT is maintained. Additional upward dose rate adjustments after reaching 12 u/kg/hr (if needed) should be by 1u/kg/hr to avoid overshooting the aPTT goals which could increase the risk of hemorrhage. **The maximum hourly rate of the LDIVH infusion should be 1800 units / hour regardless of patient weight.**

The reason for the dose escalation is to mirror a previously reported protocol (Maryland LDIVH protocol) where LDIVH has been administered safely to over 100 aSAH patients treated with craniotomy and clipping without any hemorrhagic complications. The

divergence from that procedure (8 to 9 to 10 u/kg/hr) is to currently initiate the infusion at 8 units/kg/hr and then increase to 10 u/kg/hr and then again to 12 u/kg/hr. This modification was put into effect and is currently being used at the University of Maryland due to the recent (October 2009) reevaluation of UFH potency by the FDA and the United States Pharmacopeia (USP) that resulted in an approximate 10% reduction in heparin potency within the United States in October 2009 compared to previous years. [<http://www.fda.gov/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/ucm184502.htm>]

The other area of divergence is that the Maryland LDIVH protocol set a specific target infusion rate (10 or 12 units/kg/hr) regardless of the drug's effect on an individual patient. Variability in aPTT test values in these patients highlighted that the rate of metabolism of UFH varies among patients and therefore we expect the clinical effect to likewise vary among patients when a steady rate of infusion is maintained for all patients. **Our current protocol allows for adjustments of the rate of infusion up or down from 12 units/kg/hr to achieve a target aPTT for each patient that is institution specific and correlates with an anti-Xa heparin assay value of 0.1 – 0.3 IU/ml. The highest rate of heparin that consistently allows subjects at an institution to remain within the desired aPTT goal range is preferred such that the upper end of the target range is more desired over the lower end of the target range. Adjusting the rate by less than 1 unit/kg/hr is not allowed.** The anti-Xa values recommended for heparin anticoagulation therapy (for DVT or PE treatment therapy) are between 0.3 to 0.7 IU/ml. Therefore targeting aPTT values that correlate with 0.1 - 0.3 IU/ml anti-Xa values will provide a consistent, low-level, continuous systemic heparin effect while minimizing the risk of hemorrhagic complications. Routine adjusting of the LDIVH drip will be done with aPTT testing and not with Anti-Xa testing.

Maintaining the aPTT in the target range is important to avoid potential hemorrhagic complications while attempting to maximize the potentially beneficial anti-inflammatory effects of the study drug. Hemorrhagic complications could occur secondary to an anticoagulation effect in aSAH patients treated with coil embolization secondary to the femoral arteriotomy access site (i.e., risk of life threatening retroperitoneal hemorrhage) or inadequate embolization of the ruptured cerebral aneurysm, or secondary to ventriculostomy placement. The anticoagulation effect maintained below the anticoagulation therapeutic window should reduce the chance of a hemorrhagic complication in these subjects.

The time of treatment initiation and duration was selected for several reasons. We elected to start the infusion 12 hours following the procedure to minimize any complications related to temporal proximity to the procedure and to allow time for adequate post-procedure neurological assessment following general anesthesia / extubation. This delay in infusion from the procedure time was used in the Maryland LDIVH protocol in post-craniotomy patients. Delaying initiation of the study drug longer may reduce any treatment benefit as the inflammatory process likely begins to establish itself soon after the hemorrhage event and it may be easier to modulate this inflammatory response in earlier phases before the peak intensity of the inflammatory

response is reached. In the majority of aSAH patients who develop cerebral vasospasm and delayed cerebral ischemia, vasospasm begins to significantly improve by the 14th day following the hemorrhage ictus. It is believed that the majority of the delayed neurological deficits that do occur happen within this window of time, therefore a maximum 14 day LDIVH infusion seems to have the maximal opportunity to benefit the study patients. Previous retrospective human studies of UFH using the Maryland LDIVH protocol showed benefit with infusions up to 14 days.

5.3 Study Drug Supply, Accountability And Preparation

The investigational research pharmacy at each clinical site will be responsible for purchasing and mixing the UFH from stock solution or powder to the appropriate concentration (100 units per mL) into an intravenous saline infusion bag. Pharmacies also have the choice to purchase premixed unfractionated heparin from a manufacturer, as long as the concentration is equal to 100 units per mL. Optimal cost-effective management of the study drug would involve mixing a 250 or 500 ml bag that would last approximately 24 hours (UFH in 0.9% or 0.45% saline at 100 units per mL).

The heparin solution, infusion bags, IV tubing, will be supplied by the standard vendors within each participating institution, and tracked per that individual institution's specific policies and procedures. These supplies are needed above and beyond the normal SOC and therefore should not be billed to insurance. The study subject ID number, patient name, and site specific medical record number will be on the study drug label prepared by the investigational pharmacy so that if two subjects are enrolled simultaneously at a clinical site the subjects will always receive the proper study drug.

5.4 Study Drug Blinding Procedure

Physicians, residents, nurses, and clinical staff providing clinical care during the acute hospitalization of the study subjects will be aware of the treatment allocation (unblinded) and as a result will be monitoring for safety concerns related to prolongation of coagulation parameters (aPTT and anti-Xa), thrombocytopenia, bleeding, development of DVT or PE, or other AEs or that might be related to the patient's treatment allocation (LDIVH vs. SOC).

The investigational pharmacy at each site will properly label the study drug infusion bag with all of the information including patient name, site specific medical record number, study site ID#, study patient ID#, and study drug concentration.

The study team members conducting the neurological and cognitive assessments during the 90- day and 1- year follow-up visits will remain blinded to treatment allocation. The patients will be instructed not to reveal their treatment allocation. All imaging will be de-identified and coded in a HIPAA compliant manner with the patient's study ID # and date of scan but without any other information that would allow the core imaging lab to identify the patient identity, treatment allocation or dose. The biostatistician will be aware of two treatment groups but will not be aware of

whether the group was treated with UFH during interim analysis for the DSMB unless the DSMB determines it is necessary to unblind for safety purposes. The final statistical analysis will be performed in an unblinded fashion but rigorous methodology will be utilized. The study team members conducting imaging verification and the biostatistician will not have direct patient contact so accidental disclosure by the study subjects themselves should not occur.

5.5 Treatment Compliance And Drug Accountability

Daily documentation in the patient's eCRFs that attest to the patient receiving the study drug infusion, and the rate of the study drug infusion is required for those randomized to the LDIVH arm. The investigational pharmacy will prepare each study drug infusion bag.

5.6 Treatment Of Study Drug Overdoses

If a study subject has received a study drug overdose, the infusion will be stopped and the patient monitored until it is determined that it is safe to resume the drug. If deemed necessary by the attending study physician labs (aPTT) will be monitored to ensure patient safety and restarted when deemed appropriate. The overdose, relevant labs, and study drug start/stop times will be recorded in RedCap under Protocol Deviations.

6. STUDY PROCEDURES: SCREENING THROUGH DISCHARGE FROM THE ACUTE CARE SETTING

6.1 Screening

Potential study patients will be considered to be in the screening process as soon as research personnel are notified of a potential study candidate. The potential study subjects screened will be patients presenting with a likely diagnosis of aSAH. They will be considered to be in screening until the patient is either randomized and enrolled or excluded from participation in the trial by inclusion/exclusion criteria or lack of informed consent. Screening may begin before or after the aneurysm coiling procedure.

Patients referred to study personnel presenting at participating institutions with a suspected diagnosis of aSAH will be considered potential study subjects. Study personnel will review the eligibility criteria to determine if they are candidates for this trial. Relevant snapshots of the initial head CT scan will be uploaded into Oculus imaging by the site coordinator to be reviewed by the study PI or designee and verify modified fisher grade. An alternative option is to send de-identified snapshots of the CT scan by other electronic means, such as text message.. Please see section 4.1 for detailed information on the Modified-Fisher Score including example images. The WFNS SAH scale scores are to be performed by properly trained study personnel. Please see section 4.1 for detailed information on the WFNS SAH Scale. If the patient qualifies, the patient, and/or the patient's legally authorized representative (LAR) will be approached by a designated study team member to begin the informed consent process

and be given the opportunity to participate. The patient is considered “enrolled” after they have been randomized to a study treatment allocation.

All subjects admitted with the diagnosis of non-traumatic SAH should have basic, de-identified data entered into a screening log at each institution. This will require the study team to interact with the clinical ICU and/or neurosurgical team at least daily to identify these patients (in person or by phone). A log will be maintained and the specific reason(s) for each non-enrolled potential subject who either fails to meet criteria, refuses participation, or deemed by the clinical treating team to ineligible for study due to reasons not pre-specified. These logs will be maintained at each institution and electronically entered to a central database that will maintain a log for all centers involved in the study. These logs will be reviewed on a regular basis by the PPI (RF James) to assess whether sites are meeting recruitment goals and to determine whether there are any systematic reasons for non-enrollment that may bias the study outcomes.

6.2 Informed Consent

The principles of Informed Consent, according to FDA Regulations and the International Conference on Harmonisation (ICH) guidelines on Good Clinical Practice (GCP) will be followed. Investigators and research personnel will complete CITI training (Collaborative Institutional Training Initiative; <https://www.citiprogram.org/>) related to these guidelines as a method to document their familiarity and understanding of these guidelines. CITI certificates for all members of each site’s study team will be forwarded to the clinical coordinating center (University of Louisville). The informed consent form will be approved by the IRB at each site prior to any patient enrollment at that site. All study subjects must have informed consent to participate and only the participant and/or the participant’s legally authorized representative (LAR) may provide consent. The consent document is to be cosigned by the study team member obtaining informed consent at the time of the initial consent process when the patient and/or LAR sign the document. A study investigator may sign the consent document at a later time if they were not the one conducting the initial informed consent process, but this is not required.

Study treatments or procedures will not begin, and data will not be collected for study purposes until consent has been obtained. Informed consent may be obtained before or after the aneurysm coiling procedure. If consent is obtained prior to the aneurysm coiling procedure, the patient will be randomized and enrolled following the procedure assuming they continue to meet the enrollment criteria. Enrollment is not intended to occur at the time that informed consent is obtained but rather ONLY after confirmation that the patient meets enrollment criteria and has been randomized to a treatment allocation. There may therefore be patients who have provided informed consent to participate in this study whom are never actually enrolled in the study. These patients should be informed of this event if it occurs. In the event of screen failure, patients will be assigned a screening ID number and basic, de-identified information will be entered into a separate REDCap database to document the reason for screen failure.

It is intended that the informed consent will be obtained by a member of the research team in as private a place as possible. It is intended that the informed consent document will be reviewed by a member of the research team with the patient and/or a legally authorized representative (LAR). Any questions that the patient or LAR have will be answered to the best ability of the research team member. It is intended that the patient / LAR will be given sufficient time to review the consent document and ask further questions. It is intended that the person obtaining informed consent will ask the patient / LAR questions to assure understanding. If the patient / LAR agrees to participate, the patient / LAR will be asked to sign the consent document. It is intended that a copy of the consent will be given to the patient / LAR and the original, signed Informed Consent Document (ICD) will be kept in the study records. Special care will be taken with the consent process as we are targeting a vulnerable population for our study sample. Patients and LAR will be instructed that they can withdraw from the study at any time for any reason without any change in the standard of care treatment of their medical problem.

For potential study subjects / LARs with limited or low literacy, it is intended that the consent will be read aloud to the patient and explained at the appropriate level. It is intended that questions will be asked by the study team member obtaining informed consent to ensure understanding. Questions from the patient / LAR will be encouraged. Cognitive impairment and decision-making ability will be determined by the research team member obtaining consent based on patient history, history of present illness, medications, presentation, stroke scale, etc. It is intended that identification of the LAR will follow the general statutes in place, as well as policies dictated by the institution. We recognize that if a LAR has been utilized to obtain consent, at a future encounter with the participant the research team will continue to educate the subject regarding the research, and it is intended that the research team will periodically verify the subject's wish to either continue their participation or exit from the study.

Those who do not speak and / or read English will be accommodated with an institutionally approved short-form and a foreign language interpreter. When deemed appropriate, we will seek translation of the informed consent document into other languages. Cultural and social considerations will be made including spending the time necessary for patients and their family to consider participation. Pastoral care services available at the hospital will be utilized when appropriate based on patient and family needs and requests.

The rights, safety, and well-being of trial subjects are the most important considerations and will prevail over the interests of this study, the investigators, science, and society.

6.3 Final Screening Following Informed Consent And Aneurysm Securement

A CT scan of the head obtained between 2-12 hours after completion of the aneurysm embolization procedure is required prior to enrollment in the study. A head CT scan performed at some point after the procedure represents standard of care (SOC)

following aneurysm securement. The CT scan results are to be reviewed by a physician investigator and/or neuroradiologist. If there is an indication on this CT of findings that would exclude the patient from enrollment (see exclusion criteria section 4.2) then the patient will not be enrolled.

If the patient remains eligible as indicated by meeting all enrollment criteria including appropriate findings on their post-procedure CT, and informed consent has been obtained, the patient will be randomized. If the patient has not yet been consented, the informed consent process will begin at this time with the patient and / or LAR. If consent is granted, the patient may be randomized.

6.4 Randomization Procedure

The randomization will be a blocked-stratified randomization scheme. We considered 4 factors for stratification to ensure equal distribution of factors between treatment groups: age, aneurysm location, gender, and clinical site. As age increases above 70 years old the risk for undiagnosed dementia increases and this could have a significant confounding result on our primary efficacy outcome of cognition (MoCA score). As a result we elected to limit enrollment to 70 years old or less to eliminate the need for age stratification. Given we will have 8-10 clinical sites, stratification of randomization by site would be difficult to implement while controlling for other more important factors and therefore we will do post-analysis statistical controlling for clinical site. Finally we elected to stratify with just two factors sex and aneurysm location. Aneurysm location will be a binary stratification of anterior communicating artery (ACoA) aneurysm versus all other locations. This was done because of previous reports that ACoA location SAH has a higher risk of cognitive disability than other locations and our own preliminary human data that suggests the same. Block sizes will fall within the range of 4-10 but will not be disclosed in this protocol to prevent any bias related to patient selection by investigators.

A member of the study team can randomize post-coiling subjects with informed consent who continue to meet the eligibility criteria. Patients will be randomized to one of two treatment allocation (LDIVH or Control) groups. A blocked-stratified randomization table will be created prior to initiation of the study. The REDCap database will utilize this to select treatment allocation. If the randomization feature of the EDC system is non-functional, the subject will be randomized by telephone call to the clinical coordinating center (ASTROH Project Manager). Patients should not be randomized until it is determined the patient meets all eligibility criteria, has undergone the informed consent process, AND it has been determined by the post-procedure head CT scan that the patient is still eligible to participate.

6.5 Enrollment Of Subjects

Patients are considered enrolled in the trial once they are randomized to a study treatment allocation.

6.6 Collection Of Baseline Patient Information

[May occur prior to enrollment, during screening process and may be completed after enrollment for those items not related to eligibility]

During screening, after informed consent has been obtained, or following enrollment baseline information may be collected.

The CRFs for this trial were developed as modifications of the stroke modules provided by the NIH/NINDS common data elements (CDEs) for clinical trials program.

Clinical and historical assessments will include: historical pre-morbid mRS, Assessment for Dementia and pre-morbid employment status. Assessments of NIHSS and MoCA, having occurred immediately prior to enrollment or soon after enrollment.

Baseline biomarker specimens will be obtained after enrollment but prior to initiation of study drug or within 6 hours of initiating study drug.

6.7 Baseline Blood And Biomarker Specimen Collection

Please draw all normal blood tests as you would for your normal standard of care for the treatment of an aneurysmal subarachnoid hemorrhage. hsCRP and Anti-Xa levels will be drawn for all subjects after enrollment but prior to initiation of the study drug (for those subjects in the LDIVH treatment group).

Additional blood will be drawn and processed to plasma and stored for additional biomarker analysis. This may be performed up to 6 hours after initiation of study drug for the LDIVH arm (but preferably prior to study drug initiation). Please see the section below on the collection and processing of plasma biomarker specimens Section 6.11

If the subject already has an external ventricular drain or a lumbar drain placed, then removal of cerebrospinal fluid should be performed again with processing of the specimens for biomarker analysis. This may be performed up to 6 hours after initiation of study drug for LDIVH arm (but preferably prior to study drug initiation). Please see the section below on the collection and processing of CSF biomarker specimens Section 6.11.

6.8 Initiation Of Study Drug, Standard Dose Escalation, And Duration Of Infusion

Once the patient has been randomized, if the patient has been randomized to the LDIVH treatment arm then the study drug will be initiated 12 ± 4 hours following the completion of the aneurysm treatment procedure. The LDIVH infusion will initiate at 8 u/kg/hr. The date and time of study drug initiation will be recorded in the CRF. The control group will not receive any study infusions.

The LDIVH infusion (LDIVH group only) will be initiated at 8 u/kg/hr. This rate of heparin infusion should typically be escalated twice to reach the standard goal infusion rate of 12 u/kg/hr. There should be 12 hours between each dose adjustment. This escalation should result in an increase of the rate by 2u/kg/hr to a minimum target of 12u/kg/hr. A follow-up aPTT should be checked 6 hours after dose initiation and each dose adjustment. The goal rate for the LDIVH group should be **at least** 12 u/kg/hr, however if the aPTT is still below the lower limits of the acceptable range, then 12 hours after the last dose escalation, the rate can be again increased by 1u/kg/hr beyond the minimum of 12 u/kg/hr. (Max infusion rate of 1800 units/hr). Again, LDIVH target is at least 12 u/kg/hr while maintaining an aPTT value within the range that correlates with an Anti-Xa value of 0.1-0.3 u/ml.

Example: Site A initiates LDIVH on Patient X weighing 80kg. Target aPTT for Site A is 40-60 seconds.

LDIVH gtt is initiated at 8 u/kg/hr (640 u/hr) and an aPTT is obtained 6 hours later. aPTT result 6 hours later is 42 seconds. (Even though aPTT was 42, you will always try to get to a minimum of 12 u/kg/hr minimum as long as aPTT is not above the upper goal [60 in this example])

Dose escalation needed.

12 hours after LDIVH initiation, Patient X will have the LDIVH rate increased to 10u/kg/hr (800 u/hr).

aPTT result 6 hours later is 38 seconds

Dose escalation needed.

LDIVH gtt is escalated 12 hours after last dose adjustment (approximately 6 hours after most recent aPTT value) to 12 u/kg/hr (960 u/hr).

aPTT result 6 hours later is 39 seconds

Dose escalation needed.

LDIVH gtt is escalated 12 hours after last escalation (6 hours after aPTT value) to 13u/kg/hr (1040 units/hr)

aPTT result 6 hours later is 42 seconds

No further dose adjustments needed for this patient at this time.

Routine aPTT checked 24 hours later and result is now 36 seconds and Patient X remains at 13u/kg/hr

Dose escalation needed.

LDIVH gtt rate is escalated to 14 u/kg/hr (1200 units/hr)

aPTT 6 hours later is 45 seconds

No further dose adjustments needed for this patient at this time.

3-days have passed. The Routine aPTT checked every 24 hours is now 65 seconds and Patient X remains at 14 u/kg/hr.

Blood should be redrawn (paying close attention to technique) and a new aPTT should be obtained to confirm the aPTT is too high. If the aPTT recheck is again too high, the LDIVH dose rate must be decreased by 1-2 u/kg/hr depending on the aPTT value.
Dose decrease needed.

LDIVH gtt rate is decreased to 13 u/kg/hr (1040 units / hr)
aPTT 6 hours later is 58 seconds
No further dose adjustments needed for this patient at this time.

The duration of the LDIVH drug infusion will be 14 total days unless the patient is discharged from the acute care setting prior to the ability to complete the 14 days. It is not the intent of this protocol to extend the hospital stay in order to complete a full 14-day infusion. The LDIVH infusion can be given in the ICU setting or the non-ICU setting. The LDIVH infusion should not delay the transfer of the patient out of the ICU setting.

6.9 Study Drug Infusion Safety Monitoring By Investigators

To reduce the risk of bleeding complications in the LDIVH arm of the study, close monitoring of aPTT laboratory values for the LDIVH arm will be done. The aPTT laboratory values for subjects in the LDIVH arm will be collected at regular intervals to monitor anticoagulation effects and adjust the infusion as necessary. aPTT testing will be performed prior to enrollment on all study subjects. aPTT testing will also be done 6 hours after each upwards dose adjustment and at least once daily for the duration of the study drug infusion for patients allocated to the LDIVH treatment group. aPTT testing will occur on control subjects on Infusion day #6 drawn at the same time as the Anti-Xa test listed below.

Anti-Xa testing will occur on patients allocated to both the LDIVH group and the control group at enrollment prior to initiation of the study drug and again on infusion day #6 to help correlate aPTT data between sites. An aPTT value correlating with an anti-Xa value of 0.1 – 0.3 IU/mL is considered within the appropriate target range.

For most hospitals, a CBC (complete blood count) is performed daily for aSAH during admission and the platelet count is inclusive of this test. At a minimum, platelet count will be monitored at least daily for all patients enrolled in the study. We do expect a few cases of type II heparin induced thrombocytopenia within the LDIVH group and/or control group based on the reported incidence of HIT being approximately 0.3-5%. Both the LDIVH group and the control group are at risk for this complication, though we expect the risk to be slightly higher in the LDIVH group. ***We recommend providing a diligent surveillance for this potentially dangerous complication known to be associated with heparin, heparinoid infusions and heparinoid chemical DVT prophylaxis.***

6.10 Study Drug Infusion Rate Adjustments And Emergent Stopping Of Study Drug Infusion

6.10.1 aPTT too high

If the aPTT is found to be higher than the goal target range for patients in the LDIVH study arm and there are no immediate safety concerns related to bleeding or the patient's hemodynamics, then the aPTT value should be redrawn and double-checked. It will be important to have the staff obtaining the blood waste 10 cc prior to collection if obtaining blood from the same IV line as the LDIVH study drug or from a direct puncture of a vein proximal to the IV site for the LDIVH infusion. If the aPTT value is still found to be elevated and out of the acceptable range for patients in the LDIVH arm, the UFH infusion should be decreased by 1 unit/kg/hr lower than the previous dose rate and a follow-up aPTT level should be checked 6 hours later. If this level is again proven elevated above the acceptable amount the UFH infusion should be again decreased by 1 u/kg/hr less than the previous dose and another follow-up aPTT level should be again checked 6 hours later. This process should be repeated until the follow-up aPTT level is within the acceptable range possibly requiring additional LDIVH dose rate adjustments. If the aPTT is at an extremely high, dangerous level (with or without confirmation) then the LDIVH infusion should be stopped immediately (though temporarily) until the aPTT is no longer at an extremely high, dangerous level. We will allow each site investigator to determine what is considered an extremely high, dangerous level for aPTT at their site.

6.10.2 Allergic Reaction

A documented hypersensitivity reaction or high suspicion for hypersensitivity reaction to the LDIVH infusion would necessitate ceasing the study drug infusion temporarily. If the symptoms resolve and high suspicion remains for a serious allergic reaction to the UFH, then the infusion should NOT resume. However, if the symptoms resolve and are discovered to be from an alternate cause and not related to the UFH, then the UFH infusion can be resumed, cautiously, with emergent treatments for a severe allergic response available should they be needed.

6.10.3 Bleeding Complications

Bleeding in all patients should be taken seriously and for those patients in the LDIVH treatment arm we recommend following the 2011 Clinical Practice Guide on Anticoagulant Dosing and Management of Anticoagulant-Associated Bleeding Complications in Adults; Presented by the American Society of Hematology, adapted in part from the: American College of Chest Physicians Evidence-Based Clinical Practice Guideline on Antithrombotic and Thrombolytic Therapy (8th Edition).

<https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&ved=0CB8QFjAA&url=http%3A%2F%2Fwww.hematology.org%2FClinicians%2FGuidelines-Quality%2FQuick-Ref%2F525.aspx&ei=yE2PVYj1M8Ks->

[QGYyYHgDw&usg=AFQjCNHVwPINavcTi7zKKG74RuDEnxmHyqQ&bvm=bv.96783405,d.cWw](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6111111/)

The HASHTI principles of management of anticoagulant-associated bleeding are ideal and should be followed as the clinical scenario necessitates. Details on the HASHTI principles are included in the reference above.

HASHTI

1. **H**old further doses of anticoagulant
2. Consider **A**ntidote
3. **S**upportive treatment: volume resuscitation, inotropes as needed
4. Local or Surgical **H**emostatic measures: topical agents (aminocaproic acid, tranexamic acid)
5. **T**ransfusion (red cells, platelets, FFP as indicated)
6. **I**nvestigate for bleeding source

Patients who have Major Bleeding or Clinically Relevant Non-Major Bleeding and who are allocated to the LDIVH treatment arm should have the study drug infusion stopped immediately, an aPTT level checked, and consideration of administration of protamine sulfate if there is a life-threatening bleeding event. The UFH infusion can resume after the bleeding has been controlled and any aberrant coagulation parameters have been corrected based on the judgment of the local investigator (attending physician). The continuation of the LDIVH infusion should be delayed at least 12 hours after a major bleeding event and at least 6 hours after a moderate event. These are minimum restart times and the actual duration of time to wait after any bleeding event is at the discretion of the treating physician.

6.10.4 Reversal of Heparin Infusion with Protamine sulfate

Reversal of heparin by protamine sulfate is usually dosed at 1 mg of protamine sulfate for every 100 units of heparin that needs reversal. A simple method is to calculate the total amount of heparin given over the last two to three hours and divide by 100 to get the dose of protamine sulfate necessary to reverse the majority of the heparin effect. Example: If the patient receives 1200 units of heparin per hour then the total amount of heparin over two to three hours would be 2400 to 3600 units of heparin needing reversal. Reversal of the heparin effect would require approximately 24-36 mg of protamine sulfate. The ultimate decision on whether to reverse heparin with protamine sulfate and the dose chosen will be up to the clinicians at each clinical site based on the individual clinical scenario.

Medical professionals at each clinical site will have authority to emergently stop the study drug infusion if they feel there is an urgent or emergent clinical development related to the study drug such as a drug related SAE.

6.10.5 Heparin Induced Thrombocytopenia (HIT)

If HIT is suspected all heparin medications should be stopped immediately. The management of HIT or suspected HIT is covered in **Section 8.4**.

6.11 Inflammatory Biomarker Collection and Processing

hs-CRP blood levels will be drawn and processed by each site's clinical laboratory and the results of these tests will be documented in the database CRFs for each time point which will include time of enrollment, and post-enrollment days #2, 4, 6, and 10. As such, these values will be immediately available to the DSMB and Steering Committee during the interim analysis.

hs-CRP levels will be measured in the CSF as well. CSF will be sent to the local institution's lab for analysis of hs-CRP in the appropriate tube per the lab's standard procedure.

Additional blood and CSF (when a drain is present) will be drawn at enrollment and post-enrollment days # 2, 4, 6 and 10. The blood and CSF will be processed and stored locally and then shipped to the biomarker core lab for further planned biomarker analysis to be processed in a batch fashion after all samples have been collected. Currently the planned biomarkers to be analyzed include: IL-6, TNF- α , P-Selectin, ICAM-1, HMGB-1, and MRP 8/14.

Biomarker processing kits will be mailed to each site that will include instructions, transfer pipettes, shipping packaging, specimen box, pre-filled labels, and other supplies necessary to process blood to plasma and store them in the -70°C freezer. Each site will need to supply venipuncture supplies (needles, vacutainer tubes, or butterfly needles). Each site will also need to supply their own centrifuge, ice bucket, dry ice, and -70°C freezer space.

Collection of Biomarker Blood Specimens and Processing to Plasma:

1. **Collect 9-10 ml of venous blood** using a lavender top Vacutainer (EDTA). Mix according to standard guidelines. If collecting blood from an IV, PICC, or other line, make sure there are no medications hooked up to the line (such as heparin), if there are, temporarily turn off these infusions and waste at least 5 cc of blood prior to collecting the specimen. Turn infusions back on at the same rate following the blood draw.
2. **Within 30 minutes of the blood draw, centrifuge the tube for 10 min at 500g** (approx. 2,000 rpm in a standard centrifuge). If samples cannot be processed within 30 minutes, the tubes should be kept on ice until they can be centrifuged (*no more than 2 hours delay*). **Do NOT freeze the whole blood samples!** Heavily hemolysed or lipemic samples are to be avoided.
3. **Aspirate and aliquot the plasma.** Using a transfer pipette, transfer approximately 0.9-1.0 ml of plasma (supernatant) into each of five properly-

labeled cryovials. Avoid any contamination with RBCs or WBCs at the bottom of the centrifuge tube. Cryovial tube should be labeled with the subject's study ID#, study timepoint (example: baseline, post-enrollment day #, etc), date, and time of the blood draw.

4. **Freeze the plasma aliquots.** Place cryovials in appropriate freezer boxes. A -70°C freezer is preferred for long-term storage. If not immediately available, a regular -20°C can be used for short storage periods (< 1 week) before transferring the samples to a -70°C freezer. Make sure the Freezer boxes are properly labeled. Attempt to keep cryovials for the same subject located together in the same freezer box when possible.

Collection of Biomarker CSF specimens and Processing:

1. **Collect 9-10 ml of cerebrospinal fluid** using standard sterile technique in a plain red-top vacutainer tube.
2. **Within 30 minutes centrifuge the CSF for 10 min at 500g** (approx. 2,000 rpm in a standard centrifuge). If tubes cannot be centrifuged within 30 minutes the tubes should be kept on ice until they can be centrifuged (no more than 2 hours delay). **Do NOT freeze uncentrifuged CSF samples!**
3. **Aspirate and aliquot the CSF.** Using a transfer pipette, transfer approximately 0.9-1.0 ml of CSF supernatant into each of five properly-labeled cryovials. Avoid any contamination with cells at the bottom of the centrifuge tube. Cryovial tube should be labeled with the subject's study ID#, study timepoint (see above), date, and time of the CSF collection.
4. **Freeze the CSF.** Place cryovials in appropriate freezer boxes. A -70°C freezer is preferred for long-term storage. If not immediately available, a regular -20°C can be used for short storage periods (< 1 week) before transferring the samples to a -70°C freezer. Make sure the Freezer boxes are properly labeled. Attempt to keep cryovials for the same subject located together in the same freezer box when possible.

We plan for samples to be mailed from each site in two batches to the Core Biomarker Laboratory. Commonly one shipment will occur after 6 patients have been enrolled and one shipment will occur after the last patient at the site has completed the inpatient phase. Batch shipping procedures may be modified by the CCC as necessary without altering this protocol in the future.

Shipment of Biomarker Samples:

1. Samples need to be shipped to the biomarker core laboratory in the freezer boxes packed into a Styrofoam shipping box containing dry ice. Use an overnight carrier with overnight delivery. Do not allow samples to thaw under any circumstances! Please ship on a Monday-Wednesday. Do not ship on a Thursday, Friday, Saturday or Sunday to be sure there is someone to receive the samples when they arrive.
2. Shipping address:
Attn: Chris Cunningham, Ph.D. / ASTROH Study
Research Operations Manager
Clinical Trials Unit
University of Louisville
401 East Chestnut St, Suite 460
Louisville, KY 40202
T:(502) 852-2906
F:(502) 852-2610 or 589-7310
chris.cunningham@louisville.edu
3. After receiving the shipment, the Styrofoam boxes will be immediately transferred to a -70°C freezer. Samples will be inventoried appropriately (Origin, type of sample, number of aliquots, sample Id number, location within the box and the freezer).
4. Samples will be kept at -70°C until ready to be assayed.

Plasma and CSF from all patients at all time points will be processed by the Core Biomarker Laboratory in a single batch fashion for all biomarkers being tested (except hs-CRP which will be performed at the local site clinical labs). This data will be associated with each patient's study ID# and time of collection. Biomarker lab personnel will remain blinded to treatment allocation. Unused specimens will be stored in a bio repository at the University of Louisville laboratory for up to 10 years after the completion of the study for potential future biomarker analysis without the need for additional informed consent or notification of study subjects at the discretion of the study leadership. Permission for the possibility of future genetic testing on the blood or plasma samples is included in the standard ASTROH informed consent document template provided to each study site.

Biomarker Assays:

A. CYTOKINES (IL-6, TNF α)

1. Plasma or CSF aliquots will be thawed, centrifuged at 10,000 x g for 5 minutes and the supernatants used to measure the concentrations of IL-6 and TNF α using Milliplex MAP Multiplex kits (EMD Millipore, Billerica, MA) according to the manufacturer's instructions. Samples will be assayed in duplicates.

B. ADHESION MOLECULES (sICAM1, P-SELECTIN)

1. Plasma or CSF aliquots will be thawed, centrifuged at 10,000 x g for 5 minutes and the supernatants used to measure the concentrations of sICAM1 and P-Selectin using Milliplex MAP Multiplex kits (EMD Millipore, Billerica, MA) according to the manufacturer's instructions. Samples will be assayed in duplicates.

C. OTHER INFLAMMATORY MARKERS (HMGB1, MRP8/14)

1. Plasma or CSF aliquots will be thawed, centrifuged at 10,000 x g for 5 minutes and the supernatants used to measure the concentrations of HMGB1 and MRP8/14 using appropriate ELISA kits (Biolegend, San Diego, CA). Samples will be assayed in duplicates.

6.12 Clinical Management of the Study Patient

As much as possible, the clinical management of each subject will be identical to the standard of care (SOC) at the local institution except as specifically altered by this protocol. Generally speaking the SOC at each institution should be similar to the AHA / ASA published Guidelines for the Management of Aneurysmal Subarachnoid Hemorrhage [Connolly ES et al. *Stroke* 2012;43:1711-1737] and / or the Critical Care Management of Patients following Aneurysmal Subarachnoid Hemorrhage: Recommendations from the Neurocritical Care Society's Multidisciplinary Consensus Conference [Dringier MN et al. *Neurocritic Care* 2011;15:211-240].

6.12.1 Required Clinical Laboratory Testing

Upon admission prior to or during enrollment, the following laboratory blood testing will be recommended: CBC, Comprehensive Metabolic Panel (CMP) to include Sodium, Chloride, Potassium, Bicarbonate, Blood Urea Nitrogen (BUN), Creatinine, Glucose, AST, ALT, Alkaline Phosphatase, PT, International Normalized Ratio (INR), and aPTT as part of the SOC for the treatment of aSAH patients in the acute hospital setting. We recommend a Cardiac Enzyme Panel including Troponin will be drawn on admission prior to or at enrollment and then repeated every 8 hours until a total of 3 sets of cardiac enzymes have been drawn as part of the SOC. In addition, Urinalysis upon admission or prior to enrollment including a urine toxicology screen is recommended as part of the SOC. [Cocaine and other illegal substances can affect the clinical course of aSAH patients].

The following Study related blood work is required above and beyond the SOC upon enrollment: hs-CRP and Anti-Xa levels (Funded by Study).

The following Blood testing will be performed for aSAH patients enrolled in this study from the time of enrollment until stopping of the study drug infusion during the acute hospitalization. Those items that are typically considered the SOC have been labeled as such:

1. CBC: Daily for both treatment groups (SOC)
2. aPTT: Daily for the LDIVH treatment group (Funded by Study). In addition to the enrollment aPTT level, an additional aPTT will be needed on enrollment day #6 for control patients to allow correlation to Anti-Xa values (Funded by Study). Additional aPTT testing for heparin dose adjustments will be performed and will be estimated at 4 samples per LDIVH study patient (Funded by Study). This will mean a total of 18 non-SOC aPTT values will be funded by the study per LDIVH treatment group subject and 2 non-SOC aPTT lab tests will be funded by the study per control group subject.
3. Basic Metabolic Panel: At least every other day for both treatment groups (SOC)
4. hs-CRP: Upon enrollment and enrollment day #2, 4, 6, and 10 (Funded by Study, both treatment groups)
5. Anti-Xa: Upon enrollment and enrollment day #6 (Funded by Study; both treatment groups)

6.12.2 Treatments of Clinical (Symptomatic) Vasospasm

The treatment of clinically symptomatic cerebral vasospasm will be performed per the usual SOC at each institution.

Generally speaking, frequent neurological and clinical evaluation supplemented by transcranial Doppler (TCD) monitoring is ideal for the evaluation of cerebral vasospasm. Considering all patients enrolled in this study will be “Good-Grade Patients at High Risk of Vasospasm” (per NCS consensus recommendations) patients may also benefit from monitoring with CTA/CTP or DSA even in the absence of detectable clinical symptoms and this argument could justify performing one of these imaging modalities during the peak vasospasm window as the SOC (Recommended for this study). Any CTA / DSA / MRA imaging performed in the peak vasospasm window or performed because of a high suspicion for vasospasm should be submitted to the imaging core lab for assessment as part of this study. Consensus recommendations for monitoring with TCD threshold sensitivities are < 120 cm/s for the absence of large artery vasospasm and >200 cm/s and / or an MCA/ICA (Lindegaard) ratio >6 for the presence of large artery vasospasm.

The development of new focal neurological deficits (e.g., pronator drift), change in level of consciousness not otherwise explainable, or a significant increase in TCD velocities or Lindegaard ratio should necessitate an angiographic study to help confirm the

development of arterial vasospasm and help direct therapy or therapy should be directly administered as per the Consensus Recommendations by the Neurocritical Care Society (*Neurocrit Care* 2011). When patients develop symptoms of delayed cerebral ischemia and in whom elective angiographic imaging has already been performed, initiation of medical / endovascular therapy to reverse delayed cerebral ischemia without additional imaging is reasonable (Consensus Recommendations, NCS).

For purposes of database documentation, this study will track vasospasm “Rescue Therapies”. Triple H (Hyperdynamic) therapy protocols will be used per SOC at each clinical site. For this study, hyperdynamic therapy (i.e., Hypervolemia, Hypertension) to treat clinically symptomatic cerebral vasospasm will be considered one of three “rescue therapies”.

“Rescue therapies” include:

- Hypervolemic hyperdynamic - Fluid boluses of 500 cc or more or increases in the hourly intravenous fluid rate of more than 50% for the purpose of treating clinically symptomatic cerebral vasospasm
- Hypertensive hyperdynamic - a goal systolic blood pressure (SBP) of 160 mm Hg or more is achieved with vasopressors or inotropic medications for the purpose of treating clinically symptomatic cerebral vasospasm.
- Endovascular treatment by intracranial balloon angioplasty and / or intraarterial vasodilators (such as verapamil, nicradipine, etc.) for the purpose of treating clinically symptomatic cerebral vasospasm.

“Rescue therapies” DO NOT include:

- Allowing the SBP to naturally rise above 160 without measures such as vasopressor or inotropic medications
- Volume expansion or inotropic or vasopressor drug usage for purposes of treating decreased cardiac output or hypotension that is not associated with the treatment of symptomatic clinical vasospasm (i.e., volume or pressure support for the treatment of sepsis)

All rescue therapies initiated for the treatment of symptomatic clinical vasospasm will be documented including the symptoms associated with the clinical vasospasm, the time of onset of symptoms, duration of symptoms, resolution of symptoms, type(s) of rescue therapy utilized, other cause exclusionary testing results (i.e., CT of head showing no hydrocephalus), and TCD or angiographic data supporting the diagnosis (if available).

6.12.3 Anti-epileptic Medications

Phenytoin (Dilantin) usage is reported to be associated with decreased cognitive outcomes in aSAH patients (Naidech AM et al. *Stroke* 2005;36:583-587). Considering the primary exploratory clinical efficacy outcome of this study is a cognitive measure, the utilization of anti-seizure medications must be considered. Patients with a previous history of epilepsy should not be part of this study based on the exclusion criteria.

Per the consensus recommendations of the Neurocritical Care Society on seizure prophylaxis in aSAH patients, **routine use of anticonvulsant prophylaxis** with phenytoin (Dilantin) or fosphenytoin (Cerebyx) **is not recommended** (low quality of evidence – strong recommendation) **and is strictly prohibited by this protocol**. If indicated and anticonvulsant prophylaxis is used, a short course (3-7 days) is recommended and this will be documented on the patient eCRFs. In patients who suffer seizure after presentation, anticonvulsants should be continued for a duration defined by the local SOC but our preference is to avoid phenytoin / fosphenytoin if possible.

- All anti-epileptic medications, doses, and treatment durations should be documented in the CRFs. All seizure episodes or high suspicion seizure episodes should be documented. All EEG monitoring results should be recorded including type and time of EEG recording. For patients who have had a seizure and require anti-epileptic therapy, we prefer the usage of valproic acid or Keppra over phenytoin or fosphenytoin for the reasons mentioned above.
- Status Epilepticus (SE) is considered a neurological emergency and should be treated according to well-established protocols, guidelines, and/or the SOC at each clinical site. It is not the intention of this protocol to influence the standard emergent treatment for (SE).

6.12.4 Anti-thrombotic and Anticoagulation Medications

Anti-platelet medication therapy prior to aneurysm coiling or after aneurysm coiling is not SOC and will not be permitted. However, a single dose of aspirin (325 mg or less) at the time of the coiling procedure (if SOC for the interventionalist) will be permitted when used to prevent intraprocedural thrombotic complications.

Intravenous administration of unfractionated heparin immediately prior to or during the coiling procedure is permissible per the site's SOC, but should NOT be continued after the procedure except as determined by randomization to the LDIVH treatment arm. Following the coiling procedure, practitioners will not use anti-platelet medications in either study arm during the study drug infusion period. If the need to place patients on antiplatelet medications following the aneurysm procedure is known prior to randomization this would exclude the patient from enrollment. We prefer these medications not to be given until after the 90-day follow-up, however, if they are felt to be necessary prior to the 90-day follow-up (i.e., to prevent continued thrombotic complications), their administration will need to be documented and recorded as a study

deviation. These patients will remain in the study and their outcomes will still be recorded.

UFH (or heparin analog) infusion, injection, or other route of administration will not be permissible during this study [except as allowed by this protocol] unless it is required to treat an identified DVT or PE or other potentially serious medical condition that requires anticoagulation per usual SOC. If intravenous anticoagulation is required in the LDIVH treatment group during the infusion period, then the LDIVH infusion will be stopped and replaced with anticoagulation therapy per SOC.

6.12.5 Deep Venous Thrombosis (DVT) Prophylaxis

To reduce the chance of bleeding complications, subcutaneous chemical DVT prophylaxis will not be allowed for the LDIVH treatment arm during the duration of the LDIVH infusion. All study patients will receive mechanical DVT prophylaxis using sequential compression devices (SCDs) and preferably with TED stockings during the entire study drug infusion period.

Chemical DVT prophylaxis will be resumed as needed following the completion of the LDIVH infusion.

Chemical DVT prophylaxis of 5000 units of unfractionated heparin administered subcutaneously twice per day which will correspond to a SOC treatment will be provided to all patients randomized to the control arm of this study (The dose of UFH may be higher in patients with elevated BMI, according to each site's SOC).

Lower extremity venous duplex DVT surveillance is recommended on all study patients prior to or soon after enrollment and at least weekly during study drug infusion time period (LDIVH and Controls) for a total of at least two LE venous duplex testing results (initial and at least 1 more 7 days later). This recommended (but not required) surveillance will be considered part of the SOC for each clinical site and will not be funded by the study.

6.12.6 Febrile Episodes

Fever (defined in this study as any temperature $>38.3^{\circ}\text{C}$; $\geq 101.0^{\circ}\text{F}$) commonly occurs in up to 70% of patients following aSAH and may contribute to short and long-term morbidity. Fever without concomitant infection has been implicated as a cause of worsened neurological function and cognitive disability. **Guidelines from the Neurocritical Care Society and American Heart Association recommend maintaining normothermia after aSAH.** Our hypothesis that modulating the inflammatory response will have the potential to reduce the incidence of delayed neurological deficits and cognitive dysfunction in aSAH is intimately related to clinical signs of inflammation such as fever. Blood within the cerebrospinal fluid can induce fever by the release of inflammatory cytokines in the absence of true infection. In addition, aSAH patients are predisposed to hospital-acquired infection such as urinary

tract infection, ventilator associated pneumonia (VAP), and ventriculitis associated with external ventricular drainage catheters. Other medications commonly used in aSAH patients such as phenytoin can cause drug fevers. Increasing fever burden is a long-term predictor of poor functional and cognitive recovery following aSAH. As a result, neurocritical care specialists now promote aggressive fever control in aSAH patients. In fact, a clinical trial is in development to evaluate normothermia in aSAH (NASH) and is focusing on pharmacological therapy with intravenous ibuprofen and other aggressive fever reduction methods.

It is possible that UFH will modulate the inflammatory response to the point of reducing fever burden preferentially in the LDIVH arm. This may lead to improved clinical functional measures such as perseveration of cognitive function. It is also possible that a significant muting of the inflammatory response will limit the body's natural inflammatory mechanism to fend off infectious insults resulting in the possibility of unintended infectious complications which if not recognized early in their course (i.e., no heralding fever, elevated serum WBC count) could become more difficult to treat with antibiotics. However, this theoretical risk of increased infections as a result of improved fever control was not borne out in a study undertaken at Columbia University when comparing conventional fever control methods to aggressive fever control methods (Badjatia N et al. *Neurosurgery* 2010;66(4):696-700) and was not identified in more than 100 post-craniotomy patients treated with the LDIVH protocol.

This study will monitor fever episodes and overall daily fever burden. It will also track all infectious events and consider them adverse events (AEs) as well as track any anti-microbial therapies. The following recommendations are made to merge the goals of this study with the Neurocritical Care Society's Consensus recommendations:

- **The cause of fevers should be investigated and treated aggressively per the SOC at each clinical site based on guidelines set forth by the Society of Critical Care Medicine (SCCM) and the Neurocritical Care Society** which may include antimicrobials (for proven or suspected infections), other pharmacological agents and utilization of temperature modulation devices (TMDs).
- **The overall efficacy of most antipyretics is low and we prefer avoidance of acetaminophen and / or ibuprofen for fever control unless required per the SOC at the institution as these medications could confound our study results.** If these medications are deemed necessary per the SOC at the institution, then the dose, duration, and timing of the medications will be recorded in the EDC system and **statistical analysis will be done to determine if there is any confounding influence of these medications on the study results.**
- **Surface cooling or intravascular devices are generally more effective than antipyretics and could be considered for fever control when antipyretics are not utilized. Use of these devices should be accompanied by monitoring for skin injury and venous thrombosis.**

- **Patients should be monitored and treated for shivering.**

Fever will be defined in this study as core body temperature of > 38.3° C (≥ 101° F). Core body temperature measurements are preferred and should be measured using one of the following devices when possible: pulmonary artery thermistor, urinary bladder catheter thermistor (Foley), esophageal probe, rectal probe. Pulmonary artery catheters are currently uncommonly used in the management of aSAH patients within the intensive care setting, however if the catheter is already being utilized for other purposes in the study subject, then core body temperature may be measured from this catheter as this is considered the gold-standard for core body temperature measurement.

The most common core body temperature measurement option for aSAH patients with limited invasiveness would be the urinary bladder catheter thermistor and this is the preferred method for this study due to the high accuracy of these measurements. Most aSAH patients need critical input and output measurements requiring urinary bladder catheter placement in the critical care setting. In addition, the tendency to give generous intravenous fluid volumes to these patients tends to increase the utilization of urinary bladder catheters to avoid hourly trips to the toilet or soiling of bed linens. Yet in recent years there has been a trend towards early urinary bladder catheter removal to prevent hospital acquired urinary tract infections. Therefore, while core body temperature measurements utilizing the urinary bladder catheter thermistors would be an ideal option in aSAH subjects enrolled in this study, the urinary bladder catheters should be removed when appropriate based on the SOC at each clinical site and should not be continued only for temperature measurements for this study if this would fall outside of the site's SOC.

Esophageal temperature probes in the lower 1/3 of the esophagus are reasonable core body temperature measuring devices and are acceptable for temperature measurements in this study. However, these are most common in intubated patients, and we expect very few of our enrolled patients to have endotracheal intubation during study drug infusion. On awake, non-intubated patients esophageal temperature probes can still be utilized, but may be uncomfortable to patients.

Newer central venous catheter temperature probes (Alsius/Zoll) may also be utilized. Rectal probes may also be used but tend to be slightly less accurate than urinary and esophageal probes. **If none of these probes are available, then oral temperature probes placed in the sublingual space are acceptable** but proper attention should be given to recent eating, drinking, mouth breathing and proper probe placement to improve accuracy.

Infrared ear thermometry tends to be less accurate and is not recommended for this study. Temporal artery thermometers, skin surface thermometers and axillary thermometers are not allowed for purposes of temperature data collection in this study.

Temperature measurements will be recorded into the medical record while the patient is in the intensive care unit according to each site's SOC, but at least every 4 hours. If there is more than one measurement obtained within a given hour, then the highest measurement obtained during that hour will be utilized as temperature data for this study. The method of temperature monitoring should be recorded.

Temperature logs will be collected for each subject. Missing temperature time points will be assumed to equal the most recent previously recorded temperature. A total daily fever burden will be calculated for the duration of the study drug infusion (Daily Fever Burden = Sum of hourly fever burden over 24 hours; Hourly Fever Burden = Any hourly temperature recording $> 37.0^{\circ}\text{C}$ minus 37°C ; thus hourly temperature readings of 37°C or less will result in a "0, zero" fever burden for that hour).

If the patient is not in the ICU and is having temperature measurements every 4 hours, then the 4-hour temperature burden will be calculated as temperature measured during that 4 hours window (or highest measurement if more than one measure) minus 37°C x 4. The Daily fever burden will then be the sum of the six 4-hour temperature burden calculations.

In addition, the T_{max} per 24-hour period will be documented in the study CRF/Database as well as the number of temperature values recorded as a fever ($>38.3^{\circ}\text{C}$) per 24 hour period. **For the purposes of this study, a day will run from 7 AM until 6:59 AM the following morning.**

Daily T_{max} , Daily Fever Burden, and # of fevers per day will be evaluated as an outcome measure on enrollment day # 2, 4, 6, and 10 to determine any correlation between fever and individual inflammatory biomarker levels and neurological assessments and MoCA scoring at each time point (# 6 and 10). Also, the mean of each subject's average hospitalization daily fever burden (up to 21 days) will be analyzed in relation to the mean of the biomarker levels summed over enrollment days #2, 4, 6, and 10 per study treatment group. Finally, presence of any temperature $> 38.3^{\circ}\text{C}$ and multiple febrile episodes (>2) for a study subjects will be noted and compared between treatment groups as an analysis of categorical proportions.

6.12.7 Blood Transfusions

We recommend that transfusion of packed red blood cells be avoided unless the patient has **symptomatic** anemia. Transfusion of packed red blood cells has been reported to be associated with decreased cognitive outcomes in patients following aSAH. Should a transfusion of packed red blood cells be necessary per a site's SOC, it will be recorded in the subjects' CRFs. All transfusions of other blood products will also be recorded in the CRFs. Please keep in mind that **if blood transfusions are given during a bleeding event, administration of 2 or more units will automatically designate that bleeding event as a Major bleeding event.** See section 8.5 for more information.

Recommendations based on the Neurocritical Care Society Consensus paper:

- Measures should be taken to minimize blood loss from blood drawing.
- Transfusion criteria for general medical patients should not be applied to decisions in SAH patients.
- Consider RBC transfusions to maintain hemoglobin above 8 g/dl, but for purposes of this study please only transfuse 1 unit of packed red blood cells when there is no urgent bleeding event and then reassess before additional transfusion.

6.12.8 Nimodipine

Nimodipine will be given to subjects at 60 mg by mouth every 4 hours per the SOC at each site (capsule or liquid suspension). However, reduction in dose with increased frequency of dosing to 30mg by mouth every 2 hours is sometimes utilized for patients who have serious hypotensive responses to the 60 mg dose, and is an acceptable alternative in this study. Holding Nimodipine will be done as per the SOC at each clinical site, and is usually only done in the setting of severe hypotension not responsive to mild to moderate pressors. However, whenever possible, the clinical team should avoid holding nimodipine. If nimodipine is held for severe hypotension, it should be resumed as soon as the blood pressure is corrected and inotropic medications are available to help support the blood pressure. Decreasing the total daily dose of nimodipine to less than 360 mg / day or discontinuing nimodipine should be avoided, although this eventuality will be up to the discretion of the treating physician. Nimodipine compliance will be recorded in the EDC for this study. Experience in the LDIVH post-craniotomy study suggested that co-administration of LDIVH and nimodipine may yield the best clinical results. Great care should be taken when using nimodipine in the setting of severe aSAH induced cardiac dysfunction as this could potentiate a serious hypotensive event not easily correctable with vasopressor medications.

6.12.9 Statins

Statin medications have previously been suggested to help reduce the incidence of delayed cerebral ischemia and improve outcomes in aSAH patients. However a recent meta-analysis (Vergouwen MD, et al. *J Cereb Blood Flow Metab.* 2009;29:1444-1453) showed no clinical benefit and a more recent large phase III placebo controlled trial comparing 391 patients receiving 40 mg simvastatin daily versus 412 patients receiving placebo showed no benefit in outcomes as measured by ordinal mRS analysis adjusted for admission WFNS grade (Kirkpatrick PJ et al. *Lancet Neurol.* 2014;13(7):666-675). Therefore, for purposes of this study, statin agents should not be newly initiated for aSAH study subjects. If the study subjects have previously been taking statins for other medical problems then they may continue at the previous dosing and schedule. Any statin usage in this case will be recorded in the EDC.

6.12.10 Magnesium sulfate

Magnesium sulfate has also previously been suggested to help improve outcomes after aneurysmal subarachnoid hemorrhage. Recent randomized placebo-controlled multicenter phase III trials (IMASH and MASH-2) have not demonstrated any benefit (Wong GK et al. [IMASH] *Stroke* 2010;41:921-926 and Mees SMD et al [MASH-2] *Lancet* 2012;380(9836):44-49). Therefore, for purposes of this study, magnesium sulfate infusion should not be given routinely as a prophylactic neuroprotective agent. It is acceptable to give magnesium sulfate to patients who have low magnesium levels for routine electrolyte replacement.

6.12.11 Corticosteroids

Corticosteroids with predominant glucocorticoid effect should be avoided. Symptomatic treatment of headaches or other inflammatory or meningismus related symptoms should be avoided. Steroid medications should only be used as required for stress dosing or to treat hypocortisolism. Corticosteroids with predominant mineralcorticoid effect (such as fludrocortisone / Florinef for treatment of natriuresis / hyponatremia) are acceptable. Chronic corticosteroid usage prior to admission is considered a criteria for exclusion to enrollment.

6.13 Completion Of Study Drug Infusion

The study drug infusion will be stopped after completion of 14 full days of infusion. In the unlikely event that the patient is discharged home or to a facility prior to this time point, then the study drug infusion will be stopped at least two hours immediately prior to discharge. After stopping the study drug infusion, all previous restrictions placed on the patients during the study drug infusion time period as listed in this protocol will no longer be in effect.

6.14 Post-Infusion (Pre-Discharge) Head CT

A post-infusion head CT is required by this protocol. Most centers will perform a head CT during the later phases of the acute admission to evaluate for development of delayed hydrocephalus and / or to obtain a baseline scan prior to discharge. As a result, this scan will be considered part of the SOC and will not be funded by the study. We prefer the timing of this scan to be 12 hours (\pm 12 hrs) after completion of the study drug infusion (or an equivalent time period in the control group) to allow for comparisons to the post-procedure (pre-enrollment) head CT. If the patient's hospital discharge is planned for a time period < 24 hours after the cessation of the study drug infusion, the CT scan should be obtained prior to discharge.

6.15 Imaging Processing Procedures

A study imaging processing company will be used to systematize the redaction of protected health information and confirm image quality prior to image review by the

imaging core lab. OCULUS Imaging, Inc. under the direction of Dr. Keith Woodward, an interventional neuroradiologist, Knoxville, Tennessee will provide the imaging database for the study (<https://www.oculusimaging.com>). The OCULUS imaging system is a secure HIPAA compliant system that has been used in numerous clinical trials for processing of images for imaging core lab review. Radiologists with the imaging core lab would be able to remotely log into the OCULUS imaging system to review and report on the imaging data preventing the need to mail hard copies (CDs) of the images and making it less likely for an unanticipated disclosure of protected health information.

However, should the OCULUS Imaging system not be utilized or be temporarily disabled, radiological images pertinent to the study will be sent to the Imaging Core Lab for verification of findings. This process would involve mailing CD-ROMs with de-identified images if the electronic imaging processing system is not functioning properly.

For Direct Shipping of Imaging CD-ROMs [In the event of an unlikely long-term failure of Oculus Imaging]: Shipping address:

ASTROH Imaging Core Lab
Attention: Marlene Baumeister, RN
Cerebrovascular Center
Level 4, Suite 430
Stony Brook, NY 11794-7447

Email: marlene.baumeister@stonybrookmedicine.edu

DICOM digital copies of these images will be maintained by the Imaging Core Lab or by OCULUS Imaging, Inc. for a minimum of 10 years after study completion. The storage of these images and any related data will be HIPAA compliant. The images will be labeled with the patient's assigned Study Identification Code, the date of imaging (Day # from enrollment), and type of imaging. Whenever possible these images will be in a de-identified form, however, some images have patient identifiers that may not be able to be removed. All images will be shared and disposed of in a secure manner and the collection, sharing, and storage of these images will be disclosed in the informed consent document and HIPAA Authorization. Dr. David J. Fiorella will supervise the Imaging Core Laboratory procedures and will have direct access to the Oculus Imaging Database.

6.16 Clinical Assessments Through Post-Enrollment Day #14

Study personnel at each site will be trained in the proper clinical assessments of study subjects. The team members performing the inpatient clinical assessments will NOT be allowed to perform the blinded clinical assessments at the 90-day or 1-year follow-up visit.

Study specific clinical assessments of study subjects will occur on post-enrollment day, day 6, and day 10 during the acute hospitalization.

- **Glasgow coma scale score (GCS) [5 minutes]**
- **NIH Stroke Scale (NIHSS) [10 minutes]**
- **Montreal Cognitive Assessment (MoCA) [15 minutes]**
 - This MoCA has three English versions. A different English version should be given to study subjects during each time point (Version 1: Enrollment; Version 2: post-enrollment day #6; and Version 3: post-enrollment day #10) [Note: The final MoCA assessment on the 90-day follow-up visit will be Version 1 (repeated) and MoCA assessment on the 1-year follow-up will be Version 2 (repeated)]
 - Similarly, there are other language options with three versions (i.e., Dutch, German, Portuguese, and Spanish). An identical version test distribution would be used in these other languages. Test information and other languages are available at www.mocatest.org)
 - **The administration of the MoCA at enrollment should be done at least 12 hours after removal of the endotracheal tube and recovery from any anesthesia given during the coiling procedure.** This may require that the MoCA to be administered on post-enrollment day #1 instead of on the enrollment day (post-enrollment day #0). This rule is in place to minimize post-anesthesia effects from influencing the MoCA score. In the event that the patient remains intubated past post-enrollment day 1, the MoCA test should be performed as soon as possible after extubation, once sedation has cleared.
 - **MoCA assessments will be audio recorded in order to verify accurate scoring. Recordings will be labeled with subject ID number only and destroyed at the end of the study.**

6.17 Post-Infusion / Pre-Discharge Assessment

The following clinical assessments will be performed prior to patient discharge from the acute care setting (preferably on the same day as the post-infusion, pre-discharge head CT that will be submitted to this study:

- **Glasgow coma scale score (GCS) [5 minutes]**
- **NIH Stroke Scale (NIHSS) [10 minutes]**

Adverse Event assessments will need to be performed daily by trained study personnel [See Section 8] for enrolled patients while patient is an inpatient. All adverse events (including hemorrhagic complications) will be documented, including all serious adverse events, adverse drug reactions, serious adverse drug reactions, unexpected adverse events, and unexpected adverse drug reactions. Any AE or SAEs such as bleeding, hemorrhagic, infarction, DVT, PE, or death that occur at any point from enrollment until the 90-day follow-up visit will be recorded in the EDC and will be available for the DSMB and in the final analysis of the study after study completion. SAEs must be reported to the CCC within 24 hours of the occurrence and AEs must be

reported to the DCC within 5 business days (7 calendar days) of the occurrence. These AE and SAEs should be reported to the study project manager. All adverse events will be handled according to good clinical practice. AEs will not be collected after the 90-day follow-up visit.

6.18 Discharge From Acute Care Setting

The patient will be discharged from the acute care setting per the normal SOC. The study patient is not expected to require any additional days of hospitalization or days in the intensive care unit by being enrolled in this trial. However, if the patient experiences a complication directly related to study drug infusion, there may be an increased length of stay as a result. Increased costs attributed to this unlikely event will be the burden of the patient and/or clinical site. The study's investigators and the University of Louisville (or any of its subsidiaries including the University of Louisville Research Foundation) will not provide indemnification to the clinical sites or patients against any increased expenses related to complications attributed to any patient's participation in this study. This will be listed as one of the risks of enrollment on the patient's informed consent document.

When a patient is clinically ready for discharge, they will be discharged from the hospital. Completion of the study drug infusion through the end of post-enrollment day 14 will not be an absolute requirement if the patient could otherwise be discharged. However, in our experience, it is rare for a patient with modified fisher grades 3 to be ready for discharge prior to post-enrollment day # 14. The total duration of the study drug infusion will be recorded, and any decreased length of study drug infusion because of early discharge will be noted but will not be considered a study deviation.

7. OUTPATIENT FOLLOW-UP VISITS

7.1 90 DAY (\pm 14 Days) FOLLOW-UP VISIT FROM DATE OF PROCEDURE

All patients in the study will be followed for the purposes of the study until the 90 day follow-up visit which will occur 90 days (\pm 14 days) after the SAH ictus. Thus, the primary data analysis date for the primary endpoint of the study will be after the last active patient enrolled in the trial reaches their 90-day follow-up and completes this visit.

Clinical, functional, social and cognitive evaluations are to be performed by properly trained study personnel. These evaluations are to be performed by personnel blinded to treatment allocation. Due to personnel time-off needs and vacations **we advise having at least two clinical research personnel designated and trained for these blinded assessments at each clinical site. This is above and beyond the need for a clinical research nurse / coordinator to manage the inpatient procedures.**

In the event that patients or families have already been informed of their treatment allocation, all patients and family members will be requested to not disclose this

information to the evaluators during this follow-up visit. Upon arrival at their follow-up visit, they will be given a letter reminding them not to disclose their treatment allocation.

The following evaluations will be administered during the follow-up visit: [Approximately 95 minutes-above and beyond the normal SOC]

- Montreal Cognitive Assessment (MoCA) [15 minutes]
- Modified Rankin Scale (mRS) via Rankin Focused Assessment tool (RFA) [10 minutes]
- Assessment of Return to Work Status [5 minutes]
- QOLIBRI-OS [5 minutes]
- NIH Stroke Scale (NIHSS) [10 minutes]
- Glasgow coma scale score (GCS) [5 minutes]
- CIS-f (fatigue scale) [5 minutes]
- Barthel Index [5 minutes]
- Trail Making Test Parts A&B [5 minutes]
- Lawton Instrumental Activities of Daily Living (Lawton-IADL) Score [10 minutes]
- Center for Epidemiologic Studies Depression Scale (CES-D) [5 minutes]
- Assessment for Adverse Events and Serious Adverse Events: [15 minutes]

7.2 One-Year (\pm 60 days) Follow-up Visit

The one-year (\pm 60 days) follow-up visit will be the final study-related visit and assessment.

The following evaluations will be administered during the one-year follow-up visit: [Approximately 40 minutes-above and beyond the normal SOC]

- Montreal Cognitive Assessment (MoCA) [15 minutes]
- Modified Rankin Scale (mRS) via RFA tool [10 minutes]
- Center for Epidemiologic Studies Depression Scale (CES-D) [5 minutes]
- Assessment of Return to Work Status [5 minutes]
- QOLIBRI-OS [5 minutes]
- Address of serious adverse events

Any forms used to complete the evaluations above (i.e., MoCA form which includes patient drawings, etc.) will be labeled with the Subject Identification Code, Date of Exam, and scanned to an electronic format and then uploaded into the REDCap EDC system. The hard paper copy will be included in the patient's study binder if uploading is not possible. The electronic copy will be forwarded to the CCC via the Electronic Data Capture program and/or email (ASTROH@louisville.edu) per the procedures outlined in the Manual of Operations. Some neuropsychiatric testing score sheets (MoCA) will be re-scored in a blinded fashion by D. Erik Everhart, PhD to assure data validity.

A 90-day and one-year follow-up visit after an aneurysmal subarachnoid hemorrhage are considered the SOC. Only the additional procedures (assessments) listed here are considered study related. Therefore, the study will cover the expenses for the extra time it takes the research personnel to perform these study-specific tasks above and beyond the assessments typically performed on the visit as the normal SOC.

8. MANAGEMENT OF ADVERSE EVENTS AND ADVERSE DRUG REACTIONS

Patient safety is of paramount importance in this trial and study personnel will be monitoring for adverse events throughout the trial. The Clinical Events Adjudication Committee (CEC) will review adverse events (AEs) according to the provisions of the ASTROH CEC Charter and will validate all serious adverse events (SAEs), adverse drug reactions (ADRs) and serious adverse drug reactions (SADRs). If the CEC identifies a dangerous or unexpected trend in SAEs, ADRs, or SADRs they will forward this information immediately to the chair of the DSMB.

8.1 DEFINITIONS

Adverse Drug Reaction (ADR): All noxious and unintended responses to a medicinal product related to any dose should be considered an adverse drug reaction. There must be a reasonable possibility that there is a causal relationship between the medicinal product and the adverse event.

- **Adverse Event (AE):** Any unfavorable and unintended sign (including laboratory findings), symptom or disease that occurs to a subject while enrolled in a clinical investigation. Medical conditions that exist at study enrollment are not considered an AE unless condition worsens after enrollment.

- **Serious Adverse Event (SAE) or Serious Adverse Drug Reaction (SADR):** Any untoward medical occurrence that at any dose:
 1. Results in death
 2. Is life threatening
 3. Requires inpatient hospitalization or prolongation of current hospitalization
 4. Results in persistent or significant disability/incapacity
 5. Results in a congenital abnormality or birth defect
 6. Requires a medical or surgical intervention to prevent a permanent impairment of a body function or permanent damage to a body structure.

- **Unanticipated Adverse Event (UAE) / Unexpected Adverse Drug Reaction (UADR)/or Other Unexpected Problems:** Unexpected (in terms of nature, severity, or frequency) means that:

1. The event and/or reaction was not previously described in the research procedures included in the protocol-related documents, such as the protocol, or consent document, or in other relevant sources of information, such as product labeling or package inserts.

OR

2. The event and/or reaction was not previously described given the characteristics of the participant or the participant population being studied such as natural progression of any underlying disease, disorder or condition of the participant experiencing the adverse event/reaction, and the participants predisposing risk factor profile for the adverse event/reaction.
- **Relatedness to study drug or participation in the research.** The site investigator will determine the causality of the AE or SAE (definitely not related, probably not related (unlikely), possibly related, probably related, definitely related). These designations will be adjudicated by the CEC.
 - **A problem** suggests that the research places subject or others at a greater risk of harm (physical, psychological, economic, or social harm) than was previously known or recognized.
 - **Unanticipated / unexpected problems involving risks to participants or others** are defined as meeting all of three of the following criteria:
 1. The event is unanticipated because it is not included in the currently approved research study documents, Investigator's Brochure, package insert, or study protocol or the event exceeds the described frequency or severity, or it is unexpected that it would occur given the study population described in the research. AND
 2. The event is definitely related, probably related or possibly related to procedures involved in the research. AND
 3. The event suggests that the research places the participants or others at a greater risk of harm than previously thought.

8.2 REPORTING OF ADVERSE EVENTS / REACTIONS

Adverse events are generally detected in two ways:

Clinical: Symptoms reported by the subject or signs detected on examination.

Ancillary testing: abnormalities of vital signs, laboratory tests, and other diagnostic procedures (other than outcome measures: the results of which are not being captured as AEs).

Assessment of Clinical Adverse Events

At each visit including the 90 day follow-up visit, the subject will be asked “Have you had any problems or symptoms since your last visit?” in order to determine the occurrence of adverse events. If the subject reports an adverse event, the Investigator will determine:

1. Type of event
2. Date of onset and resolution (duration)
3. Severity (mild, moderate, severe)
4. Seriousness (does the event meet the above definition for an SAE)
5. Causality, relation to investigational product and disease
6. Action taken regarding investigational product
7. Outcome

Relatedness of Adverse Event to Investigational Product

The relationship of the AE to the investigational product should be specified by the Site Investigator or the CEC, using the following definitions:

1. **Definitely Not Related:** Concomitant illness, accident or event with no reasonable association with treatment.
2. **Unlikely (probably not related):** The reaction has little or no temporal sequence from administration of the investigational product, and/or a more likely alternative etiology exists.
3. **Possibly Related:** The reaction follows a reasonably temporal sequence from administration of the investigational product and follows a known response pattern to the suspected investigational product; the reaction could have been produced by the investigational product or could have been produced by the subject’s clinical state or by other modes of therapy administered to the subject. (suspected ADR)
4. **Probably Related:** The reaction follows a reasonably temporal sequence from administration of investigational product; is confirmed by discontinuation of the investigational product or by re-challenge; and cannot be reasonably explained by the known characteristics of the subject’s clinical state. (suspected ADR)
5. **Definitely Related:** The reaction follows a reasonable temporal sequence from administration of investigational product; that follows a known or expected response pattern to the investigational product; and that is confirmed by improvement on stopping or reducing the dosage of the investigational product, and reappearance of the reaction on repeated exposure. (suspected ADR)

Recording of Adverse Events

All AEs and ADRs will be reported in the subjects’ medical record, and to the CEC by an online adverse event reporting system on the appropriate eCRF (REDCap). **Events**

that are both serious and unanticipated should be reported to the CEC within 24 hours once the site becomes aware of the event, in addition to following site-specific IRB procedures for adverse events/reactions. **All other SAE's, ADRs, and SADR's should be reported within 5 working days/7 calendar days** to the coordinating site once the site becomes aware of the event, in addition to following site-specific IRB procedures for internal reporting. Non-Serious AEs do not have a specific time reporting requirement.

If discernable at the time of completing the AE log, a specific disease or syndrome rather than individual associated signs and symptoms should be identified by the Site Investigator and recorded in the AE log. However, if an observed or reported sign, symptom, or clinically significant laboratory anomaly is not considered by the Site Investigator to be a component of a specific disease or syndrome, then it should be recorded as a separate AE on the AE log. The Site Investigator would typically identify "clinically significant laboratory abnormalities" as those that require intervention (AEs). Lab values that are out of the normal range that do not have any negative clinical effect or concern are not considered AEs.

Serious adverse events

Once a SAE is reported in REDCap the project manager monitoring the system will notify the Chair of the Clinical Events Committee as outlined in the CEC Charter. If the SAE is expected, then the SAE may be reviewed by the CEC at the next scheduled meeting time (at least quarterly). All unexpected SAEs will be addressed directly by the chair of the CEC within 10 business days of CCC notification and if the chair of the CEC feels that an impromptu full CEC meeting is necessary he will call that meeting within an additional 10 business days. The CEC may request further information if necessary. REDCap will store information on the AEs and their adjudication. The Project Manager will coordinate communications related to any adverse event in the study. If warranted, the CEC will notify the DSMB chair. The DSMB may suggest changes to the protocol or consent form to the Study Chair (PPI) as a consequence of adverse events. The DSMB has the right to halt the study at any time for reasons of patient safety.

Non-serious adverse events

Non-serious adverse events that are reported to or observed by the investigator or a member of his research team will be submitted in REDCap in a timely fashion (within 5 working days / 7 calendar days). The events will be presented in tabular form and given to the CEC chair on a quarterly basis or as requested. Local site investigators are also required to fulfill all reporting requirements of their local institutions.

The CCC will prepare aggregate reports of all adverse events (serious/not serious, expected/unexpected and relationship to the study drug) for the CEC and DSMB as needed per their charters.

A separate report detailing protocol compliance will also be available from the CCC for DSMB and/or site review every six months or as requested. The Study Leadership Team will then evaluate whether the protocol or informed consent document requires revision based on the reports.

8.3 EXPECTED (ANTICIPATED) RISKS

The following events have been identified as possible complications related to the diagnosis and treatment of aSAH including the coil embolization procedure as well as possible complications related to infusion of LDIVH. These events are anticipated as possible complications for patients enrolled in this trial but are not necessarily caused by the study intervention:

- Additional procedures
- Adrenal Hemorrhage / Insufficiency
- Alopecia
- Arrhythmia
- Aneurysm re-rupture
- Bruising
- Cardiac ischemia / infarction
- Cerebral ischemia
- Coagulopathy
- Cognitive deficits
- Coma
- Death
- Deep vein thrombosis
- Delayed neurological deficits
- Dissection or pseudoaneurysm
- Drug reaction (i.e., allergic) to contrast, antiplatelet medication, heparin
- Electrolyte imbalance
- Emboli (air, tissue, thrombus, or foreign body)
- Gangrene
- Gastrointestinal disturbance
- Headache
- Hematoma, pain, and/or infection of the surgical access site
- Hemorrhage
- Heparin induced thrombocytopenia
- Hepatic Injury
- Hormone imbalance
- Hypertension
- Hypotension
- Inability to resume gainful employment
- Infection
- Liver function test elevation
- Memory problems
- New neurological symptoms
- Ovarian hemorrhage
- Pain
- Paralysis
- Radiation burn or injury
- Rash or dermatitis
- Renal insufficiency/failure
- Respiratory distress/failure
- Retroperitoneal Hemorrhage
- Seizure
- Personality changes
- Pulmonary embolism
- Stroke
- Surgical intervention
- Syncope
- Transient ischemic attack (TIA)
- Thrombocytopenia
- Vasospasm
- Vessel occlusion
- Vessel perforation or rupture
- Vessel thrombosis

8.4 DEFINITION AND MANAGEMENT OF HEPARIN INDUCED THROMBOCYTOPENIA (HIT)

There are two types of HIT, Type I demonstrates a fall in platelets (100,000-150,000/ μ l) that returns to normal despite continued heparin exposure, it is asymptomatic and benign. Type II HIT is characterized by thrombocytopenia, a significant drop in platelets (<100,000/ μ l or <50% of baseline), increasing the risk for thrombotic events. Type II HIT occurs in ~0.3-5% of those treated with heparin, occurring typically 5-10 days after heparin exposure has begun, and 20-50% of these develop thrombotic complications.³⁷ The disruption of platelets indicative of HIT result in the formation of blood clots, thus symptoms of deep vein thrombosis (DVT), pulmonary embolism, heart attack, and stroke should be monitored for.³⁹ Symptoms of DVT include pain, tenderness, sudden swelling, skin discoloration, large visible veins, and skin that is warm to the touch.³⁹ Symptoms of pulmonary embolism include shortness of breath, dizziness, sharp chest pain, change in heart rate, anxiety, and sweating.³⁹ Skin changes to monitor for that may be associated with HIT include bruising/blackening around heparin injection sites, fingers and toes, and nipples that may become gangrenous.³⁹

Diagnostic and management process for this study to identify and treat HIT in study subjects should follow the site's own SOC. Some suggested management options are included below:

Management of HIT includes immediate discontinuation of the study drug infusion (UFH), any other heparin, including heparin coated catheters and heparin-containing flushes, and prompt administration of an alternative type of anticoagulant if clinically indicated (direct thrombin inhibitors, Factor Xa inhibitor, or Vitamin K antagonist) including, but not limited to argatroban, bivalirudin, lepirudin, hirudin, fondaparinux, and warfarin.^{38, 39} Platelet transfusions are contraindicated.³⁹

Suspicion of HIT type 2 will require stopping all heparin including the LDIVH infusion as above. If deemed necessary, suspected HIT type 2 may be confirmed via an immunoassay to detect antibodies to heparin/PF4. If it is later determined that the patient does not have HIT type 2, then the patient may resume their UFH treatments as indicated assuming acceptable platelet count levels per the judgment of the local investigator and / or the patient's attending physician.

8.5 DEFINITIONS AND MANAGEMENT OF BLEEDING / HEMORRHAGE EVENTS

We have chosen to follow formal guidelines set forth by the International Society of Thrombosis and Haemostasis for bleeding complications (Schulman S, et al. *J Thromb Haemost* 2005;3(4):692-694). The normal rate of major bleeding or clinically relevant non-major bleeding in aSAH patients with protected aneurysms is estimated at approximately 10-20% (EVD insertion/tract hemorrhages, femoral artery access complications, GI bleed, fall out of bed, nasopharyngeal feeding tube insertion, etc.).

Bleeding complications are defined as:

Any development of clinically relevant non major bleeding or major bleeding, as defined by the International Society of Thrombosis and Haemostasis (ISTH):**a) Major Bleeding (≥ 1 of the following in 24 hr time period):**

- i) Bleeding causing a fall in hemoglobin level of 2 g/dL or more, or leading to transfusion of two or more units of whole blood or red cells.
- ii) Symptomatic bleeding in a critical area or organ, such as intracranial, intraspinal, intraocular, retroperitoneal, intraarticular or pericardial, or intramuscular with compartment syndrome, and/or
- iii) Fatal bleeding

b) Clinically Relevant Non-Major Bleeding

- i) Acute or sub-acute clinically overt bleeding that does not satisfy the criteria for major bleeding and leads to physician-guided medical or surgical treatment for bleeding, or a change in antithrombotic therapy (including study drug) for bleeding.

c) Minor Bleeding

- i) All acute clinically overt bleeding events not meeting the criteria for either major bleeding or clinically relevant non-major bleeding are classified as minor bleeding.

8.6 DATA SAFETY MONITORING BOARD (DSMB)

A Data Safety Monitoring Board (DSMB) charter will be created that will specify all of the rights and duties of the DSMB. The DSMB Chair and the PPI will draft the DSMB charter with input from the biostatistician. This protocol is not meant to supersede that document and the terms of that document will take priority over any conflicting terms or statements listed within this study protocol.

The DSMB will meet (in person or virtually) periodically (at least annually) to review the progress of this study (e.g enrollment, site performance, data quality, meeting risk factor targets) as well as data on the safety of both treatment arms (e.g. complications of LDIVH infusion). Before the study begins, the DSMB will decide on the frequency and timing of interim safety analyses. The DSMB may recommend termination of the study if the LDIVH treatment arm is found to be unsafe. The DSMB may also recommend protocol revisions to address enrollment, safety, etc. There will be no interim analysis of efficacy by the DSMB. The DSMB may also recommend modifications to the protocol if reversible safety issues are identified. The primary principal investigator (RFJ) may attend the DSMB meetings as a resource to the DSMB, but will not be allowed to vote and may be dismissed from the meeting(s) at anytime by the DSMB chair. The primary principal investigator will always be dismissed if there is any discussion of unblinded outcome measures. After each meeting, the DSMB Chair will prepare a letter to the study's primary principal investigator (RFJ), Steering Committee, and site investigators, which will document the safety review that took place at the meeting. This letter will indicate whether or not there are any safety concerns. This letter will be provided to any of the IRBs at participating sites at their request. Safety of study participants is of the utmost importance.

9. CRITERIA FOR INTERVENTION DISCONTINUATION

9.1 TEMPORARY INTERRUPTION OF THE STUDY DRUG

It may become necessary to temporarily stop the study drug infusion during the course of this study.

- The attending physician investigator responsible for the patient will give permission to stop the study drug.
- For minor procedures not involving the CNS (e.g., tracheostomy, central line) the LDIVH infusion does not need to be stopped as long as the last set of coagulation labs were considered to be at a safe level to perform the procedure.
- For a minor procedure involving the CNS (e.g., ventricular drain, lumbar puncture, lumbar drain) the LDIVH infusion will be discontinued 3-6 hours before performing the procedure then resumed at least 3-6 hours after the procedure is completed if there were no bleeding complications associated with the procedure. For all ventricular drain placements or ventricular drain repositioning a new head CT will need to be obtained that shows no hemorrhagic complication prior to restarting the LDIVH. If there is a new asymptomatic intracranial hemorrhage related to ventricular drain insertion or positioning, then a follow-up head CT will need to be obtained 12 hours later. If the hemorrhage is stable on follow-up head CT then the LDIVH infusion can be resumed.
- Suspensions in administration of the LDIVH will be noted, and an adverse event/adverse drug reaction form will be completed if the drug is held due to hemorrhagic complication.
- LDIVH infusion can be resumed 12 hours after craniotomy assuming a head CT following craniotomy shows no concern.
- Resumption of LDIVH after any other procedures will wait a minimum of 3 hours.

All interruptions of the study drug infusion will be documented on the appropriate CRF. This will include the date of the interruption, the duration of the interruption, the reason for the interruption, and the rate of infusion at which the study drug was resumed.

9.2 EARLY DISCONTINUATION OF THE STUDY DRUG

It may become necessary during a patient's enrollment that the study drug infusion be discontinued for patient safety reasons. Reasons for early discontinuation of the study drug infusion will be recorded in the appropriate CRF, as well as any AEs, SAEs, or UAEs that occurred.

9.3 WITHDRAWAL FROM THE STUDY

Subjects may withdraw from the study at any time for any reason with no penalty or loss of benefits they are otherwise entitled to. The patient will suffer no change in their non-study related medical care as a result of this decision. They will not be penalized in any way either directly or indirectly. If a patient chooses to withdraw from the study, the study team will be notified as soon as possible so that additional data collection will terminate.

10. DATA ANALYSIS AND CONSIDERATIONS

10.1 Sample Size Justification and Primary Clinical Effect Analysis

The sample sizes justification is based on the combination of the pilot data that we have previously observed (RFJ Unpublished) and results based on Schweizer et al. (2012). Two main outcomes, MoCA and MMSE, were compared by Schweizer et al (2012) and their validity was compared. The two measures have slightly different cut-offs for defining impairments (MoCA score <26 and MMSE score <27). In this study we will use MoCA as a primary outcome measure. Based on published data, MoCA has mean (SD) of 25.4 (2.8) with n=32. In our pilot data with small sample sizes, we observed an overall mean of 24.6 (5.4) with n=47. The separate group means were 26.4 (2.3) with n=25 and 22.7 (7.0) with n=22 in the treatment and control arms, respectively. Because the two groups were heterogeneous, we assume SD=2.5 in treatment group and SD=7.0 in control group. As hypothesized and as documented in the preliminary data, MoCA is higher in the treatment arm than in the control arm, and so we will use one-sided test procedures. The study design is that of a two group, multicenter randomized clinical trial.

Table S1: Sample sizes based on having alpha=5% and SD1=7.0 and SD2=2.5 to detect 3 unit (effect size=0.5 SD) using one-sided two-sample t test with unequal variances.

Power	N1	Total N
80%	40	80
85%	46	92
90%	54	108

Given the number of centers participating, we plan to enroll 40 evaluable subjects in each arm. Assuming 8 centers, a sample size of 80 would be realized by recruiting 10 per center. Note that we need to balance the number of treatment and controls to be equal at each center, but not necessarily having equal number of subjects at each center. However, the numbers in the tables do not adjust for loss to follow up, drop outs, the ability to adjust for covariates, or for the loss of degrees of freedom for the centers in a test or model. The discussion below addresses further aspects of the sample size consideration.

Based on a desired total of 40 patients per arm, and an assumed dropout rate of 10%, the trial would require 44 patients in each arm for a total enrollment plan of 88 patients distributed over 8 sites, or on average 11 patients per site. There are multiple covariates that may confound the findings and this needs to be addressed in the design of the study. The primary factors are: center, risk associated with anterior communicating artery (ACoA) aneurysm location (1 versus others), age (≤ 70 versus > 70) and sex. The other factors are: fever (yes, no), transfusion (yes, no), vasospasm (yes, no). Due to limited resources only primary factors (ACoA, group and sex) will be considered to balance the allocation of treatment and control in 1:1 ratio. Thus, the randomization will be balanced within each of 6 strata. Advanced age will be controlled for by eliminating enrollment for patients over 70 years. Effect of the remaining factors will be addressed in the multi-variate model.

We will also compare the frequency of severe cognitive impairment (MoCA ≤ 20) in the two arms. Previous data demonstrated a rate of 0% (0/25) severe cognitive impairment in the heparin treatment group and approximately 32% (7/22) in the control group. In our currently planned study, we will assume a 5% rate of severe cognitive impairment in the heparin treatment group and a 32% rate of severe cognitive impairment in the control group. This will also allow a demonstration of power = 80% with alpha at 0.05 for our previously calculated sample sizes ($n_1=40$ and $n_2=40$) using a two-sample one-sided binomial test. This binomial calculation provides a second sample size estimate that supports our previous estimate of needing 40 patients per group. Therefore the final sample size estimation will be placed at a total of 88 patients to account for a 10% dropout rate leaving a final number of patients available for primary outcome analysis of 80 patients. Drop-out will be monitored during the enrollment process. If we approach 8 patients lost to follow-up then additional enrollments (beyond 88) will be considered if funding is available.

10.2 Correlation Analysis

In addition to testing the correlations with one sample (treatment + control groups together), we can identify an absolute correlation of 0.27 from no correlation at the same design parameters. When comparing within each treatment group the detectable correlation will be at least 0.37. For comparing correlations between two groups (treatment and controls), the difference of detectable correlation is at least 0.50 at the same design parameters. In all three types of effect size calculations, the detectable effect sizes are moderate-to- large (Cohen, 1988). Note that sample size justification does not take into account the multiple but correlated outcomes within same subjects. Sample size calculation is based on methods described in Chow et al (2008) and Cohen (1988).

10.3 Accrual Rate and Duration

Based on our experience we plan to enroll 45 patients per year. It will take approximately 2.0 years to complete the enrollment of 88 patients. Given the relatively

short follow-up time (90 days), all patients will be followed for up to 90 days once enrolled.

10.4 General Statistical Plan

Patients enrolled will be presented. On-study protocol violations will also be presented. Patients who do not complete the required observations will be listed and evaluated separately as necessary. Reasons for study discontinuation and date of withdrawal from study will be presented.

Descriptive statistics related to the participant characteristics, treatment, and prognostic factors will be reported. For the continuous variables mean (95% confidence interval), median (minimum and maximum) will be reported and for discrete variables frequency and percentages will be reported. For continuous variables a two sample t-test/rank sum test will be used to compare between groups. A Chi-square test will be used for establishing association between categorical variables. For example MoCA will be compared between two groups using a contrast based t test in addition to simple two sample test either based on a linear regression or Mixed model approach when multiple observations on sample subject is considered. A discrete outcome such as prevalence of 'have any fever' between treatment and control arms will be tested using a logistic regression analyses. In order to examine the significant prognostic factors, we will use the multiple linear and logistic regression models in multi-variable setting.

A significance level of 5% will be used to declare results significant. For selected primary outcome variables we will report adjusted p-values for multiplicity in addition to unadjusted p-values. All calculations will be performed with methods described in Walker et al. (2010), Rosner (2010) and with SAS statistical software (SAS, 2003).

10.5 DSMB Statistical Monitoring Rules

Safety monitoring of the accumulated outcomes data is designed to ensure the continuing safety of the currently enrolled participants and participants not yet enrolled. This is achieved by stopping the trial early to reduce the number of participants exposed to a harmful or ineffective treatment.

10.5.1 Non-efficacious Treatment (Futility)

Due to small sample size and somewhat longer evaluation time it is not practical to stop the trial for futility. Therefore we do not use a group sequential approach for monitoring efficacy (Cytel, 2002).

10.5.2 Toxic Treatment (Safety)

We plan to monitor toxicity within each arm. The cumulative number of grade 3 or 4 toxic events will be monitored after each person is enrolled (Ray and Rai, 2011 & 2012). If the cumulative number of toxic events produces enough evidence to conclude that the

true toxicity rate is greater than or equal to 33% ($Pt0 = 0.33$) then the trial will be stopped early for safety reasons. The cumulative number of toxic events after each patient is treated will be compared to the boundary values in Table S2. If the cumulative number of toxic events after person i is treated is greater than or equal to the associated boundary value bi then the combination treatment is rejected for safety considerations. With this rule, there is only a 5% chance of stopping the trial early for lack of safety if the true toxicity rate is less than 33%. Continual assessment of the toxic events ensures we do not expose an undue number of patients to a harmful treatment. This is a conservative approach. Another approach will be using the method of Ray and Rai (2013) when monitoring will be done at the time of DSMB reports, which will be flexible and used at that stage the proportion of subjects experiencing SAEs.

Table S2. Toxicity Boundaries, N = 44, Pt0 = 0.33, and alpha=0.05

Minimum Number of Subjects	Maximum Number of Subjects	Number of Toxicities
4	4	4
5	6	5
7	7	6
8	9	7
10	11	8
12	14	9
15	16	10
17	18	11
19	19	12
20	22	13
23	23	14
24	27	15
28	28	16
29	30	17
31	32	18
33	34	19
35	37	20
36	40	21
41	42	22
43	44	23

11. SITE MONITORING

Central monitoring of clinical sites will be performed during the study by query monitoring of the EDC system and with periodic phone calls to the site study coordinators to ensure all aspects of the protocol are being followed appropriately and to address areas of concern. Original source documents will be reviewed for verification of data in the electronic database. The Investigator/participating institution guarantees direct access to original source documents (kept in subject binders) by sponsor personnel, their designees, and appropriate regulatory authorities as needed during the

monitoring process. The study may also be subject to a quality assurance audit by the sponsor or its designees, as well as inspection by appropriate regulatory authorities. It is important that the Investigator and relevant study personnel are available during the monitoring visits and possible audits and that sufficient time is devoted to the process.

12. HUMAN SUBJECTS REGULATORY REQUIREMENTS

The study will be conducted in accordance with the U.S. Food and Drug Administration's (FDA) Code of Federal Regulations (CFR 21), and Good Clinical Practice (GCP) guidelines.

Each site will obtain Institutional Review Board (IRB) approval for the protocol and Informed Consent forms, prior to initiating the study. In addition, each investigator will sign an Investigator Agreement. Protocol amendments are not allowed by any site investigator without prior approval from the Study's Primary Principal Investigator (PPI) or the Steering Committee. All changes to the protocol should be submitted to the site's IRB for review and approval as appropriate.

12.1 Institutional Review Board (IRB)

Prior to initiating the study, each Investigator must submit the following to an Institutional Review Board (IRB); the informed consent, this protocol and materials used to recruit subjects for this clinical trial. No study subjects may be recruited until documented IRB approval is obtained. Any addendums to the above documents must be resubmitted for review and approval. Each investigator will follow the requirements of their IRB on periodic reporting of the progress of the study, reporting of serious or unexpected adverse events, safety monitoring reports, and termination of the study.

12.2 Subject Confidentiality

Efforts will be made to maintain patient confidentiality for subjects enrolled in this trial. After agreeing to participate some patient identifiers will be recorded, including, but not limited to the subjects name, date of birth, telephone number, first three digits of zip code, date of admission, discharge, dates of procedures, and the subjects assigned Subject Identification Code. This information will be kept in patient binders as well as recorded in the respective eCRF. Images collected will be as de-identified as possible. Specimens collected will be labeled with the Subject Identification Code, and the date and time of collection, though they may be matched with basic demographic information later to look for possible correlations. Only approved study personnel and those included in the HIPAA Authorization will have access to identified, partially identified, and coded data. Data and specimens will be collected, stored and transported with consideration for subject privacy and confidentiality in mind. Data used for publications or dissemination of findings will be stripped of all identifiers.

12.3 Study Modification/Discontinuation

It may be necessary to modify this study protocol during the trial. All minor and major modifications to the study protocol will be approved by the Steering Committee. The DSMB Chair will be informed of any planned major modifications to the study protocol that would be considered serious alterations to the study design.

It is possible this study could be discontinued or halted by any of the study oversight bodies such as the DSMB, or the Steering Committee. If such an event occurs, clinical sites will be notified immediately, and enrollment of subjects will cease. Study related LDIVH administration and drawing of labs for study purposes will be discontinued. All study forms (paper and electronic) must still be kept and made available. If the study is discontinued prematurely, subjects who have received LDIVH as a part of this study, will still complete their 90 day follow-up visit and data collection may still occur on this visit to continue to monitor for adverse events.

12.4 Medical Safety Monitor (MSM)

An independent neurocritical care physician will serve as a medical safety monitor for this study. The MSM responsibilities will include reviewing the first 5 cases enrolled and evaluating for any safety issues. The MSM will also provide the immediate evaluation and potential management recommendations for any patient safety concerns as they are raised and as they may relate to the study procedures and this protocol. Patient safety will take priority over the scientific goals of this study. The ASTROH Project manager may request the MSM to review any situation for advice on the best way to maximize patient safety. Additionally, individual sites may obtain patient management advice from the MSM for patients enrolled in this study by contacting the MSM directly without having to go through the clinical coordinating center (University of Louisville). If there is a patient safety emergency and the MSM is not available, then Dr. Kevin Sheth (Neurocritical care physician and National Co-PI for ASTROH) will serve as a back-up for the MSM. If both the MSM and Dr. Sheth are unavailable then Dr. Robert James (National Primary Principal Investigator-PPI) will address the emergent safety concern. The PPI will be informed of all serious safety concerns that are managed by the MSM or Dr. Sheth. The MSM has the ability to directly bring concerns to the attention of the DSMB Chair at any time.

13. PUBLICATION AND PRESENTATION OF RESEARCH FINDINGS

All data from this study are owned jointly by Robert F. James, MD and J. Marc Simard, MD, PhD. All publications and presentations of data obtained as a result of this clinical trial are to be jointly approved by a majority of the Study Leadership Team which include the Primary Principal Investigator (PPI), Robert F. James, M.D. and the Co-Principal investigators: J. Marc Simard, MD, PhD, Kevin N. Sheth, MD, and J Mocco, MD, MSc. All publications or presentations that arise based on any data generated from this clinical trial, no matter who is the primary author, should include all of these investigators as co-authors.

This study will have a Writing/Publication Committee that will be tasked with producing and reviewing the manuscript submitted for initial publication of the primary findings of this study. Drs. James, Simard, Sheth, and Mocco will all be part of the writing/publication committee and this committee will be co-chaired by Drs. James and Simard. Additional membership to the Publication Committee will be determined by Dr. James and Dr. Simard. All members of the Publication Committee will be listed as authors on any manuscripts written and reviewed by the Publication Committee. The Publication Committee will determine all additional co-authors for the manuscripts it creates or reviews based on the contribution criteria that it will devise. Drs. James and Simard will jointly determine the order of authors listed for any publications related to this clinical trial. We anticipate members of the Steering Committee and the PI for each clinical site that meets recruitment goals will be included as co-authors on the initial publication of the primary findings of this study. The primary findings of this study will be submitted for publication regardless of the results of the study (favorable or unfavorable for LDIVH). Voting members of the DSMB, CEC, and the MSM will not be listed as authors for the initial publication of the primary findings of this study to prevent any conflict of interest.

Individual sites may not publish data related to this trial from their individual site until the study has been finalized and the primary initial findings have been published online or in print.. The individual sites must also obtain prior approval from Dr. James or Dr. Simard to submit an abstract or manuscript for consideration of presentation or publication with their site's data.

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15. APPENDICES

15.1 Schedule of Events

	0-48 Hrs Post Ictus	2-12 hrs Post Coiling	Upon initial Enrollment (Enrollment Day 0, 1/12 Hrs post-coiling)	Enrollment Day 0.2 (12 hours post Day 0.1)	Enrollment Day 1	Enrollment Day 2	Enrollment Day 3	Enrollment Day 4	Enrollment Day 5	Enrollment Day 6	Enrollment Day 7	Enrollment Day 8	Enrollment Day 9	Enrollment Day 10	Enrollment Day 11	Enrollment Day 12	Enrollment Day 13	Enrollment Day 14	Discharge	90 Day Follow-up	1 Year Follow-up
Review	X*	X*																			
Informed Consent	X*	X*																			
Randomization		X																			
Demographic Data	X*		X*																		
Medical History	X*		X*																		
Baseline Labs	SOC*		SOC*																		
CBC (with platelet)	SOC*		SOC*		SOC	SOC	SOC	SOC	SOC	SOC	SOC	SOC	SOC	SOC	SOC	SOC	SOC	SOC			
Anti-Xa			RES							RES ^D											
hsCRP			RES			RES		RES		RES				RES							
processing for Serum & CSF Biomarker Analysis			X			X		X ^A		X ^A				X ^A							
Sample shipment																			X ^B		
CT	SOC	SOC																	SOC		
Angiography	SOC								SOC**	SOC**	SOC**	SOC**	SOC**	SOC**							
Coiling Procedure	SOC																				
Send Images to Core			X																X		
Administration ^C			X		X	X	X	X	X	X	X	X	X	X	X	X	X	X			
aPTT ^C	SOC*		SOC*		RES	RES	RES	RES	RES	RES	RES	RES	RES	RES	RES	RES	RES	RES			
Monitor aPTT/Adjust Dosage ^C				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
WFNS SAH Scale	X	X																	X	X	
Glasgow Coma Scale	X	X								X				X					X	X	
NIH Stroke Scale	X	X								X				X					X	X	
MoCA			X							X				X					X	X	
Modified Rankin Scale	X***																		X	X	
Return to Work																			X	X	
QOLIBRI-OS																			X	X	
Barthel Index																			X	X	
Trail Making Tests																			X	X	
Lawton-IADL																			X	X	
CES-D																			X	X	
CIS-F																			X	X	

* Not duplicated, can be performed at either time point

** Any of these time points would be acceptable to satisfy this requirement, these are not duplicates of the angiography test but represent ONE angiography over this range of dates

*** Historical mRS

^A: Baseline and day# 2 biomarkers should be drawn on exact date. Day 4, 6 and 10 should be drawn as close as possible to the exact date, but may have +/-24 hours to accommodate for lab processing schedule

^B: Batch shipment. (Not per subject) First shipment will contain all samples from first 5 patients enrolled. Second and final shipment from each site will contain samples from all additional enrolled patients.

^C: Events only performed on subjects randomized to the LDIVH treatment arm (on average 1 out of 2 enrolled subjects will require these events). An aPTT will be drawn on Day #6 on the control group per ASTROH protocol

^D: The control group should have the Anti-Xa drawn within 4 hours of their next scheduled DVT prophylactic SOC subcutaneous heparin dose