The Role of Hyperoxia in the Emergency Department Treatment of Acute Ischemic Stroke

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Study Protocol and Statistical Analysis Plan

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1. PURPOSE OF STUDY

Every year in the United States alone, approximately 795,000 people will experience a new or recurrent stroke, with the vast majority of these being an acute ischemic stroke (AIS)[1]. For every minute that a stroke goes untreated, 1.9 million neurons die, underscoring the importance of early identification and treatment[2]. Additionally, it is estimated that half of all stroke patients enter the healthcare system via Emergency Medical Services (EMS)[3]. As such, evaluating potential interventions in the prehospital and emergency department (ED) phases of care is a key, yet often ignored, component of stroke treatment. Administration of 15L/min oxygen via a partial non-rebreather facemask to create systemic hyperoxia is a relatively low cost, potentially beneficial part of stroke care that could be implemented early in the prehospital and ED phases of care. However, this therapy has had conflicting results, with little data evaluating its potential benefits early after stroke onset, in the ED and prehospital phases of care, where most preclinical data suggests a potential benefit. *The central hypothesis of this study is that hyperoxia during the acute ischemic period of a stroke prior to potential recanalization decreases infarct volume and improves patient outcomes.* This clinical trial will evaluate the potential therapeutic role of hyperoxia when applied in the immediate ischemic period following a stroke in the controlled ED setting and will evaluate the effects of hyperoxia in stroke patients on the production of markers of free radical damage and inflammatory markers associated with hyperoxic lung injury.

2. BACKGROUND AND RATIONALE

Hyperoxia in stroke – therapeutic mechanism and biomarkers: Ischemic strokes are characterized by an infarct core comprised of cells having suffered irreversible damage in the setting of hypoxia that will progress to complete necrosis. This is surrounded by a "penumbra" of brain tissue, which is not yet irreversibly destined for cell death[4]. This penumbral zone contains cells whose metabolic and structural integrity have been compromised, but the damage is not irreversible should the cells be provided sufficient oxygen and metabolic substrates in a timely fashion. After the initial ischemic event, the penumbral tissue immediately surrounding the infarct core undergoes depolarization and subsequently increases its metabolic rate. Coupled with compromised cerebral blood flow and low oxygen supply, this creates a hypoxic environment that perpetuates tissue destruction and infarct size[5,6]. Tissue damage resulting from this hypoxia is mediated in part by a shift to anaerobic metabolism which in turn leads to production of excess amounts of oxygen and nitrogen free radicals and ultimately lipid peroxidation, protein denaturation, DNA damage and activation of apoptotic pathways resulting in cell death[7,8].

The utility of biomarkers to lend insight into the molecular pathophysiology of stroke and to act as potential biomarkers of diagnosis, hemorrhagic conversion, and ultimately stroke severity remains an area of active research. In stroke, it is difficult to directly measure the production of reactive oxygen species (ROS) given their short half-life within brain tissue itself, let alone in peripheral serum. Previous groups have shown that increased production of downstream products of free radical-induced lipid peroxidation leads to neurologic deterioration[9]. MMP-9 is one matrix metalloproteinase that is activated downstream of these oxidative stress pathways and contributes to destruction of the blood brain barrier and increased cerebral edema after stroke. MMP-9 also correlates with infarct volume, clinical stroke severity, and hemorrhagic transformation after thrombolysis[10,11]. IL-6 is an inflammatory cytokine that is increased during and after AIS in both astrocytes and microglia

and in peripheral leukocytes. Increased peripheral concentration of IL-6 in stroke correlates with stroke severity and the development of post-infarction infections[12,13]. One potential mechanism of hyperoxia therapy based on data in rodent models suggests that the provision of increased levels of PaO_2 will raise the amount of oxygen available to the ischemic tissues and prevent further progression down this pathway toward cell death (Figure 1).

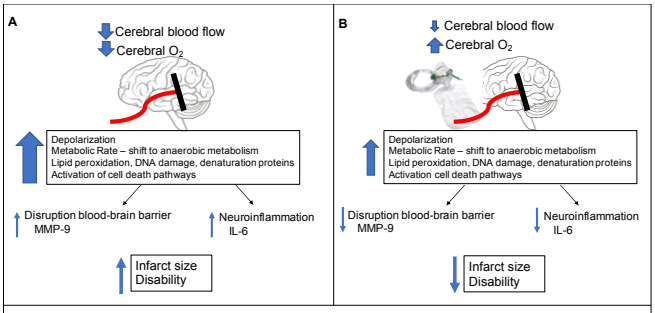


Figure 1: Proposed mechanism of hypoxic injury in AIS (A) and the mitigation of these effects with hyperoxia (B).

Hyperoxia in preclinical models: In rodent models of middle cerebral artery occlusion (MCAO), hyperoxia supplied shortly after the initiation of ischemia results in return of penumbral brain tissue oxygenation levels to near pre-ischemic levels and increases cerebral blood flow to ischemic tissues[14-19]. Furthermore, there is no increase in production of markers of ROS. In other rodent models, hyperoxia even blunted the production of matrix metalloproteinase-9 (MMP-9) and subsequently increased levels of two tight junction proteins involved in maintaining the blood brain barrier[20,21]. Rodent models also correlated this decrease in ROS with decreases in markers of cell apoptosis[14,22]. However, in gerbil brain ischemia models, hyperoxia was shown to increase ROS and lipid peroxidation[23]. This potential mechanism for adverse effects of hyperoxia has not been evaluated in humans. We will examine the effects of hyperoxia in AIS on these biomarkers (IL-6 and MMP-9) and others.

While the above beneficial effects of hyperoxia were observed in rodents using a transient MCAO model to carefully control for the potential negative effects of oxygen during reperfusion, using a permanent middle cerebral artery occlusion (pMCAO) model also saw beneficial effects of hyperoxia[24]. This model even showed an increase in systemic vascular resistance similar to prior observations in humans. However, despite the increase in systemic vascular resistance, hyperoxia still showed improved outcomes compared to controls and did not show a significant difference in cerebral blood flow to the contralateral hemisphere.

Furthermore, the window in which hyperoxia is supplied is a key determinant of the potential therapeutic benefit. Mice treated with hyperoxia during the acute ischemic phase of MCAO showed improved outcomes including increased partial pressure of oxygen in the penumbral tissue, improved function on neurobehavioral testing, and lower levels of necrosis[25,26]. Importantly, these benefits were lost when hyperoxia was extended into the period of reperfusion where hyperoxia was associated with increased ischemic tissue damage and cerebral edema[27].

Hyperoxia in humans: A pilot study in which patients were treated with 45L/min humidified oxygen via facemask started within 12 hours of their last known well (LKW) and continued for 8 hours demonstrated reduced markers of anaerobic metabolism and increased markers of neuronal integrity and mitochondrial function in the hyperoxia treated group[28]. Hyperoxia was also associated with an initial improvement in neurological function (measured by the NIHSS) and a significant difference in penumbral salvage at 4 hours (~70% in controls vs 120% in hyperoxia)[29]. However, the difference did not meet significance at 24 hours When hyperoxia was applied for even longer periods of time there was also no significant neurological improvement[30]. Thus, like in the preclinical models, the duration of the hyperoxia may influence outcomes. Additionally, the dose of “hyperoxia” likely affects the potential therapeutic benefit. In patients treated with “hyperoxia” in the form of 3L nasal

cannula for the first 24 hours after admission, there was no difference in neurological outcomes[31,32].

As a group, these human studies were relatively small studies with an overall positive effect of hyperoxia when given at high levels and started relatively early after AIS onset. However, when initiated at lower supplemental oxygen levels and/or treated further from the onset of ischemia, there was no significant improvement in patient outcomes nor evidence of harm. Hyperoxia therapy is most likely to benefit patients in the early stages after initial ischemia by limiting progression to final infarct volume while awaiting spontaneous recanalization. This is supported by the most promising studies which used 45L/min oxygen therapy for 8 hours in patients presenting within 12 hours from LKW. Thus, we have designed our study to focus on early administration, prior to potential reperfusion, of high levels of supplemental oxygen, targeting a PaO_2 of 200-400mmHg as in the rodent models.

Hyperoxia in other diseases – safety concerns: The role of hyperoxia in stroke remains surrounded by controversy based on data from other disease states. Initial studies on the role of hyperoxia in myocardial infarction (MI) showed a reduction in the degree of myocardial necrosis[33-35]. However, further studies demonstrated potential harm through decreases in coronary blood flow, myocardial oxygen consumption and cardiac output, as well as through increases in systemic vascular resistance and myocardial reperfusion injury[36-39].

Looking at a broader population of cardiac patients, a meta-analysis of patients with PaO_2 ranging from 234-604mmHg who presented with heart failure, coronary artery disease (CAD), recent coronary artery bypass grafting (CABG) surgery, or sepsis found that compared to controls, hyperoxia overall resulted in decreased heart rate, decreased cardiac output and increased systemic vascular resistance[40]. However, these changes were not consistent amongst all disease entities. CAD and post-CABG patients showed decreased heart rates, but this was not seen in heart failure or in sepsis patients. Cardiac output was decreased in CAD and heart failure patients but not in post-CABG nor in septic patients. Thus, while there continue to be concerns regarding potential harmful hemodynamic and cardiovascular effects of hyperoxia, these risks appear somewhat disease-state specific. At best, in states with a baseline impaired cardiac function, hyperoxia has no overall benefit, with the possibility for negative hemodynamic consequences.

Hyperoxia is associated with cerebral vasoconstriction in healthy subjects. Thus, it is also important to evaluate hyperoxia in the context of other brain injury states in which this potential vasoconstriction could be detrimental, such as traumatic brain injury (TBI). In TBI, hyperoxia provided in the first 48 hours increases the partial pressure of oxygen in brain tissue and reduces brain tissue lactate levels, corresponding to an improved prognosis[41]. In a clinical trial of severe TBI patients, hyperoxia resulted in: 1) improved cerebral metabolic rates of oxygen, 2) decreased lactate levels, 3) increased brain tissue oxygen levels, and 4) no increase in markers of hyperoxic lung injury (as measured by surrogate lung inflammatory biomarkers, IL-6 and IL-8)[42]. Despite these findings, safety concerns stemming largely from the potential for acute oxygen toxicity leading to the accumulation of ROS and free radicals leading to lung injury and lipid peroxidation of brain tissue limit the use of hyperoxia in TBI. While this remains the leading argument for not using hyperoxia, a recently published pilot study examining the use of hyperoxia in severe TBI, again showed no difference in the production of ROS[43].

The most commonly proposed mechanism of potential harm from hyperoxia therapy is the increased production of ROS that could not only contribute to reperfusion injury, but might also lead to hyperoxic lung injury. This issue has also been evaluated in rodent and human models of critically ill patients, through induced endotoxemia. Here, brief hyperoxia with a PaO_2 of ~400mmHg for 3.5 hours in humans resulted in no change in the ability to

mount a cytokine and inflammatory response[44]. Both hyperoxic and control subjects had similar patterns of expression of tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), IL-8, or IL-10 in response to endotoxemia. Additionally, hyperoxia treatment did not clinically alter the leukocyte population produced in response to endotoxemia. Hyperoxic lung injury has been demonstrated in patients exposed to hyperoxia for long periods of time but has not been evaluated in those with brief hyperoxia exposures[45,46]. Furthermore, rodent MCAO models suggest that this risk is low in stroke given there was no evidence of such effects when therapy was instituted immediately after the ischemic event[14-19]. Thus, by limiting the oxygen therapy in this clinical trial to no more than 4 hours, the study will minimize this potential risk.

Potential risks of hyperoxia in stroke: Further safety concerns regarding possible increased mortality resulting from hyperoxia therapy were raised in a multicenter retrospective study that determined the 28-day mortality in mechanically ventilated intensive care unit (ICU) stroke patients (including ischemic stroke, subarachnoid hemorrhage (SAH), and intracerebral hemorrhage (ICH)). This study found an increased mortality in stroke patients found to be hyperoxic at 24 hours after admission[47]. These patients were exposed to longer durations of hyperoxia than proposed in this current study. Additionally, upon subgroup analysis, increased mortality was only seen in the hyperoxic SAH and ICH groups compared to normoxic controls, and there was no significant difference in mortality in the AIS patients exposed to hyperoxia[47]. This was further supported by a second study of ICU stroke patients being mechanically ventilated that failed to demonstrate an association between oxygenation level (PaO_2) and mortality[48]. The safety of hyperoxia in AIS patients was also questioned in a recent unpublished clinical trial (NCT00414726) studying hyperoxia, that randomized patients to room air or 45L/min via facemask. The trial was terminated early, after enrolling only 85 participants, for safety concerns[49]. However, upon closer blinded review of the results, only one death in each group was determined to be possibly related to the therapy. The remainder (16/43 deaths in the hyperoxia group, 6/41 deaths in normoxia group) were determined to be unrelated to therapy. Taken together, these data suggest that the safety of hyperoxia therapy is disease-specific and short exposures to hyperoxia prior to recanalization in AIS patients are safe. However, the beneficial immediate and long-term effects of brief hyperoxia in AIS are not fully understood.

The results of the proposed study have important implications for the acute treatment of stroke. Oxygen therapy is a simple, low cost treatment that could be instituted at the point of first contact with the healthcare system for many patients, in the prehospital and ED phases of stroke care. While the European PROOF trial of hyperoxia for acute stroke recently began enrollment, it randomizes patients to receive 40L/min supplemental oxygen, a level that is not currently feasible in the prehospital setting and that lacks data to suggest it is necessary for a beneficial effect. Additionally, this therapy is to begin within four hours of subject's non-contrast CT-head and extends further into the period of potential reperfusion. Thus, understanding the effects of hyperoxia provided at more feasible and tolerable levels and proximate to the onset of symptoms has the potential to impact many patients who are otherwise lacking potential therapeutic options, some of whom are already being exposed to oxygen therapy in the prehospital setting. The proposed project will inform the design of a future clinical trial of hyperoxia and will provide the needed experience and preliminary data to develop a career in clinical emergency medicine research emphasizing the treatment of acute stroke.

3. ADMINISTRATIVE ORGANIZATION

This study will be conducted at the University of Colorado Hospital (UCH) Anschutz Medical

Campus and the UCH-Memorial Central Hospital. Subjects will be recruited, enrolled, and therapy initiated upon presentation to the Emergency Department. Arterial Blood Gas (ABG) samples will be drawn and immediately processed per their standard protocol for routine ABG testing. Venous serum samples will also be collected and processed for storage in the Emergency Medicine Research Lab or the clinical labs of the UCH-Memorial and subsequently transferred to their research storage. All serum biomarker analyses will occur in aggregate at the end of subject recruitment.

4. STUDY DESIGN

This is a multicenter, prospective, randomized, controlled, feasibility study to evaluate the feasibility of early brief hyperoxia therapy for AIS in the ED and to gather preliminary data on the potential therapeutic and adverse effects of such intervention. It is hypothesized that global hyperoxia treatment in the acute ischemic period of a stroke prior to potential recanalization will decrease infarct volumes and improve neurologic outcomes in AIS patients without increasing the production of markers correlating with ischemic-reperfusion injury and the hyperoxic lung injury. To test this hypothesis, subjects will be randomized equally between the placebo (medical air) and hyperoxia (supplemental oxygen) treatment groups.

For the purpose of this study, we define normoxia as a pulse oximetry of 94% or greater, although per AHA guidelines, supplemental oxygen is recommended only in patients with a pulse oximetry of less than 94%[50]. Based on rodent models that targeted systemic hyperoxia with a PaO2 of 200-400mmHg, hyperoxia will be defined as a PaO2 of >200mmHg. Previous literature demonstrated that 10-15L/min facemask resulted in the PaO2 of 425 +/- 43mmHg in patients undergoing cardiac catheterization found to have clean coronary arteries and 403 +/- 49mmHg in those with CAD[51]. Thus, treatment with 15L/min should be sufficient to achieve this level of hyperoxia in this study population, and will be confirmed through ABG sampling at 4 hours after initiation of treatment intervention.

Patients who are found to require supplemental oxygen according to AHA guidelines will be provided such intervention with careful documentation of the timing, duration, and dose of supplemental oxygen provided.

The design will proceed as follows: (see Figure 2)

- Randomize subjects between placebo and supplemental oxygen via partial non-rebreather facemask at 15 L/min.
- Collect study measurements
- Perform 3-month follow up for mRS
- The study duration is estimated at 30 months to allow sufficient time for subjects enrollment and completion of their 3 month follow-up

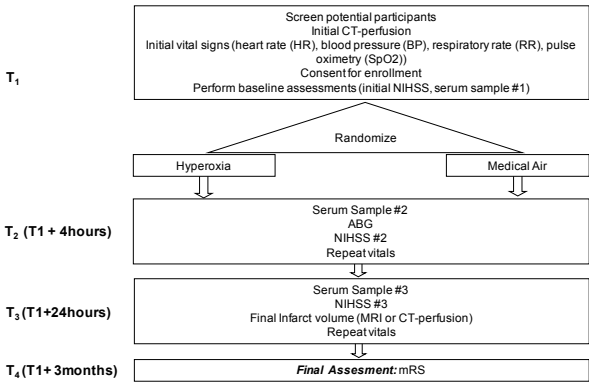


Figure 2: Schematic of study design and outcome measurements

In order to minimize bias, efforts will be made to blind the observers to the control versus treatment group. However, medical air and oxygen have different ED wall supply connectors that could be potentially identified by both the patient and the study personnel. Thus, full blinding cannot be guaranteed. To minimize bias, ED staff will initiate a given intervention and they will be instructed to not tell the patient which was started on the patient. To avoid bias with regards to infarct lesion size,

the automated RAPID software will be used to perform lesion measurements and screening for enrollment based on target mismatch. The CT-Perfusion and automated CT-ASPECT (Alberta Stroke Program Early Computed Tomography Score) have been previously used to identify patients who are early in their acute stroke trajectory with a small core infarct volume and remaining area suggestive of salvageable tissue[52]. Both are standardized quantitative or semi-quantitative measurements that can be automatically computed by RAPID software, thereby eliminating the subject assessments of providers to identify patients with potentially salvageable penumbral tissue. The assessment of the NIHSS will be extracted from the initial stroke team consult note and subsequent assessments will be video-recorded by study personnel. Videos of the assessment will be stored using a unique file name and stored in the secure School of Medicine Server. They will be reviewed in groups in a blinded fashion for scoring. At the conclusion of the study and required time for maintaining study data, all videos will be destroyed. In the case where videotaping is not permitted by a performance site, NIHSS-trained personnel (RN, MD, or PA) who are caring for the patient, will be ask to complete and score the NIHSS. In the event that the clinical team is unable to complete this assessment, the research team, also NIHSS-trained, will complete this assessment. In all cases, who/how the NIHSS was completed will be documented and blinding to the study intervention will be attempted when possible.

The primary study outcome is to determine the feasibility of enrolling and randomizing AIS patients to a therapeutic intervention within less than one hour of ED presentation. Secondary outcomes of potential therapeutic effect to inform future clinical trial(s) include penumbral salvage at repeat imaging, change in NIHSS over the first 24 hours, and 3-month mRS. To assess potential adverse effects on markers of worsening stroke severity and possible hyperoxic lung injury, levels of MMP-9, IL-6, and IL-8 will be measured at initiation, 4 hours and 24 hours. Further analysis of additional biomarkers will be considered in the future.

4.1. SUBJECT POPULATION

Eligible subjects will include acute ischemic stroke (AIS) patients aged 18 years or older who present to the UCH ED. There will be no specific selection of subjects based on sex, race or ethnicity, age, or socioeconomic status. We anticipate the study population to be representative of that of the greater Colorado Springs and Aurora, CO area with the exception that stroke prevalence increases with age and this will likely be reflected in the final study population.

Subjects with a suspected stroke who are normoxic at baseline (room air SpO2$\geq$94%) and have either 1) a target mismatch lesion on CT-perfusion [defined by RAPID automated software demonstrating a ratio of $PWI_{Tmax>6}$ lesion volume/Infarct volume (with cerebral blood flow <30% normal) greater than 1.2 or 2) a non-contrast CT-head RAPID ASPECT score of 10 (suggesting a patient has not completed their infarct), a NIHSS$\geq$ 1, and a LKW $\leq$ 12 hours will be screened for eligibility. While some vulnerable populations (i.e. children and prisoners) will be excluded, AIS by the nature of the disease has the potential to impair a subject's capability to give informed consent and places them in the category of a vulnerable population. All subjects will be evaluated for the ability to give informed consent through use of the University of California, San Diego, Brief Assessment of Capacity to Consent (UABCC) Form[53]. In cases where the subject is determined to lack the ability to provide consent, the subject's legally authorized representative (LAR) will be approached. Consent forms describing in appropriate detail the study interventions, study procedures, and risks will

be given to the participant and/or their LAR. If and when the study subject regains capacity, they will be approached for continued participation. If the subject/LAR consents for participation he/she will sign the informed consent form prior to any study procedures being done.

In terms of potential pregnant women, it is anticipated that most women will be older than likely childbearing age. While supplemental oxygen use in pregnant women experiencing fetal distress is routine, the effects of maternal hyperoxia on a developing fetus are unknown. Those of likely childbearing potential will be excluded. If a subject withdraws for any reason from the study, he/she will be replaced to meet the enrollment goal when possible. Our target enrollment will be 200 patients (~100 per arm).

4.2. INCLUSION / EXCLUSION CRITERIA

Inclusion Criteria: In order to be eligible to participate in this study, an individual must meet all of the following criteria:

1. Provision of signed and dated informed consent form from patient or legal authorized representative (LAR)
2. Subject or LAR stated willingness to comply with all study procedures and availability for the duration of the study
3. Males and female (of unlikely childbearing capacity) aged over 18 years
4. Exhibiting signs and physical examination findings suggestive of an acute ischemic infarction (with either):
    a. A target mismatch profile of CT-perfusion or MRI determined by RAPID automated software to have a ratio of $PWI_{Tmax>6}$ lesion volume/DWI lesion volume >1.2 and NIHSS $\geq$1[54]
    b. A RAPID automated software calculated non-contrast CT-head ASPECT score of 10 and LKW $\leq$ 12 hours (in patients with symptoms discovered upon waking, the LKW is defined at the midpoint between going to sleep and awakening based on previous studies that suggest most strokes during sleep occur close to awakening)[55]
5. Normoxic; a pulse oximetry of 94% or greater at time of screening without the use of supplemental oxygen

Exclusion Criteria: An individual who meets any of the following criteria will be excluded from participation in this study:

1. Current use of supplemental oxygen
2. Prisoner
3. Pregnancy or those of likely childbearing potential (i.e. age <50, no documented negative pregnancy in last 24 hours, no documented history of hysterectomy)
4. Documented blood glucose <70mg/dL
5. Concurrent treatment with another investigational drug or other intervention
6. Documented history of any of the following chronic respiratory illness that require pulmonary vasodilators or supplemental oxygen at baseline: Chronic Obstructive Pulmonary Disorder (COPD), Emphysema, Interstitial Lung Disease, Restrictive Lung Disease, Pulmonary Hypertension, Pulmonary Fibrosis
7. Documented history of any of the following autoimmune diseases: systemic lupus erythematosus, rheumatoid arthritis, scleroderma, primary biliary cholangitis, multiple sclerosis, inflammatory bowel disease

8. Currently being treated for an acute myocardial infarction and/or decompensated heart failure at the onset of initial ED presentation as reported by the ED provider
9. Plans for treatment with either IV tPA (alteplase) or endovascular therapy

4.3. STUDY INTERVENTIONS

Enrolled subjects will be randomized to receive either brief hyperoxia treatment (defined as 15L/min supplemental oxygen) or medical air (at 15L/min) via a partial non-rebreather mask for four hours from the time of study initiation (T_1). Supplemental oxygen is routinely used to correct and/or prevent hypoxemia (low blood oxygen content, defined as an arterial oxygen content of less than 60-75mmHg). To avoid invasive monitoring of arterial oxygen content, pulse oximetry is routinely used to monitor for hypoxia with values less than 90-94% considered low in most clinical scenarios. Unfortunately, there are limitations with pulse oximetry and based on the oxyhemoglobin dissociation curve and a SpO_2 of 97% could represent any PaO_2 above ~90mmHg. Thus, pulse oximetry alone is not an ideal mechanism to determine hyperoxia. Based on mouse models, this study targets a PaO_2 of 200-400mmHg in the hyperoxia treatment group. In a previous study of cardiac catheterization patients, delivery of 10-15L/Min facemask oxygen resulted in average PaO_2 in the 400s.

In this study, oxygen is being used as a drug. However, an IND exemption is requested. We feel that the use of oxygen in this scenario to prevent hypoxemia in the ischemic tissue by creating a global hyperoxia environment meets the criteria for an IND exemption. Specifically, this protocol will: be conducted in compliance with requirements for IRB review and the requirements concerning the promotion and sale of drugs; not be in support of a new indication for use or change in labeling or advertising of oxygen; not invoke exemption from informed consent; not involve a route of oxygen administration or dosage level that significantly increase the risks of the drug product. Additionally, there is no consistent predetermined dosage of oxygen that can be universally applied to all clinical contexts to determine who needs supplemental oxygen and at what dose. This decision is left to the clinical judgement of the practicing medical provider. For example, in patients undergoing procedural sedation in the ED, supplemental oxygen may be provided despite having a pulse oximetry reading greater than 92-94% to prevent desaturation. According to the AHA guidelines for stroke patients, hypoxia that they suggest warrants supplemental oxygen use is defined as a pulse oximetry of less than 94%, but the data behind this exact recommendation is also very weak[50].

Supplemental oxygen wall connections and/or oxygen tanks are standard in every hospital room and ambulance. Additional oxygen tanks are available for patients during transport as needed. Oxygen will be administered using standard oxygen tubing and a partial non-rebreather facemask. It is approved for the treatment and preventions of hypoxia and hypoxemia. Hypoxia is often measured non-invasively by a pulse oximetry and thresholds to begin supplemental oxygen therapy vary based on disease entity and provider discretion. Medical oxygen can be supplied in numerous methods, but for the purpose this study it will be supplied at flow rates of 15L/min via a non-rebreather facemask. This generally is considered to provide a fraction of inspired oxygen (FiO_2) of 0.45-0.9 in most patients and should result in systemic hyperoxia with a PaO_2 >200mmHg[56].

5. RECRUITMENT METHODS

The target sample population will include adults over the age of 18 years with an acute ischemic stroke. The demographics of the sample will reflect that of Denver and Arapahoe Counties, the major geographical recruitment area – approximately 49.8% and 50.4% female, 54.5% and 59.9% White, 29.7% and 19.5% Hispanic, respectively. There will be no specific recruitment strategies for specific demographic populations. However, given that older age is associated with increased stroke incidence, it is estimated that most subjects will be elderly.

All suspected stroke patients undergoing a rapid stroke evaluation with a CT-perfusion or non-contrast CT-Head with RAPID ASPECT score and evaluation by the stroke team at the University of Colorado Hospital will be considered for enrollment. Patients will be screened upon arrival to the ED by a trained Emergency Department Research Assistant or other study personnel using a standard recruitment script. Consent, enrollment, and initiation of treatment will be performed by a study personnel. At UCH-Memorial, only the study research assistants who are trained in the how to conduct clinical research, including the more rigorous consent process associated with research compared to routine clinical care will screen, consent, and enroll subjects. The site-PI and site-sub-I will be available for clinical questions. These research team members will undergo yearly training provided by the study PI to ensure familiarity with the consent process and study activities. Additionally, the study PI will review consent documents, screening logs, and subject assessments on a monthly basis to ensure compliance and she will be available in real time, by video chat or phone for any questions.

During the treatment, the study team will stress the importance of the 3-month follow-up phone interview and two potential phone contacts will be gathered if possible. The remainder of the study activities will occur while the patient remains in the ED or hospitalized. No specific recruitment strategies will be employed for historically underrepresented minorities.

This study will not include vulnerable populations with the exception of potentially cognitively impaired subjects due the possibility of altered mental status secondary to the ischemic injury from their stroke and elderly patients with cognitive impairment from age-associated diseases such as dementia. However, in order to protect these subjects, they will only be included when a LAR is available for consent. Given the short duration of intervention while patients remain hospitalized or in the ED and follow up limited to 3 months, we anticipated only a few subjects deemed to have capacity at enrollment will become classified as vulnerable populations (i.e. develop cognitive impairment or become incarcerated) during the study. However, all subjects will be reevaluated at 4 hours by study personnel for possible changes in their capacity to consent.

Given that subjects will be committing to a follow-up phone interview, subjects will be provided a $25 gift card for their participation at the T_3 time point.

6. CONSENT PROCESS

Upon identification of a subject meeting all inclusion and no exclusion criteria, subjects will be approached by study research personnel (either the PI or a Research Assistant) to obtain informed consent prior to initiation of any study activities. This will occur while patients are in the ED and under the primary care of the ED physician. Given the goal of randomization within one-hour of presentation to the ED, care will be taken to not interfere with ongoing medical care while still ensuring timely approach for enrollment. Consent forms describing in

appropriate detail the study interventions, study procedures, and risks will be given to the participant and/or their LAR. Consent forms will be Institutional Review Board (IRB)-approved and the subject/LAR will be asked to read and review the document. A verbal explanation will also be provided in terms suited to the subject's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants will have the opportunity to discuss the study with their family or surrogates and think about it prior to agreeing to participate. The participant/LAR will sign the informed consent document prior to any study procedures being done. Subjects/LARs will be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures. A witness signature will be not be included on the consent form. However, a signature of the study personnel completing the consent is included and required. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

When the patient's capacity for informed consent is questioned due to the nature of the stroke itself or other mental impairment, capacity will be determined using the UABCC Form. This method of determining capacity consists of a 10-item questionnaire that can be delivered in 5-10minutes and has been found to have high sensitivity and specificity and good interrater reliability[53]. If a subject is determined to not have capacity for consent, the LAR will be identified and approached for consent. If and when the study subject regains capacity, they will be approached for continued participation. If the subject/LAR consents for participation he/she will sign the informed consent document prior to any procedures being done specifically for this study.

After a patient consents for participation in the study, he/she will be randomized to either hyperoxia or medical air treatment. Randomization will occur in blocks of 20 using excel and subject treatment assignments will be determined prior to initiation of the study and enrollment of subjects.

In addition to the traditional paper consent process, the ongoing COVID-19 pandemic has necessitated the use of an optional electronic consent (eConsent) process. Bringing a paper consent form and pen to the bedside of a patient with known or suspected COVID-19 and then taking these out of the room would violate infection control principles and policies. Additionally, the current pandemic presents challenges to obtaining consent from the LAR who may not be physically present in the ED at the time of consent due to a variety of reasons including limited visitation policies and/or the need to self-quarantine. Therefore, an eConsent process will also be used. In these cases, if a subject has decisional capacity and/or the LAR is physically present in the ED, but there is concern for COVID-19 infection, the research team will call the subject on their hospital room phone or personal phone and request to email the subject/LAR a copy of the eConsent form. The same process for informed consent, including explanation of the consent process and study, and opportunity for questions, will occur via telephone. At the end of the conversation, the subject/LAR will be asked to complete the eConsent Survey via REDCap. The research team and witness signatures will also be documented via REDCap. The same process will be used if the subject lacks decisional capacity and the LAR is not physically present in the ED for consent.

As blood will be collected as part of the study protocol for analysis of serum biomarkers of stroke, subjects will also be consented to have any remaining serum maintained in CU repository in the Emergency Medicine Research Lab. Blood sample collection for research purposes will not be optional for subjects participating in the study, as reflected in the consent form. However, use of the blood sample for future research is optional. Blood samples will be obtained from consenting subjects and immediately processed for storage in freezers with unique sample identifiers. Subjects will not have access to any individual results from this study or such future analysis. Future analysis of blood samples markers will be subject to COMIRB requirements, and approval for the initiation and continuance of such activities. Upon completion of this study, blood values and correlating clinical information will be linked and all information will be de-identified. All blood analyses will be completed in aggregates, not at the individual subject level.

7. STUDY PROCEDURES

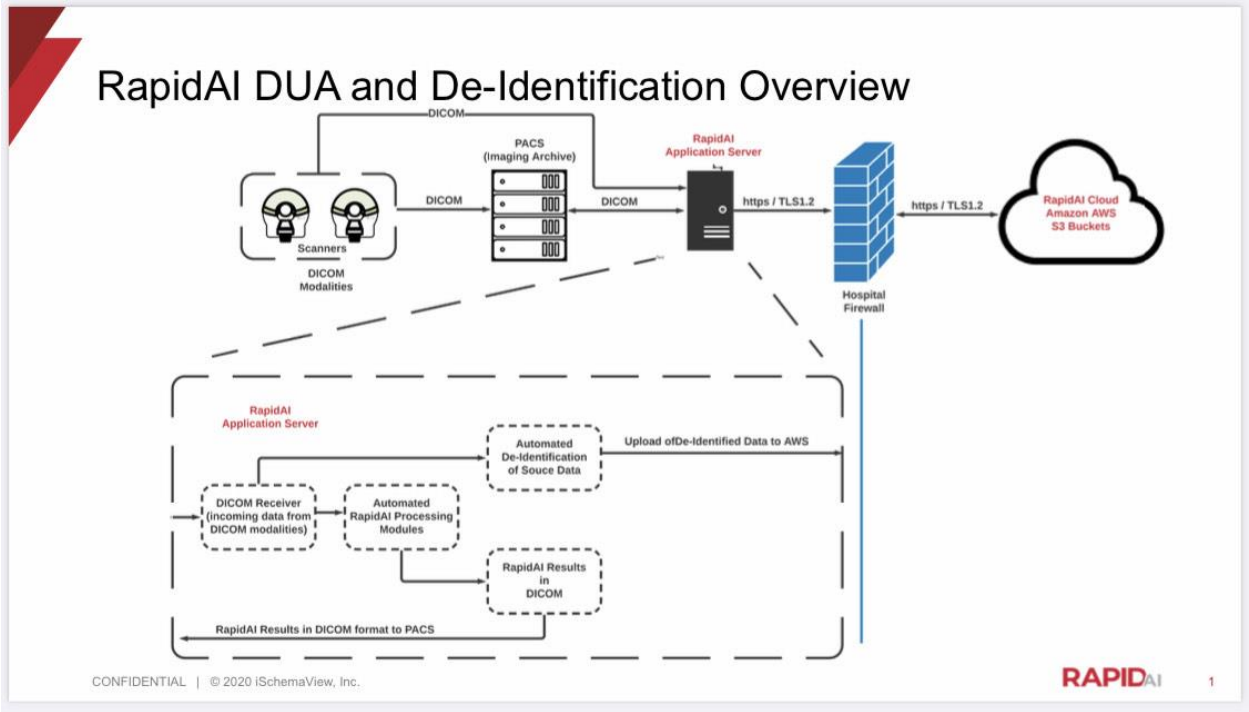
As part of the screening and enrollment process, patients with suspected AIS will have either a non-contrast CT head with RAPID calculation of ASPECT score or a CT-perfusion head. Using standard stroke alert treatment protocols at this institution, the RAPID software is used in most potential stroke patients at our institution and will be used to calculate infarct and penumbral volume and targeted diffusion/perfusion mismatch for this study. As part of the screening process, medical charts and EMS reports will be accessed by study personnel as needed to ensure potential subjects meet all inclusion/exclusion criteria. The screening and enrollment procedures along with collection of initial vitals will occur almost simultaneously given the very acute nature of the presentation and desired short interval from ED presentation to randomization. No data will be collected during the screening process that is not part of routine patient care prior to subject enrollment. Routine patient demographics, past medical history, medications, and prehospital assessments will be obtained from chart review. No research data that is not already part of routine patient care will be entered into the subject medical records. Randomization will occur in a 1:1 fashion between the two treatment groups. The estimated study duration is 30 months to allow for full enrollment of subjects and the 3-month mRS assessment. Each subject will be followed for a total of 90 days from enrollment to final assessment. No results from this study will be shared with subjects.

Once consented and enrolled in the study, subjects will have the following procedures: (See Table 1 for Schedule of Activities).

1. *Infarct progression:*
    a. Initial CT-perfusion head or non-contrast CT-Head with RAPID ASPECT score – multimodal CT-head imaging will be obtained upon ED presentation according to local stroke protocols. Targeted mismatch criteria will be based on previous standards identified in the DEFUSE 3 trial, using RAPID generated CT maps (RAPID, iSchema View, Menlo Park, CA)[57]. This study measurement will be completed as part of the screening process to ensure eligibility with regards to a target mismatch lesion. However, upon completion of the CT-perfusion or non-contrast CT-Head, the total volume of at-risk tissue (both penumbral and infarcted tissue) will also be determined and recorded. This is part of the routine medical treatment for acute stroke patients.
    b. Follow-up MRI-head, or a repeat CT-head in patients unable to obtain an MRI, will be obtained at roughly T3 according to standard stroke imaging protocols. The total volume of infarcted tissue will be determined and recorded. Repeat

imaging usually between 24-72 hours is part of the standard of care for most stroke patients to ensure there has not been hemorrhagic conversion. Only these repeat imaging studies that were routinely obtained for standard patient care will be used for further determination of infarct progression. Specific study-dedicated imaging will not be obtained.

c. The ratio of the volume of at-risk tissue (T0 – penumbral and infarcted tissue) to the volume of final infarct at subsequent reimaging will be compared between the placebo and hyperoxia groups. Volume assessments will be generated from both the CT-perfusion or non-contrast CT-Head at screening and the subsequent MRI using RAPID (iSchema View). These image files contain PHI, but are de-identified prior to processing by RAPID under the below process and PHI remains within the UCHealth firewall.



2. *Analysis of serum markers of free radical ischemic-reperfusion injury and hyperoxic lung injury*:

a. Whole blood and serum samples will be collected using routine phlebotomy and venipuncture procedures. Serum will be collected in standard blood collection tubes, subsequently isolated from whole blood and stored in small aliquots for further analysis. All samples will be labeled with unique study identifiers to ensure blinded analysis but still allowing for correlation with specific patient outcomes. To decrease variability between analysis of serum markers, serum will be collected and stored in the ED cold storage facility until samples can be run in batches.

b. Analysis of specific markers (MMP-9, IL-6 and IL-8): Serum levels will be determined using the Millipore custom-designed Milliplex® multiplex assays in accordance with the manufacturer recommended protocol. This assay uses bead-based immunodetection of multiple biomarkers simultaneously from 25uL of serum in 96-well plates. The technology also contains internal controls to help ensure accurate determination of serum levels of biomarkers. All sample analysis will be performed on a blinded basis in batches, using a single Luminex xMap

core instrument. Further undetermined biomarkers may also be analyzed separately and/or in conjunction with these selected biomarkers. This technology has the ability to analyze related biological pathways that may be related to underlying pathophysiology that is being assessed through the analysis of MMP-9, IL-6 and IL-8.

3. *Functional Outcomes:*
    a. NIHSS score: The NIH Stroke Scale (NIHSS) is a standardized tool to measure stroke severity. Study observers will be required to complete the National Stroke Association NIHSS certification online course. As part of the training, interrater reliability between study observers will be determined. This assessment is part of all initial stroke evaluations by ED and/or neurology physicians. Subsequent NIHSS assessments at T_1 and T_2 will be video-recorded by trained study personnel and/or EDRAs. However, in the case the videotaping is not permitted by a performance site, NIHSS-trained personnel (RN, MD, or PA) who are caring for the patient, will be asked to complete and score the NIHSS. In the event that the clinical team is unable to complete this assessment, the research team, also NIHSS-trained, will complete this assessment. In all cases, who/how the NIHSS was completed will be documented and blinding to the study intervention will be attempted when possible. The preference will be to have the score determined in a blinded manner upon review of the assessments at a subsequent time point.
    b. mRS scoring: The modified Rankin Scale, mRS, is another functional scale that assesses for neurologic disability on a scale of 0-6 (0-asymptomatic; 6-death). A historical mRS will be extracted from the patient chart and/or calculated at patient presentation. Patients and/or their caregivers will be interviewed by phone at 3months using a series of standardized questions regarding their neurologic disability to determine this score.

4. *Arterial puncture and ABG* – Hyperoxia (PaO2 >200mmHg) will be confirmed by an ABG sample from subjects. These will be obtained by licensed medical personnel trained to perform arterial punctures (i.e. nurses, physicians, respiratory therapists). Standard procedures for obtaining an arterial blood sample will be employed to reduce the risk of complications. To decrease variability between ABG analysis, ABGs will be performed using standard operating procedures. The site of the arterial puncture and correlating extremity will be evaluated for potential complications (increased pain, discoloration, erythema, hematoma formation, bleeding). If there are any concerning findings on these exams, the study PI will be notified and the subject will be monitored again at T_2 and/or until the event is felt to resolve.

5. *Vital signs* - The determination of need for additional supplemental oxygen in a patient that becomes hypoxic (pulse oximetry<94%) will be determined by the subject's treating physician irrespective of study interventions. Should a patient be found to require additional supplemental oxygen while enrolled in the study, this will be noted and the patient will continue through the final study assessments. The patient's treating physician will also be responsible for routine monitoring and interpretation of other vital signs such as blood pressure and heart rate. If at study assessment time points the study personnel note a subject to have significant vital sign abnormalities (SpO2<94%, SBP>180mmHg, DBP>110mmHg, SBP<90mmHg, HR>120, HR<50), the study PI and patient's treating physician will be notified to ensure proper documentation. Interventions will be left to the discretion of the patient's treating physician.

Table 1: Schedule of Activities:

Procedures	Baseline/Enrollment ED presentation -T0 (ED arrival +/-60min)	T1 (T0+4hours +/- 20min)	T2 (T0+24hours +/- 4hours)	T3 - Final Assessment (T0+3mo +/- 2weeks)
Informed consent	X			
Demographics	X			
Medical history	X			
Randomization	X			
Administer study intervention		X		
Vital signs: HR, BP, RR, SpO2	X	X	X	
ABG		X		
CT-perfusion or CT-head with ASPECTS	X			
Repeat MRI-head (CT-head if unable to get MRI)[a]			X	
Serum sample[b]	X	X	X	
NIHSS	X	X	X	
mRS	X			X
Adverse event review and evaluation		X	X	
[a] only routine re-imaging will be obtained [b] tested for selected biomarkers (IL-6, IL-8, and MMP-9)				

Assessment of study intervention adherence:

1. Compliance with study interventions will be directly observed at the given time points. If at any observation period a subject is found not to be compliant with the study intervention, medical records will be reviewed to determine the timing of termination of the supplemental oxygen/medical air.
2. All cases of non-compliance with study interventions will be recorded.

8. RISKS TO SUBJECTS

Hyperoxia has been explored in various disease states. While meta-analysis in myocardial infarction did not show an increase in mortality, hyperoxia has been shown to have negative cardiovascular and hemodynamic effects in cardiac patients. In some, but not all cardiac disease states, there were decreases in heart rate and cardiac output and increases in systemic vascular resistance[40]. These potential decreases in cardiac output and increases in

systemic vascular resistance could negatively impact stroke patients, especially those with perfusion dependent lesions.

The most commonly proposed potential harm from hyperoxia therapy is the increased production of reactive oxygen and nitrogen species that not only may contribute to reperfusion injury, but may also lead to hyperoxic lung injury. Rodent MCAO models suggest that this risk is low given there was no evidence of such effects when therapy was instituted immediately after the ischemic event[14-19]. This issue has also been evaluated in rodent models and in human models in critically ill patients. In an endotoxemia model (induced by the administration of lipopolysaccharide derived from E.coli), brief hyperoxia with a PaO2 of ~400mmHg for 3.5 hours in humans resulted in no change in the ability to mount a cytokine and inflammatory response[44]. Both hyperoxic and control subjects had similar patterns of expression of tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), IL-8, or IL-10 in response to endotoxemia. Additionally, hyperoxia treatment did not clinically alter the leukocyte population produced in response to endotoxemia, nor the ability for neutrophils to undergo phagocytosis. Hyperoxic lung injury has been demonstrated in patients exposed to hyperoxia for long periods of time but has not been evaluated in those with brief hyperoxia exposures[45,46]. Thus, by limiting the oxygen therapy in this clinical trial to no more than 4 hours, the study will minimize this potential risk.

Further safety concerns regarding possible increased mortality resulting from hyperoxia therapy were also raised with a multicenter retrospective study that determined the 28-day mortality in all mechanically ventilated intensive care unit (ICU) stroke patients (including ischemic stroke, subarachnoid hemorrhage (SAH), and intracerebral hemorrhage (ICH)). This study found an increased mortality in all stroke patients found to be hyperoxic (defined as a 24-hour post-admission PaO2 >300mmHg)[47]. However, upon subgroup analysis, increased mortality was only seen in the hyperoxic SAH and ICH groups compared to normoxic controls, and there was no significant difference in mortality in the AIS patients exposed to hyperoxia (43% in-hospital death at 28 day in normoxic patients vs 57% in hyperoxic patients, p=0.13)[47]. This was further supported by a second study of ICU stroke patients being mechanically ventilated that failed to demonstrate an association between oxygenation level (PaO2) and mortality[48]. The safety of hyperoxia in AIS patient was also questioned in a recent unpublished clinical trial (NCT00414726) studying hyperoxia, that randomized patients to room air or 45L/min via facemask. The trial was terminated early, after enrolling only 85 participants, for safety concerns[49]. However, upon closer blinded review of the results, only one death in each group was determined to be possibly related to the therapy. The remainder (16/43 deaths in the hyperoxia group, 6/41 deaths in normoxia group) were determined to be unrelated to therapy. However, the exact cause of death was not reported. Additionally, there similar rates of cardiac disorders between the hyperoxia vs normoxia groups (4/43 vs 5/41) and pulmonary related adverse events (3/43 vs 2/41). As such, a similarly designed clinical study in Europe began recently. Taken together, the data suggests that the safety of hyperoxia therapy is disease-specific and short exposures to hyperoxia in AIS patients is safe. However, the exact immediate and long-term effects of brief hyperoxia in AIS are not fully understood.

In this study, the patients will have both a venipuncture for serum samples and an arterial blood gas (ABG) analysis performed. While venipuncture for various laboratory analyses is part of the standard of care for stroke patients, only a select number of patients would normally be exposed to this procedure – usually limited to patients with questionable respiratory status. ABGs carry the risk of pain associated with the needle puncture and increased risk of bleeding and/or hematoma formation, arterial vasospasm causing distal

ischemia that affects the distal circulation in patients without good collateral blood flow, and infection. These are significant risks compared to venipuncture. However, these complications from an ABG are still rare when performed by trained personnel. Additionally, while there are no alternatives to verify arterial oxygen content, the number of ABGs performed is limited to a single draw after the institution of hyperoxia treatment to verify hyperoxia.

10. POTENTIAL BENEFITS TO SUBJECTS

Preclinical and limited human data suggests that this treatment may improve neurologic outcomes in a disease that is a leading cause of world-wide morbidity and mortality. The study intervention has the potential to increase cerebral blood flow to the penumbral tissue at risk for cell death, decrease the production of free radicals leading to reperfusion injury, help maintain the blood-brain barrier, and thereby decrease the final infarction volume and improve neurologic outcomes in stroke patients[14-17,20-22,26,28,58]. Additionally, in a study that provided supplemental oxygen via a 40% venturi mask compared to nasal cannula in severe stroke patients, there was also a non-significant trend toward lower rates of in-hospital mortality and co-morbidities such as fever, pneumonia, and respiratory failure[58].

By participating in this study, patients will be part of research that furthers the understanding of the potential therapeutic role of hyperoxia. Additionally, to date, there are limited potential therapeutic interventions for stroke, with few patients being eligible for tPA or presenting with a lesion and timeframe that is amenable to endovascular therapy. This study will shed light on these potentials for this novel therapy for stroke.

11. COSTS FOR PARTICIPATION

There will be no additional costs to the subject resulting from study activities.

12. PAYMENT FOR PARTICIPATION

While participation in the study is voluntary, subjects will be asked to agree to a follow-up telephone interview 3-months after their enrollment. To compensate them for their time, subjects completing all study interventions including this 3-month interview will receive a $25 gift card or check. This will be disbursed after the completion of the interview by mail. If a subject is unable to complete the study, they will not be paid for their participation. There will be no other forms of compensation and/or reimbursement.

13. SUBJECT WITHDRAWALS

Due to the brief nature of the intervention limited to only 4 hours of therapy, it is not anticipated that many (if any) subjects will be withdrawn by the investigator from the research without their consent. An investigator may discontinue the study intervention for the following reasons: significant study intervention non-compliance (more that 20 minutes of non-compliance with study intervention); if any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant; If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation.

Discontinuation of hyperoxia treatment arm does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by the study protocol. If a clinically significant finding is identified (including, but not limited to: an alternative diagnosis besides stroke, evidence of hypoxia prior to enrollment, baseline requirement of supplemental oxygen use) after enrollment, the investigator or qualified designee will determine if any change in participant management is needed. Any new clinically relevant finding will be reported as an adverse event (AE).

The data to be collected at the time of study intervention discontinuation will include the following: reason for discontinuation; time of discontinuation; medically necessitated interventions as a result of discontinuation.

Participants are free to withdraw from participation in the study at any time upon request. The reason for participant discontinuation or withdrawal from the study will be recorded on the Case Report Form (CRF). Subjects who sign the informed consent form, are randomized and receive the study intervention, and subsequently withdraw, are withdrawn or discontinue from the study, will be not be replaced.

14. PRIVACY AND CONFIDENTIALITY OF SUBJECTS AND RESEARCH DATA

Participant confidentiality and privacy will be ensured through careful data handling procedures. All paper forms for study documentation will be stored in locked cabinets, and only research staff will have access to the documents. Serum samples will be immediately coded into a secure, HIPAA-compliant database (either REDCap or a password-protect secure server at the University of Colorado). All study data will be de-identified after chart extraction and stored in the database to maximize the security of the electronic data. All data will be secured in locked offices and all electronic data will be stored on encrypted servers, as per institutional policy. All computer systems at the University of Colorado Anschutz Medical Campus have the level and scope of security exceeding that established by the HIPAA Security Rules. Imaging data is processed using RAPID AI. In cases where images are not automatically processed by RAPID, i.e. subsequent MRI, these raw DICOM files containing PHI will be processed with RAPID technology within the hospital firewall (see figure above regarding RAPID DUA and de-identification overview). No data will be shared with anyone who is not directly involved with the study, and findings will only be reported in aggregate.

In order to obtain required clinical information, study personnel will require access to the subject's medical charts through eRecord. Only the predetermined information will be reviewed and collected. Subjects will not be re-contacted outside of the scheduled study activities if they have chosen to opt-out of future contact. Data and unused samples will be stored for a minimum of 6 years after the completion of the study.

15. DATA / SAMPLE STORAGE FOR FUTURE USE

The field of biomarkers in stroke research is used to help predict outcomes and better understand the pathophysiology behind stroke is rapidly changing. To ensure simplicity of study design and to enhance the feasibility of this study, only three biomarkers, MMP-9, IL-6 and IL-8 have been chosen upon initial review to be included in biomarker analysis. However, additional biomarkers may also be chosen for further analysis and serum will be stored within the ED Cold Storage space for future analysis that could improve the understanding of the biological mechanism underpinning the effects of hyperoxia in AIS.

These samples will not be analyzed on an individual basis, but rather analyzed and reported in aggregates. Thus, it represents minimal additional risk to study subjects to store the serum for future biomarker analysis. The physical samples will be de-identified and coded such that they may be linked to the clinical information for the patient, which will also be de-identified after all data collection for the study has been completed. All samples will be stored in a HIPAA-compliant, restricted access ED Cold Storages space. Only study personnel will have access to the samples. Samples will aliquoted into smaller vials to help limit degradation of samples through repeated freeze-thaw cycles for up to 6 years. A password protected document on a UC secure server will be used to track the usage of individual aliquots of samples.

During the conduct of this study, an individual participant can choose to withdraw consent to have biological specimens stored for future research. However, withdrawal of consent with regard to biosample storage may not be possible after the conclusion of this study once samples have been de-identified. Samples will only be released for future analysis after a formal request process. The process will include a request for a COMIRB approved-protocol and/or protocol amendment from an individual or team of individuals associated with this original study. The released sample and accompanying clinical information will be de-identified.

16. DATA AND SAFETY MONITORING PLAN

Only trained research assistants at UCH-Memorial will be responsible for consent of subjects, with support of the site-PI, site-sub-I and/or PI as needed (see consent section for details). The research assistants at UCH-Memorial will be responsible for completion of patient assessments and reporting of potential safety issues and/or protocol violations to the site-PI, site-sub-I and PI. In addition, the PI will review data assessments uploaded to REDCap monthly for completion. Site screening/enrollment logs will be reviewed monthly. Study personnel at both sites will undergo an initial training by the PI at onset of study which will include review of the study protocol, inclusion/exclusion criteria, the consent process, methods of sample collection and processing, patient assessments, and standardized chart review methods. The trained research assistants at both sites will have complete required human subjects research trainings and specific training to ensure accuracy of the NIHSS scoring prior to completing these assessments on any subjects. A standard operating procedures (SOP) manual will also be provided for references. Training will be refreshed annually or sooner if there are protocol changes and/or any compliance concerns.

Safety oversight will be under the direction of a Safety Monitoring Committee (SMC), composed of individuals with the appropriate expertise, including stroke, neurocritical care, and emergency medicine. Members of the SMC will include members of the investigative team. However, measures will be in place to minimize perceived conflict of interest. The SMC will meet at least semiannually to assess safety and efficacy data on each arm of the study.

Assessment of safety measures and adverse events:
Study personnel and the subject's treating physician will regularly monitor subjects for safety measures and adverse events. This includes practices that are associated with routine patient care (i.e. vital sign assessments, assessment of neurologic deterioration, development of respiratory compromise). Specific monitoring includes the following:

1. Vital signs - The determination of need for additional supplemental oxygen in a patient that becomes hypoxic (pulse oximetry < 94%) will be determined by the subject's treating physician irrespective of study interventions. Should a patient be found to require additional supplemental oxygen while enrolled in the study, this will be noted and the patient will continue through the final study assessments. The patient's treating physician will also be responsible for routine monitoring and interpretation of other vital signs such has blood pressure and heart rate. If at study assessment time points the study personnel note a subject to have significant vital sign abnormalities (SpO2<94%, SBP>200mmHg, DBP>110mmHg, SBP<90mmHg, HR>120, HR<50), the study PI and patient's treating physician will be notified to ensure proper documentation. Interventions will be left to the discretion of the patient's treating physician.
2. Arterial puncture and ABG – Hyperoxia (PaO2>200mmHg) will be confirmed by obtaining an ABG in subjects. Personnel trained in obtaining arterial punctures (i.e. nurse, physician or respiratory therapist) will be notified and available to obtain the ABG four hours after initiation of study intervention. Standard procedures for obtaining an arterial blood sample will be employed to reduce the risk of complications. To decrease variability between ABG analysis, ABGs will be performed using standard operating procedures. The site of the arterial puncture and correlating extremity will be evaluated for potential complications (increased pain, discoloration, erythema, hematoma formation, bleeding) immediately after obtaining the ABG and at T2.
3. Hemorrhagic conversion – as part of the standard neurological care, patients are monitored for neurological deterioration. Patients exhibiting neurological decline will receive a CT-head and/or further imaging for evaluation of potential hemorrhagic conversion at the discretion of the treating physician. Based on previous studies in AIS, it is expected that between 13-43% of subjects will undergo spontaneous hemorrhagic conversion on imaging[59]. Any such cases will be reported as an adverse event.
4. Development of respiratory compromise – While patients are continuously assessed by their treating medical team, study personnel will specifically assess study subjects at the T0+4hour and T0+24hour evaluations for worsening respiratory status and the need for additional supplemental oxygen. At the final 3-month assessment, patients' medical records will also be reviewed for evidence of respiratory deterioration while the patient was hospitalized and up to this final assessment including: need for additional supplemental oxygen, need for non-invasive positive pressure ventilation, mechanical ventilation, development of pulmonary edema, development of pneumonia.
5. Development of post-stroke infections – Neuroinflammation after a stroke also results in immunosuppression. As such, this places patients are increased risk of post-stroke infections, the two most common being pneumonia and urinary tract infections which may account for up to 30% of deaths after an AIS[60]. Additionally, there may be sex-based differences in post-stroke infections that could be related to underlying hormone status[61]. As such, patients will be screened at the 3-month assessment to determine if they experienced any post-stroke hospitalizations and/or treatment for pneumonia, urinary tract infections, sepsis, or other infections. In addition to asking subjects directly at this assessment, their medical record will also be reviewed for re-admissions while it is being reviewed for the above respiratory compromise.

Definition and Classification of Adverse Events: An adverse event means any untoward medical occurrence, unintended sign, symptom, or disease temporarily associated with the use of an intervention in humans, whether or not considered intervention-related. All AEs will be documented and analyzed further. Subjects will be monitored for signs of an AE at T0+4hr and T0+24hours.

An adverse event (AE) or suspected adverse reaction is considered "serious" if it results in any of the following outcomes: death, a life-threatening adverse event, prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

For adverse events (AEs), the following guidelines will be used to describe severity.

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning and/or the need for further supplemental oxygen or antibiotic treatment.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events may include need for mechanical ventilation, vasoactive infusion, are usually considered potentially life-threatening or incapacitating.

The PI will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

The following AEs are considered to be expected:

1. Bleeding and/or hematoma formation at sight of arterial puncture
2. Pain at the sight of arterial puncture lasting after collection of ABG
3. Hemorrhagic conversion in up to ~40% of study subjects

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study observation time points or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate case report form (CRF). Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study intervention (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as medical history and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

The study PI will record all reportable events into a database with start dates occurring any time after randomization until completion of the study. At T0+24hours, study personnel will inquire about the occurrence of AE/SAEs since the last observation point. Solicited and unsolicited events will be captured. Unsolicited AEs will be identified by asking participants if they "have noticed anything different since beginning the treatment after the onset of your stroke." Solicited AEs will include: 1) any trouble breathing, 2) development of pneumonia, 3) need for additional oxygen therapy, 4) hemorrhagic conversion of the stroke. Events will be followed for outcome information until resolution or study completion.

17. DATA ANALYSIS PLAN

The primary endpoint of this study is feasibility. We hypothesize that the challenges of enrolling and randomizing AIS patients to a therapeutic intervention within less than one hour of ED presentation can be addressed. The number of potential subjects meeting the inclusion criteria and the relative rates of enrollment over time will be tracked. Additionally, the timing to enrollment and initiation of treatment intervention will be monitored. The primary endpoint of this study is feasibility as measured by the mean time from presentation to the ED to randomization and initiation of intervention. We hypothesize that we will be able to enroll, randomize and initiate treatment intervention in less than one hour for 80% of the enrolled subjects.

This study is not powered to detect significant differences in secondary or exploratory outcomes. However, analysis of secondary outcomes will occur both on an intention-to-treat and a per-protocol basis. Penumbral salvage will be reported as an average and as a standard deviation of the mean. We plan to enroll a total of 200 patients. Based on previous data that showed a decrease in the relative infarct volume growth (infarct volume at presentation/infarct volume at 4 hours) in patients treated with hyperoxia (87.8 +/-22%) compared to controls (149.1 +/- 41%)[29], 5 patients per arm would be needed to detect a similar difference with a power of 90% and type I error rate (α) of 0.05[62]. However, this effect was seen in a small study with less than 10 patients per treatment arm. Thus, such dramatic changes in infarct volume may not be expected, but even a smaller reduction in infarct volume will be clinically important as small reductions in infarct volume can have profound clinical effects if it is in a key cerebral territory. If you assume 20% of patients for some reason do not receive standard follow-up imaging to allow for calculation of infarct progression, a standard deviation of 41%, 80% power and α of 0.05, the proposed sample size will be able to detect an absolute difference in infarct volume growth of 18% between the control and hyperoxia.

In terms of effects on clinical patient outcomes, both the effect on 3-month mRS and NIHSS will be determined. The DEFUSE-3 study found the median NIHSS score in AIS patients evaluated for possible extended-window thrombectomy was 16 with an interquartile range of 10-20[63]. Based on these results and our proposed sample size for feasibility and a 29% loss to follow-up, we will be able to detect a two-point difference in NIHSS between the hyperoxia and control groups at 24hours with 80% power and a type I error (α) of 0.05. Given the large potential number of confounding events between the time of study intervention and a 3-month mRS, this will be analyzed on an exploratory basis. The average 3-month mRS in both the hyperoxia and control groups will be compared. Finally, an exploratory analysis of the effects of hyperoxia on biomarkers of stroke severity and hyperoxic lung injury will be evaluated over time. The change in level of biomarkers (MMP-9, IL-6, and IL-8) over time will be analyzed and reported using the mean and standard deviation and will be depicted graphically.

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