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NEUROCHEMICAL FACTORS ASSOCIATED WITH THE INITIAL PATHOPHYSIOLOGICAL REACTION TO LARGE VESSEL OCCLUSION STROKE

Sarah R. Martha
University of Kentucky, sarah.martha@gmail.com
Author ORCID Identifier:
https://orcid.org/0000-0002-1882-1419
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Sarah R. Martha, Student

Dr. Terry A. Lennie, Major Professor

Dr. Debra K. Moser, Director of Graduate Studies

NEUROCHEMICAL FACTORS ASSOCIATED WITH THE INITIAL PATHOPHYSIOLOGICAL REACTION TO LARGE VESSEL OCCLUSION STROKE

DISSERTATION

A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in the
College of Nursing
at the University of Kentucky

By
Sarah Rose Martha
Lexington, Kentucky
Director: Dr. Terry A. Lennie, Professor of Nursing
Lexington, Kentucky
2019

ABSTRACT OF DISSERTATION

NEUROCHEMICAL FACTORS ASSOCIATED WITH THE INITIAL PATHOPHYSIOLOGICAL REACTION TO LARGE VESSEL OCCLUSION STROKE

Ischemic stroke is the leading cause of disability world-wide and affects over 800,000 people per year in the United States. The majority of these strokes are ischemic due to a blockage of blood flow to the brain. Damage to the brain occurs at the onset of stroke, neuronal cell death is irreversible and therefore, quick treatment to remove blockage is critical factor in the recovery from stroke. Mechanical thrombectomy as a treatment for ischemic stroke provides an ideal opportunity to collect blood distal and proximal to the cerebral thrombus to examine neurochemical changes occurring during stroke.

The purpose of this dissertation was to explore the trajectory of neurochemical changes that occur in response to ischemic stroke during the first 72 hours and the physiological response from stroke patients to improve stroke outcomes. The specific aims were to: 1) to determine whether venous blood gases predict infarct volume and/or mortality in acute ischemic stroke in young male rats; 2) determine whether venous blood gases predict infarct and edema volume, and/or mortality in acute ischemic stroke in aged male and female rats; 3) compare the presence and relative concentrations of acid/base and electrolytes in static blood distal to thrombus and in peripheral blood drawn from adults who received thrombectomy for ischemic stroke and identify associations to post-reperfusion functional outcomes.

Specific Aim One was addressed by evaluation of young (three-month old) Sprague-Dawley rats that underwent permanent or transient middle cerebral artery occlusion (MCAO). Pre- and post-MCAO venous samples from permanent and transient models provided pH, carbon dioxide, oxygen, bicarbonate, glucose, hematocrit, hematocrit, and electrolyte values of ionized calcium, potassium and sodium. The analyses indicated that mean differences in the blood gas and electrolytes between pre- to post-MCAO and pH and iCa2+ were predictors of infarct volume in the permanent MCAO model. The second aim was addressed by evaluation of aged (18 month old) male and female rats pre-MCAO, post-MCAO, and at 72 hours of permanent MCAO venous

blood gas samples (pH, carbon dioxide, oxygen, bicarbonate, glucose, hematocrit, hematocrit, and electrolyte concentrations of ionized calcium, potassium and sodium). Changes in pH (from pre-MCAO to post-MACO and post-MCAO to 72 hours) and changes in Na+ and iCa2+ (from post-MCAO to 72 hours) were predictors of infarct volume and edema volume, respectively in both sexes. Cox regression revealed there was a 3.25 times increased risk for mortality based on changes (cut-off range within -2.00 to -7.00) in bicarbonate levels (pre- to post-MCAO). The third aim was addressed by evaluation of acid/base balance (pH, carbon dioxide, oxygen, bicarbonate, ionized calcium, potassium and sodium) of ischemic stroke patients who underwent mechanical thrombectomy. Our results suggests sex differences matter in ischemic stroke populations. Significant differences occur within proximal blood between the sexes. Additionally, females had approximately 2.5 hour increased time between stroke symptom onset to thrombectomy completion time (described as infarct time). Changes in bicarbonate and base deficit were predictors of infarct time, but only in our female population.

KEYWORDS: Acid/Base Balance, Electrolytes, Large Vessel Occlusion, Ischemic Stroke

Sarah Rose Martha

(Name of Student)

April 24, 2019

Date

NEUROCHEMICAL FACTORS ASSOCIATED WITH THE INITIAL PATHOPHYSIOLOGICAL REACTION TO LARGE VESSEL OCCLUSION STROKE

By
Sarah Rose Martha

Dr. Terry A. Lennie
Director of Dissertation

Dr. Debra K. Moser
Director of Graduate Studies

April 24, 2019
Date

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CHAPTER 1. INTRODUCTION

Background

Ischemic stroke is the leading cause of disability in the United States and affects over 800,000 Americans per year [1]. It is the third leading cause of death for women, occuring in about 55,000 more women than men each year [1]. African Americans have twice the risk of having a first stroke when compared with Caucasians, in addition to an increased mortality rate from stroke [1]. In Kentucky, stroke is the fifth leading cause of mortality [2]. Given the pervasiveness of stroke, it is critical to develop a more complete understanding of the pathophysiology at the time of stroke onset; these insights may reveal potential targets for stroke treatment.

The majority of strokes (87%) are ischemic and result from a thrombus obstructing a major artery in the brain [1]. The blockage caused by the thrombus produces initial hypoxic damage that is followed by ischemia and inflammation [3, 4]. As neurons die, they produce an infarct core. The penumbra, the area surrounding the infarct, also includes neurons and tissue at risk of cell death, and will expand to adjacent neurons if reperfusion does not occur [5]. Shifts occur within the cerebral blood flow (CBF) leading to disturbances in brain metabolism. Water, ionic concentrations, and other biochemical properties begin to change due to the obstruction and the decreasing CBF. Disruption in blood flow reduces oxygen (O_2) and glucose, and leads adenosine triphosphate (ATP) to be mismatched with use and production [6]. Oxygen availability to the brain is the product of CBF and the O_2 content of arterial blood. Cerebral ischemia occurs when metabolic demand for O_2 is not met.

The presence of the thrombus decreases CBF resulting acidosis due to a lack of glucose and an increase in carbon dioxide (CO_2). A lack of glucose triggers cellular glycolysis increasing of lactic acid production and a lowering in pH disrupting normal acid/base balance within the brain [7, 8]. During ischemia, CO_2 from neural cells accumulate in intracellular spaces, which lead to a further reduction in pH levels [9].

When blood pH decreases, electrolyte concentrations of sodium (Na^+), calcium (Ca^{2+}) and potassium (K^+) which maintain cellular structure and function are affected [6]. Dysregulation of ion gradients within neuronal cell membrane transport system occurs in Na^+/K^+ and Na^+/Ca^{2+} pumps. Under normal conditions Na^+ is pumped out of the cell and K^+ into the cell, but without a supply of ATP, the pump stops working properly. The increased concentration of intracellular Na^+ leads to cytogenic edema. The disruption of the Na^+/Ca^{2+} pump leads to an increased concentration of intracellular Ca^{2+}, which is detrimental in several ways [10]. It causes excitotoxicity by the release of glutamate neurotransmitters, which spurs neighboring neurons to also become overexcited. It also activates lipases that break down neuron cell membranes allowing other ions to enter the neuron. Finally, this process produces an increase of cytoplasmic calcium causing mitochondrial dysfunction, leading to the generation of free radicals and reactive oxygen species (ROS) that are responsible for neural cell death [10].

These initial pathophysiological changes can produce irreversible neuronal cell death and therefore, time is critical in the treatment of stroke. Current treatments break up or remove the thrombus using tissue plasminogen activator (tPA) and/or mechanical thrombectomy [11-13]. tPA catalyzes the conversion of plasminogen to plasmin and is the primary mechanism for thrombolysis. The use of intravascular tPA is time dependent

and patients can only be administered tPA within 4.5 hours after stroke onset. Mechanical thrombectomy is an intraarterial procedure to remove a thrombus and re-establish blood flow for emergent large vessel occlusions (ELVO) [14, 15]. Recently, the American Heart Association/American Stroke Association changed the time window for mechanical thrombectomy; the recommendation moved from a 6-hour treatment window to 24 hours [16-18].

Interestingly, thombectomy has also provided researchers with a rare opportunity to examine neurochemical changes that occur during stroke. It allows researchers to isolate blood within the artery immediately distal and proximal to the thrombus (systemic arterial blood in the cervical carotid artery) [19]. The analysis of these blood samples may help us better understand early neurochemical changes after stroke onset to assist in the development of adjunctive therapies to compliment current stroke treatments.

Acid/Base Balance and Electrolyte Concentration in Large Vessel Occlusion

Researchers have examined the relationship of venous and arterial blood gas parameters in critically ill patients [20-25] and in rodent models [26, 27]. These studies demonstrate that venous blood gas values in rodents and humans are similar and therefore comparable. Flores et al. (2011) examined arterial blood gases and electrolyte concentrations in distal blood adjacent to the cerebral thrombus and peripheral blood from the carotid artery during mechanical thrombectomy of ischemic stroke patients ($n = 16$), with results indicating significant differences between the two blood samples in partial pressure oxygen (distal 73.9 ± 14.9 and proximal 78.9 ± 16.3, $p = 0.007$) and oxygen saturation (distal 93.2 ± 4.4 and proximal 94.3 ± 3.9, $p = 0.003$) [28]. Back et al.

(2000) assessed pH regulation in rodents within 30 minutes and six hours of permanent middle cerebral artery occlussion (MCAO) model. They found acidosis (pH = 6.03) in the infarct core and alkalosis (pH = 7.32) in the penumbral area [8]. Together, these studies provide initial insights into acid/base balance and electrolyte concentrations. The gap in the knowledge is that we do not have blood gas values at baseline or stroke onset in large vessel occlusion and how these contribute to subsequent physiological changes like infarct and edema volumes and eventually outcomes. This study uses larger sample sizes of both rodent models and individuals diagnosed with ischemic stroke to examine associations of blood gases and electolyte concentrations to infarct and edema volumes, mortality, and infarct time.

Researchers have also examined electrolyte changes that occur during stroke. A lowering in total serum calcium concentrations in peripheral venous blood in stroke patients were associated with more severe clinical symptoms following stroke onset, including worse functional outcomes and an ishemic stroke to hemorrhagic conversion after thrombectomy [29-31]. Higher total serum calcium levels from peripheral venous blood were detected on patient admission or within 24 hours of stroke onset were associated with improved functional outcomes and decreased infarct volumes [32-34]. Serum Na^+ levels from peripheral venous samples were associated with stroke severity but the results are inconclusive. Rodrigues et al. [35] and Soiza et al. [36] found patients' with lower sodium levels or hyponatremia were associated with increased stroke severity and risk for mortality. Others have observed higher venous serum sodium concentrations were associated with stroke incidence and neurological worsening [37-39]. Serum Na^+

levels in the healthy group of controls were lower than in patients with transient ischemic attack and with ischemic and hemorrhagic strokes [40].

Purpose and Overview of Dissertation

The purpose of this dissertation was to explore the trajectory of neurochemical changes that occur in response to large vessel occlusion during the first 72 hours in rodent models and from ischemic stroke patients to improve stroke outcomes. The aims of dissertation were to: 1) to determine whether venous blood gases predicted infarct volume and/or mortality in acute ischemic stroke in young male rats; 2) determine whether venous blood gases predicted infarct volume, edema volume, and/or mortality in acute ischemic stroke in aged male and female rats; 3) compare acid/base balance and electrolytes in static blood distal to thrombus and in peripheral circulation drawn from adults who received thrombectomy for ischemic stroke and identify associations to post-reperfusion outcomes (infarct and edema volumes, and infarct time), after controlling for gender, BMI, and admission National Institutes of Health Stroke Scale NIHSS.

To date, no other study has examined acid/base balance and electrolyte changes this early after occlusion in permanent and transient-MCAO rat models or examined these changes in aged male and female rats. In addition, this is the first time these changes were tested as predictors of infarct volume, edema volume, and/or mortality in rats. We also evaluated blood gas and electrolyte values in the distal and peripheral blood adjacent to the cerebral thrombus at early time points after stroke in humans. These findings will aid in reverse translation from human to rodent models, so stroke models can focus on the most appropriate clinical endpoints and patient population. The results of this dissertation will increase our knowledge of stroke pathology and assist in

optimizing translational research seen in both rodent models and in humans. This work has the potential to fundamentally change how we develop treatment strategies for the stroke patient population.

Chapter Overviews

Chapter Two is a descriptive correlational design assessing acid/base balance (pH, carbon dioxide, oxygen, bicarbonate, base excess, glucose, hematocrit, and hemoglobin) and electrolyte concentrations (ionized calcium, potassium and sodium) changes that occur within a few minutes of focal ischemia in permanent middle cerebral artery occlusion (MCAO) and transient MCAO stroke models in young (three-month-old) male rats. The gap in the knowledge is that we do not have literature comparing acid/base and electrolyte concentrations in two focal ischemia models. These animal models mirror two stroke situations – a patient receiving stroke treatments and the natural history of a stroke patient. Additionally, there is no existing literature that evaluates early acid/base and electrolytes in focal ischemia and uses these values for predictors of stroke outcomes (infarct volume and/or mortality). Data from these models were analyzed using paired samples t-tests, Kaplan-Meier survival curve, and multiple linear regression analyses. This chapter is a published manuscript, *Translational Evaluation of Acid/Base and Electrolyte Alterations in Rodent Model of Focal Ischemia,* in Journal of Stroke and Cerebrovascular Diseases (JSCVD) (published 08/03/2018) [41].

Chapter Three reports a descriptive correlational study of acid/base balance and electrolyte changes occurring within a few minutes of focal ischemia and occlusion in the permanent middle cerebral artery occlusion (MCAO) model in aged (18-month-old) male and female rats. The blood gases were collected 1) pre-MCAO, 2) post-MCAO

(approximately seven minutes) and at 3) 72 hours post-MRI and prior to euthanization. The gap in the knowledge is that most stroke studies use young male rats as their population, and this does not reflect the age or sex balance of the patient population that suffers from strokes. This study included aged male and female rats to evaluate early acid/base and electrolyte values, as well as to determine predictors of infarct volume, edema volume, and mortality in older animals. Additionally, we compared our results to those reported in Chapter Two, and assessed the sex differences in our aged animals. Data from these models were analyzed using a one-way repeated measures ANOVA, multiple linear regression, and Cox regression analyses. This chapter is a published manuscript, *Early Acid/Base and Electrolyte Changes in Permanent Middle Cerebral Artery Occlusion: Aged Male and Female Rats*, in the Journal of Neuroscience Research (JNR) on 4/4/2019 [42].

Chapter Four reports a descriptive correlational design study of acid/base and electrolyte concentrations in distal and proximal arterial blood (relative to the thrombi) from ischemic stroke participants at the time of mechanical thrombectomy. The gap in the knowledge is that there is little evidence of arterial blood gases at the time of ischemic stroke and during mechanical thrombectomy, especially related to sex differences. This study compares acid/base balance and electrolyte concentrations between male and female ischemic stroke participants. Additionally, we were interested in predictors of stroke outcomes and infarct time, and whether these were sex dependent. The data for this study were analyzed using a one-way repeated measures ANOVA and moderated multiple linear regression. This manuscript, *Evaluation of Sex Differences in*

Acid/Base and Electrolyte Alterations in Acute Large Vessel Occlusion is in preparation for the submission to Journal of Neurointerventional Surgery (JNIS).

Chapter Five is a discussion of how each manuscript contributes to the state of the science on large vessel occlusion stroke. In addition, there is a discussion of the limitations, clinical implications, and future directions for research based on this work.

CHAPTER 2. TRANSLATIONAL EVALUATION OF ACID/BASE AND ELECTROLYTE ALTERATIONS IN RODENT MODELS OF FOCAL ISCHEMIA

Abstract

Background and Purpose: Acid/base and electrolytes could provide clinically valuable information about cerebral infarct core and penumbra. We evaluated associations between acid/base and electrolyte changes and outcomes in two rat models of stroke, permanent and transient middle cerebral artery occlusion.

Methods: Three-month old Sprague-Dawley rats underwent permanent or transient middle cerebral artery occlusion. Pre- and post-middle cerebral artery occlusion venous samples for permanent and transient models provided pH, carbon dioxide, oxygen, glucose, hematocrit, and hemoglobin, and electrolyte values of ionized calcium, potassium and sodium. Multiple regression determined predictors of infarct volume from these values, and Kaplan-Meier curve analyzed morality between permanent and transient middle cerebral artery occlusion models.

Results: Analysis indicated significant differences in the blood gas and electrolytes between pre- to post-middle cerebral artery occlusion. A decrease in pH and sodium with increases in carbon dioxide, potassium, ionized calcium, and glucose changes were found in both middle cerebral artery occlusion models; while hematocrit and hemoglobin were significant in the transient model. pH and ionized calcium were predictors of infarct volume in the permanent model, as changes in pH and ionized calcium decreased, infarct volume increased.

Conclusion: There are acute changes in acid/base balance and electrolytes during stroke in transient and permanent rodent models. Additionally, we found pH and ionized calcium changes predicted stroke volume in the permanent middle cerebral artery

occlusion model. These preliminary findings are novel, and warrant further exploration in human conditions.

Introduction

Approximately 87% of strokes are ischemic, and are characterized by blockage of blood flow in the brain from a thrombus or embolus [1]. Neurological insult occurs immediately when the cerebral artery is occluded, as neural tissue is deprived of oxygenated blood, glucose, and other nutrients [2]. The penumbra, the area surrounding the infarct, also includes tissue at risk of cell death, and will expand to adjacent areas if reperfusion does not occur [2]. When there are instabilities in cerebral blood flow (CBF), disturbances in brain metabolism occur, causing shifts in water and ion concentrations. With decreasing CBF, the blood downstream of the occlusion undergoes biochemical changes. Disruption in blood flow reduces oxygen (O_2) and glucose, and leads adenosine triphosphate (ATP) to be mismatched with use and production [3]. Cerebral ischemia occurs when cerebral O_2 supply fails to meet cerebral metabolic demand. Reduction in CBF causes lactic acid and carbon dioxide (CO_2) accumulation [4-6]. Furthermore, electrolyte concentrations such as sodium (Na^+), calcium (Ca^{2+}) and potassium (K^+) are sensitive to pH changes to maintain cellular structure and function [3]. Dysregulation is additive, as disruption of the Na^+/Ca^{2+} pump leads to an increased concentration of intracellular Ca^{2+} [7]. Increased calcium can trigger apoptosis, depolarization that activates lipases to break down neuron cell membranes, and mitochondrial dysfunction, leading to the generation of free radicals and reactive oxygen species (ROS) [7].

Several studies have investigated the relationship of venous and arterial blood gas parameters in critically ill human patients [8-13], and in rodent models [14, 15]

demonstrating correlations between the blood gas values. Knowledge of blood gases could provide clinically valuable information about the cerebral infarct core and penumbra. To date, few studies have evaluated acid/base balance and electrolyte changes occurring within a few minutes of focal ischemia and occlusion. The aim of our study was to evaluate these changes, and use these changes as predictors of infarct volume and/or mortality in two different rat models of stroke: permanent middle cerebral artery occlusion (MCAO) and transient MCAO. The permanent-MCAO model would mimic the natural history of large vessel occlusive stroke, while the transient-MCAO model accounts for recanalization seen in current treatment states that aim to re-establish flow.

Materials and Methods

Permanent and Transient MCAO Model

Three-month old Sprague-Dawley rats (ENVIGO, Indianapolis, IN) were used for all procedures. The rats weighed between 300 and 350 grams. The study was conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and study protocols were approved through our Institutional Animal Care and Use Committee (IACUC). Animals were kept in a climate controlled room on a 12-hour light and dark cycle (0700 - 1900) with free access to food and water. For both models, animals were administered vehicle of phosphate buffered saline (PBS) at 7.4, at 6, 24, and 48 hours after MCAO.

For the permanent middle cerebral artery occlusion (pMCAO) procedure, as previously described [16], animals were placed in an induction chamber and anesthetized with 5% isofluorane/oxygen. A constant flow of 3 - 4% isofluorane in 100% oxygen at a rate of 1 L/min was maintained during the procedure. A vertical incision was made near

the sternum to expose the right common carotid artery, and then a dissection was performed to isolate the common carotid and its branches. The first clamp was placed on the internal carotid prior to the pterygopalatine/internal cerebral artery (ICA) bifurcation, while the second clamp was placed on the common carotid artery. The placement of the second clamp further in the posterior direction allows more room for maneuvering during filament insertion. The external carotid was isolated, and used to access the arterial system. A 40mm nylon monofilament was fed distally into the intracranial artery to approximately 25mm, and then sutured in place to obtain permanent occlusion of the middle cerebral artery (MCA), M1 segment. The incision was closed with the filament in-place. A Laser Doppler (Moore Lab Instruments, Farmington, CT) monitored blood flow during the process. Animals that did not show 60% reduction in perfusion of blood flow after placement of the monofilament were excluded from the study. The transient MCAO (tMCAO) followed the same procedure, but the monofilament was removed and flow re-established after 60 minutes. The transient-MCAO rats (n = 19) were euthanized at 72 hours. The permanent-MCAO rats were euthanized at either 72 (n = 7) or 96 hours (n = 11). Brains from both models were harvested for infarct measurements.

Blood gas samples were collected and analyzed pre- and post-MCAO. An internal jugular line was inserted, prior to MCAO monofilament placement but after anesthesia induction, and an approximately 0.5mL of venous blood sample was collected (representing the pre-MCAO sample). These venous systems of the brain drains into the sinus of confluence which splits to form the internal jugular veins, which is the final collecting port before returning to the heart [17]. The majority of the venous blood samples were drawn on the ipsilateral side (affected MCAO side) of the animal. When

the blood samples at the affected site were unable to be obtained, the blood draw occurred on the contralateral side (unaffected MCAO side). While the ipsilateral side location is preferred for consistency in our blood draw protocol, the blood is analogous due to the venous anatomy. It takes approximately 30 minutes to obtain the blood sample given the details of the MCAO procedure and anesthesia induction (represented by the shaded box for the variable time points, Figures 2.1 and 2.2). The sample was analyzed using iSTAT Portable Clinical Analyzer (Abbott Laboratories, Abbott Park, IL). After pMCAO (approximately seven minutes), rats remained anesthetized on the operating table and venous blood sample was again collected (representing the post-MCAO sample) and analyzed with the iSTAT. pH, carbon dioxide (pCO_2), oxygen (pO_2), bicarbonate ($HCO3^-$), base excess of extracellular fluid (beecf), glucose, and serum electrolytes, including ionized calcium (iCa^{2+}), potassium (K^+), and sodium (Na^+) were analyzed. In the case of permanent MCAO, the post-MCAO sample was taken on average seven minutes after induction of the MCAO, while, in the case of the transient MCAO, the post-MCAO sample was taken approximately one hour and seven minutes after MCAO.

Euthanasia and Tissue Preservation

Animals were euthanized with Ketamine and Xylazine mixture (75 mg/kg and 7.5 mg/kg), at 72 or 96 hours after MCAO. They were perfused with 0.9% saline, then with 4% paraformaldehyde in PBS to remove blood from circulation and to preserve tissue. Brains were harvested in 4% paraformaldehyde, and then shifted to 20 to 30% sucrose for cryopreservation. Six coronal brain sections were cut (30μm) with a cryostat and taken from +1.7 to -3.3mm bregma coordinates. Coronal sections were mounted on glass slides

and stored at −20°C. To label degenerating neurons Fluoro-Jade staining (Histochem, Jefferson, AR) was used. This method has been previously detailed [18]. To rehydrate the coronal sections, 100% ethanol was used for three minutes, followed by a minute each in 70% ethanol and deionized water. Sections were then Fluoro-Jade stained (0.001% solution in 0.1% acetic acid) for 30 minutes. Slides were rinsed and dried at 45°C for 20 minutes, and then coverslipped with DPX mounting medium (Electron Microscopy Sciences, Ft. Washington, PA).

Infarct Volume Analysis

Coronal brain sections (bregma coordinates +1.7 through −3.3mm) were collected from rats in both models. A Zeiss AxioSkop2 microscope (model 801572) with a Zeiss AxioCam Color camera (model 412-312, Oberkochen, Germany) was used with OpenLab imaging software (Improvision Ltd., Lexington, MA) to capture images. Total infarct volumes were measured with NIH Image J software by taking the damaged ipsilateral hemisphere and dividing the total area of the contralateral hemisphere (six sections of each brain) measurement and multiplying by 100 to obtain a percentage. Infarct volume measurement equation: ipsilateral/contralateral x 100 = percentage.

Statistical Analyses and Sample Size

Before conducting statistical analyses, variables were transformed to meet assumptions of normality. Data from both MCAO models were analyzed using SPSS, version 24 software (SPSS, Inc., Chicago, IL). Data analysis began with a descriptive examination of all variables, including frequency distribution, means, and standard deviations. Paired samples t-tests were used to compare venous blood gas differences in mean scores between pre- and post-MCAO samples. Multiple linear regression with

backward variable entry was performed in the permanent and transient models to determine predictors based on venous blood gas and electrolyte changes to infarct volume. The variables were entered into the model at once. The adjusted R^2 was used to determine the variance explained in infarct volume. The P-to-P plot was used to assess the changes in pH and iCa^{2+} data sets for normal distribution. Multicollinearity was assessed by examining correlation coefficients and tolerance/variance inflation factor values. A Kaplan-Meier curve using Mantel-Haenszel log-rank test analysis was performed to analyze mortality between permanent and transient-MCAO models. A p-value of 0.05 was set a priori to determine statistical significance.

The total sample size for the permanent MCAO model was 18. These animals are furthered categorized into those that survived to 72 hours (n = 11), and those that died prior to 72 hours (n = 7). In comparison, the total sample size for transient MCAO was 19. These animals are also divided into those that survived to 72 hours (n = 17), and those that died before 72 hours (n = 2). Total sample size for both models was reduced during the iSTAT blood sample collection due to individual readings errors. Errors were excluded from statistical tests.

Results

Relationship Between MCAO Model and Mortality

A Kaplan-Meier curve and Mantel-Haenszel log-rank test was used to analyze event-free survival between permanent and transient MCAO models (p = 0.045). Approximately 39% mortality was seen in the permanent model, while an average of 11% occurred in the transient model. The mortality rates were collected at four different time points; 6 hours, 24 hours, 48 hours, and 72 hours. In the permanent model (n = 18),

seven rats died at 24 hours and 11 rats survived up to 72 hours. By comparison, the transient model (n = 19) rats exhibited greater survival, with one death at 6 hours and one other at 24 hours; the remaining animals (n = 17) survived up to 72 hours (Figure 2.3). The permanent-MCAO infarct volumes ranged from 24.40 to 90.79% (Figure 2.4). The transient-MCAO infarct volumes ranged from 12.07 to 33.34% (Figure 2.5). There is no significant difference in infarct size between transient and permanent occlusion.

Permanent MCAO Model

In the permanent MCAO model, there were several significant differences in the blood gas analysis between pre- and post-MCAO (Figure 2.6). These included significant decrease in pH ($p = 0.035$), Beecf ($p = 0.001$), and Na^+ ($p < 0.001$), as wells as increases in pCO_2 ($p = 0.036$), K^+ ($p = 0.006$), iCa^{2+} ($p = 0.045$), and glucose ($p < 0.001$). Multiple regression analysis in the permanent-MCAO model (n = 11) showed that the changes in pH and in iCa^{2+} were significant predictors of infarct volume ($F(2,8) = 16.582$, $p = 0.001$). This indicates a negative coefficient between pH and ionized calcium together with the extent of the infarct, as changes in pH and calcium decreased, infarct volume increased. These variables explained 76% of the total variance in the model, as predictors of infarct volume (Figure 2.7 and 2.8).

Transient MCAO Model

In the transient MCAO model, there were also several significant differences in blood gas analysis between pre- and post- MCAO (Figure 2.9). These included decreases in pH ($p = 0.004$) and Na^+ ($p = 0.002$), as well as increases in pCO_2 ($p = 0.024$); K^+ ($p < 0.001$), iCa^{2+} ($p = 0.015$), glucose ($p = 0.047$), hematocrit ($p < 0.001$), and hemoglobin ($p < 0.001$). Unlike the permanent model, Beecf ($p = 0.397$) was not significantly altered.

None of the acid/base changes or electrolyte changes in the transient-MCAO model were predictors of infarct volume.

Discussion

To our knowledge our study demonstrates, for the first time, rapid changes in venous blood gases and electrolytes occurring immediately following cerebral ischemia in two separate MCAO rat models (permanent and transient). Furthermore, we establish a statistically significant predictors between some of those changes and infarct volume. Significant differences were seen between the two models for mortality. Our blood analyses follow the biochemical changes reported in other cerebral occlusion studies in animals and humans. We report that significant systemic changes in blood chemistry occurs very rapidly after occlusion with an average of seven minutes in the permanent MCAO model, and approximately one hour and seven minutes in the transient MCAO model. To our knowledge, we have not found any other studies that examined these changes so early after occlusion and in two separate MCAO models.

We used the permanent and transient MCAO models for two reasons. First, we aimed to identify ultra-early changes in a permanent model of ischemia. While there are a host of signaling cascades that occur in multiple pathways to induce injury, apoptosis, and necrosis after stroke, we evaluated the relationship between these ultra-early changes and eventual outcome. Second, we wanted to plot the course of blood gas and electrolyte changes upon early reperfusion. As recanalization strategies such as tissue plasminogen activator (tPA) and mechanical thrombectomy have become standard of care, it is important to understand pathologic changes in stroke in the context of acute recanalization. The permanent model is an evaluation of large vessel occlusive stroke in

its natural history [19]. The majority of patients who experience an ischemic stroke do not receive tPA and/or mechanical thrombectomy [20, 21]. The transient model is an evaluation of the effect of treatment, because it mimics the human condition for receiving tPA and/or mechanical thrombectomy and the reperfusion event. The transient model evaluates the persistency of changes in acid/base and electrolytes immediately after reperfusion.

This study combined all blood measurements to determine association with infarct volume. In evaluating the early changes in acid/base and electrolytes in the permanent model the multiple linear regression showed significance in changes of pH and iCa^{2+} to infarct volume in the permanent model. These two variables clearly have an interrelationship to infarct volume, but causality needs to be furthered evaluated in future studies.

In human stroke patients, lower venous total serum calcium concentrations were associated with more severe clinical symptoms following stroke onset, worse functional outcomes, and hemorrhagic transformation post-thrombectomy [22-24]. Conversely, higher venous total serum calcium levels detected on admission or within 24 hours of stroke onset in acute ischemic stroke patients were associated with better functional outcomes and reduced cerebral infarcts [25-27]. These findings reflect increased serum calcium levels are linked to positive stroke outcomes. Our study indicates an inverse relationship with changes in ionized calcium and infarct volume; however, this is based on immediate ionized calcium (iCa^{2+}) changes, not total calcium serum. Furthermore, our measurements were within minutes to hours of the infarct induction, while the previous studies above included levels drawn within 24 hours of admission. Therefore, there may

be a calcium concentration curve that is temporal in nature as it relates to infarct. Further temporal studies are needed to map this relationship.

While we found no predictors between changes in Na^+ and infarct volumes, several reports with contradictory results show serum Na^+ levels are associated with stroke severity in human patients. Rodrigues et al. [28] and Soiza et al. [29] found lower sodium or hyponatremia was associated with stroke severity and mortality. Other studies have observed higher venous serum sodium concentrations were associated with stroke incidence and neurological worsening [30-32]. Serum Na^+ levels in the control group was lower than in patients with transient ischemic attack, and with ischemic and hemorrhagic strokes [33]. Our findings are similar in showing changes in serum Na^+ levels from pre-to post- pMCAO, but the significance of these changes needs to be elucidated. Again, the timing of sampling may have an effect on the power of the association. It may be the case that our early-timed assay does not reflect changes that occur in the first 24 to 48 hours.

The transient MCAO model did not show predictors in the multiple linear regression model. We believe this is due to the magnitude of the changes of acid/base and electrolytes may be masked by the benefit of reperfusion. While we saw many similar differences in changes from pre- to post-MCAO (compared to the permanent model), the animals did not have any predictors of infarct volume. This may reflect the overpowering effect of recanalization and reperfusion on dictating final infarct volume. Further studies will need to be conducted to address these issues.

Another interesting difference between the two models was noting the significant differences of increased hematocrit and hemoglobin pre- to post-MCAO in the transient model, most likely due to hemoconcentration of fluid losses (urination, temperature or

insensible losses), which is often seen in human ischemic stroke patients. This was not observed in the permanent model perhaps due to the timing of the second iSTAT collection, where animals may not have had an opportunity to compensate yet; these are issues that need to be addressed in future studies.

The transient model had better survival rate than the permanent model. The permanent model shows animal deaths occurred at various time points post-MCAO with the majority occurring at 24 hours. The 24-hour time point is critical in human ischemic stroke patients because this is the period when patients experience edema, herniation, and hemorrhagic conversion resulting in increased mortality [34] We did not find this in the transient model, which verifies the importance of reperfusion in ischemic stroke and survival outcomes.

Conclusion

In this study, we demonstrated early significant changes in blood gases and electrolyte concentrations after induction of a middle cerebral artery occlusion stroke. This study shows that blood chemistry in the systemic circulation responds rapidly to the ischemic event in the brain. Other studies examined one stroke model and one blood gas parameter or electrolyte concentration, while we analyzed two separate MCAO models in rats and the whole blood gas and electrolyte panel. Previous researchers have shown variability in their results, however, our study shows the initial changes in pH and ionized calcium are predictors of infarct volume in the permanent MCAO model. Our future studies will examine aged male and female rats to address whether age and sex affects this issue. Eventually we are planning to expand our blood gas studies to humans to

determine if these results translate to human stroke patients, which would allow early prediction of the stroke outcome.

Table 2.1: Permanent Model
Venous blood paired samples T-Test in pre- and post- MCAO in young Sprague Dawley male rats.

	Permanent-MCAO				
Parameters	*n*	Pre-MCAO Mean ± SD	Post-MCAO Mean ± SD	Δ Mean ± SD	P-value
pH	14	7.249 ± 0.071	7.217 ± 0.075	0.033 ± 0.052	**0.035**
pCO_2 (mmHg)	14	68.660 ± 9.397	75.890 ± 14.190	-6.229 ± 9.944	**0.036**
pO_2 (mmHg)	14	105.929 ± 18.269	107.357 ± 21.685	-1.429 ± 18.711	0.780
BEecf (mmol/L)	14	4.360± 1.008	3.070 ± 1.328	1.286 ± 1.069	**0.001**
HCO_3^- (mmol/L)	14	31.380 ± 1.196	31.550 ± 2.008	-0.171 ± 1.082	0.564
Na^+ (mmol/L)	14	133.570 ± 1.222	131.930 ± 1.439	1.643 ± 1.216	**<0.001**
K^+ (mmol/L)	14	4.450 ± 0.231	4.711 ± 0.408	-0.261± 0.296	**0.006**
iCa^{2+} (mmol/L)	14	1.091 ± 0.138	1.190 ± 0.087	-0.099 ± 0.168	**0.045**
Glu (mg/dL)	14	290.290 ± 31.067	368.790± 58.380	-78.500 ± 47.383	**<0.001**
Hct (% PCV)	14	40.214 ± 1.424	40.429 ± 1.651	-0.214 ± 0.893	0.385
Hbg (g/dL)	14	13.679 ± 0.474	13.743 ± 0.543	-0.064 ± 0.295	0.430

Data are presented as mean ± standard deviations. Blood gas and electrolyte parameters: pCO2: Partial pressure of carbon dioxide (mmHg); pO2: partial pressure of oxygen (mmHg); HCO3-: bicarbonate (mmol/L); BD: base deficit (mmol/L); BE: base excess (mmol/L); sO2: oxygen saturation (%); Na+: sodium (mmol/L); K+: potassium (mmol/L); iCa^{2+}: ionized calcium (mmol/L); Glu: glucose (mg/dL); Hct: hematocrit (% PCV); and Hbg: hemoglobin (g/dL).

Table 2.2: Permanent Model
Multiple regression was performed to determine the correlations of venous blood gas and electrolyte changes to infarct volume. As changes in pH and ionized calcium decrease, infarct volume increases. These variables explained ≈ 76% of the total variance in the model as predictors of infarct volume in the permanent model.

Variable	B	SE B	β	p value
ΔpH	-86.155	21.050	-0.640	0.003
ΔiCa^{2+}	-99.878	22.994	-0.679	0.002

R^2 = .0806, adjusted R^2 = 0.757, *df* = 2, F = 16.582, Durbin-Watson = 1.730

Table 2.3: Transient Model Venous blood paired samples T-Test in pre- and post- MCAO in young Sprague Dawley male rats.

	Transient-MCAO				
Parameters	*n*	Pre-MCAO Mean ± SD	Post-MCAO Mean ± SD	Δ Mean ± SD	P-value
pH	12	7.263 ± 0.060	7.106 ± 0.143	0.157 ± 0.152	**0.004**
pCO_2 (mmHg)	9	71.490 ± 14.155	93.530 ± 26.149	-22.044 ± 23.760	**0.024**
pO_2 (mmHg)	12	139.000 ± 26.823	149.833 ± 42.734	-10.833 ± 41.772	0.388
BEecf (mmol/L)	9	4.440 ± 1.810	4.110 ± 1.537	0.333 ± 1.118	0.397
HCO_3^- (mmol/L)	9	31.310 ± 1.745	32.270 ± 2.502	-0.956 ± 1.731	0.136
Na^+ (mmol/L)	12	133.83 ± 1.030	131.830 ± 1.749	2.000 ± 1.758	**0.002**
K^+ (mmol/L)	12	4.342 ± 0.144	4.933 ± 0.485	-0.592 ± 0.414	**<0.001**
iCa^{2+} (mmol/L)	12	1.104 ± 0.151	1.222 ± 0.114	-0.118 ± 0.141	**0.015**
Glu (mg/dL)	12	305.330 ± 27.003	372.080 ± 114.099	-66.750 ± 103.660	**0.047**
Hct (% PCV)	12	39.000 ± 1.595	41.750 ± 2.137	-2.750 ± 1.138	**<0.001**
Hbg (g/dL)	12	13.250 ± 0.525	14.200 ± 0.727	-0.950 ± 0.397	**<0.001**

Data are presented as mean ± standard deviations. Blood gas and electrolyte parameters: pCO2: Partial pressure of carbon dioxide (mmHg); pO2: partial pressure of oxygen (mmHg); HCO3-: bicarbonate (mmol/L); BD: base deficit (mmol/L); BE: base excess (mmol/L); sO2: oxygen saturation (%); Na+: sodium (mmol/L); K+: potassium (mmol/L); iCa^{2+}: ionized calcium (mmol/L); Glu: glucose (mg/dL); Hct: hematocrit (% PCV); and Hbg: hemoglobin (g/dL).

Figure 2.1 Permanent-MCAO Timeline.

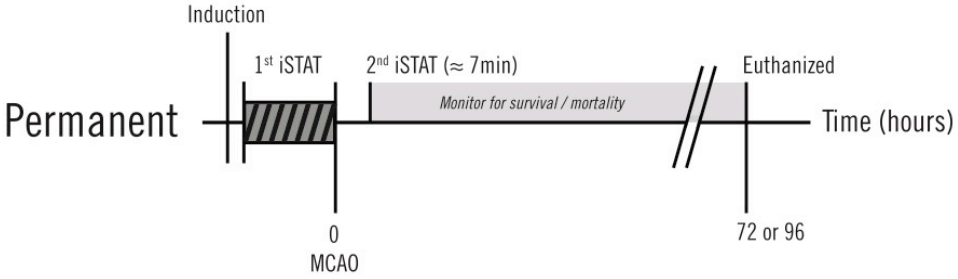


Figure 2.2 Transient-MCAO Timeline.

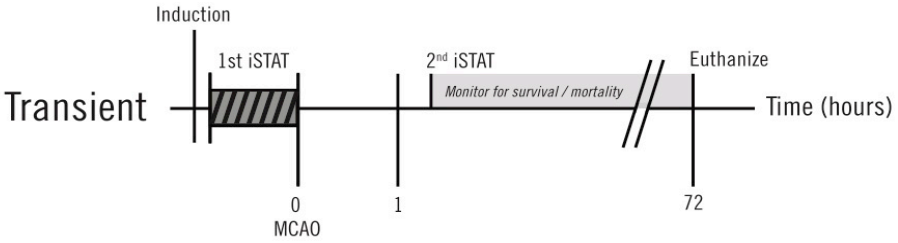


Figure 2.3 Mortality data expressed using the Kaplan-Meier curve and analysis using the Mantel-Haenszel log-rank test revealed significant difference in survival between permanent and transients (p = 0.045). The numbers in parentheses represent the mortality of rats at 24 hours and 48 hours. The surviving rats in the permanent model were n=11. The surviving rats in the transient model were n=17.

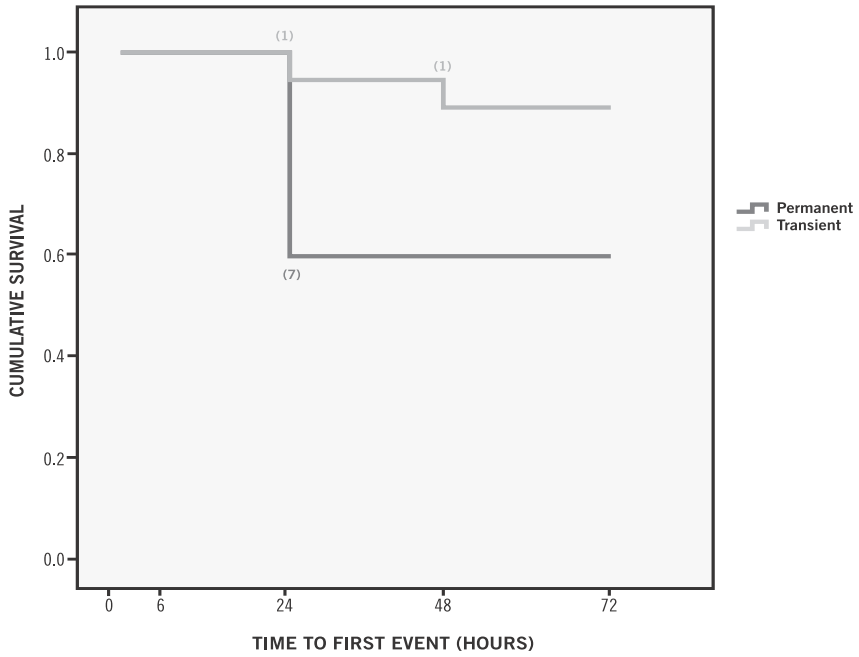


Figure 2.4 Representative permanent-MCAO infarct volume image. Fluoro-Jade staining was performed on cryosections from rat brains to identify the damaged neurons 72 hours post-MCAO. The range for permanent-MCAO infarct volumes were 24.40 – 90.79%. Scale bars =150μm.

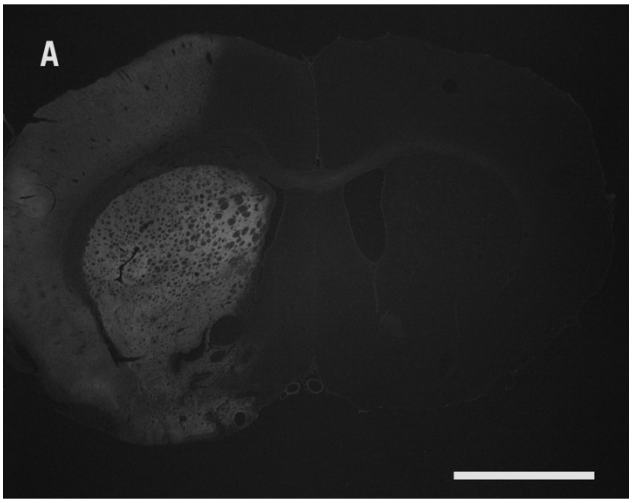


Figure 2.5 Representative transient-MCAO infarct volume image. Fluoro-Jade staining was performed on cryosections from rat brains to identify the damaged neurons 72 hours post-MCAO. The transient-MCAO infarct volumes ranged from 12.07 – 33.34%. Scale bars =150μm.

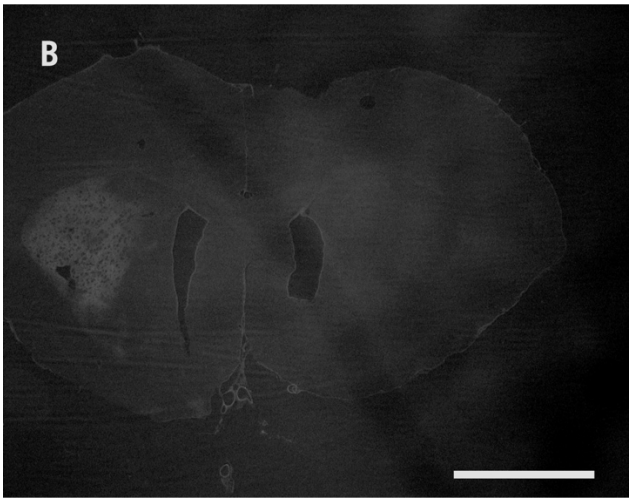
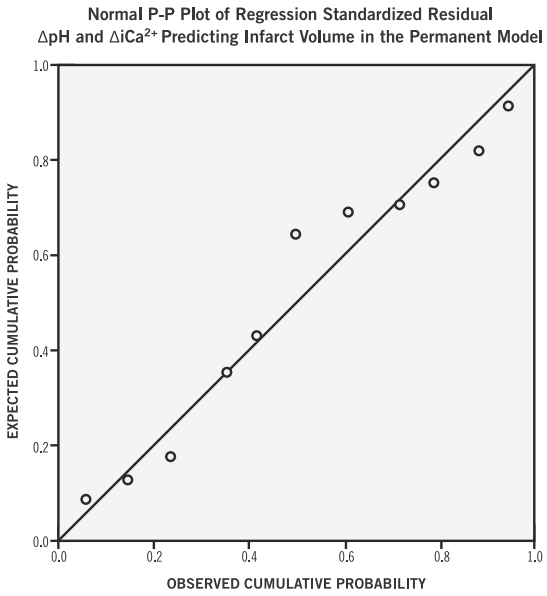


Figure 2.6: Permanent Model
P-P plot of residuals for change in pH and iCa^{2+} predictors of infarct volume.



CHAPTER 3. EARLY ACID/BASE AND ELECTROLYTE CHANGES IN PERMANENT MIDDLE CEREBRAL ARTERY OCCLUSION: AGED MALE AND FEMALE RATS

Abstract

Background: Early changes in acid/base and electrolyte concentrations could provide insights into the development of neuropathology at the onset of stroke. We evaluated associations between acid/base and electrolyte concentrations, and outcomes in permanent middle cerebral artery occlusion (pMCAO) model.

Methods: 18-month old male and female Sprague-Dawley rats underwent pMCAO. Pre-, post- (7 minutes after occlusion), and at 72 hours of pMCAO venous blood samples provided pH, carbon dioxide, oxygen, glucose, hematocrit, hemoglobin, and electrolyte values of ionized calcium, potassium and sodium. Multiple linear regression determined predictors of infarct and edema volumes from these values, Kaplan-Meier curve analyzed morality between males and females at 72 hours, and a Cox regression model was used to determine predictors for mortality.

Results: Analysis indicated significant differences in acid/base balance and electrolyte levels in aged rats not dependent on sex between the three time points in the pMCAO model. Changes in pH (from pre- to post and post- to 72 hours) and changes in sodium and ionized calcium (from post- to 72 hours) were predictors of infarct volume and edema volume, respectively. Cox Regression revealed there is a 3.25 times increased risk for mortality based on changes in bicarbonate (pre- to post-MCAO).

Conclusions: These early venous blood changes in acid/base balance and electrolytes can be used to predict stroke outcomes in our rat model of stroke. This study provides potential biomarkers to be examined in the human condition that could provide profound prognostic tools for stroke patients.

Introduction

Stroke remains the leading cause of death and disability worldwide [1]. Edema and electrolyte imbalance contribute to brain swelling, dysfunction, and death. Currently, there are no early serum tests to predict risk of death from stroke-induced edema. While MRI will show large infarcts early, it is no longer typically used in the emergent presentation due to delaying urgent treatment. Therefore, identifying an easily obtainable clinical marker to predict cerebral edema and death would be a major advancement in stroke care. Many strokes are emergent large vessel occlusion (ELVO), in which there is an acute occlusion of one of the vessels of the Circle of Willis. Accounting for 20-40% of ischemic strokes, it is the most disabling form [2]. The permanent MCAO model best represents the natural history of emergent large vessel occlusion (ELVO) stroke that is untreated with thrombolytic therapy or mechanical thrombectomy [3]. Most patients with ELVO currently do not receive either of these treatments, which results in a high mortality rates or severe disabilities [4, 5].

Blockage of the large vessel artery reduces cerebral blood flow (CBF) below functional levels depriving neurons of oxygenated glucose and blood. The penumbra, the brain area adjacent to the infarction, is at risk of infarction if reperfusion does not occur [6]. Oxygen (O_2) and glucose are reduced in the blood downstream of the occlusion. A deprivation in O_2 and glucose contribute to the failure of adenosine triphosphate (ATP) production and cerebral ischemia occurs when O_2 supply fails to meet metabolic demand [7]. Cells are forced into anaerobic glycolysis which leads to lactic acid accumulation, which lowers the pH [8, 9]. Carbon dioxide (CO_2) builds up and accumulates in extracellular and intracellular spaces leading to decrease in pH [10]. The acidic pH

impacts electrolyte concentrations such as sodium (Na^+), calcium (Ca^{2+}) and potassium (K^+) which regulate cellular structure and function [7]. Increased concentration of intracellular Na^+ causes cytotoxic edema. Additionally, the disruption of the Na^+/Ca^{2+} pump results in an increased concentration of intracellular Ca^{2+} which initiates apoptosis, causes mitochondrial dysfunction, and generates free radicals and reactive oxygen species (ROS) [11]. These acid/base and electrolyte changes are associated with the severity of the stroke [12], a greater understanding of the blood gas parameters allows us further insight into how these factors influence stroke outcomes.

Previously, we have reported a change in blood gases and electrolytes within a few minutes of focal ischemia in young male rats and these changes predict infarct volume [12]. This study uses aged male and female rodents that are better aged-matched to the typical age of the human stroke patient. Therefore, we aimed to examine venous blood gas values in male and female aged rats at three time points: (1) pre-permanent middle cerebral artery occlusion (pMCAO), (2) post-pMCAO, and (3) at 72 hours before euthanization to determine whether venous blood gas values predict infarct volume, edema volume, and/or mortality in pMCAO. This study identifies early changes in blood gases/electrolytes that predict stroke outcomes in an experimental rodent model of stroke.

Materials and Methods

Ethics Approval and Animals

Aged male and female rats (18-month old Sprague-Dawley rats (ENVIGO, Indianapolis, IN) were used for all procedures. The aged female rats on average weighed between 245 and 425 grams, and aged male rats approximately weighed between 505 to 705 grams. The study was conducted in accordance with the National Institutes of Health

(NIH) Guide for the Care and Use of Laboratory Animals and study protocols were approved by University of Kentucky's (UK) Institutional Animal Care and Use Committee (IACUC). Animals were housed in a climate controlled room on a 12-hour light and dark cycle (0700 - 1900) with access to food and water. Per Division of Laboratory Animal Resources (DLAR) cage requirements at UK's vivarium facility, the animals can be paired in one cage if the animal weight is under 650 grams. We typically house two animals (males or females) per cage upon arrival to DLAR. Once the rats are over 650 grams, they are then split into a separate cage by themselves. Animals were administered vehicle of sterile filtered phosphate buffered saline (PBS) of pH 7.4, at 6, 24, 48, and 72 hours after permanent middle cerebral occlusion (pMCAO). The rats that survived to 72 hours (n = 16) underwent Magnetic Resonance Imaging (MRI) for Diffusion Tensor Imaging (DTI) images and T2 weighted images to measure infarct and edema volumes and then euthanized.

Permanent Middle Cerebral Artery Occlusion

During the pMCAO procedure, as previously described [13], animals were placed in an induction chamber and anesthetized with 5% isofluorane/oxygen. During the procedure, a constant flow of 3% isofluorane in 100% oxygen at a rate of 1 L/min was maintained. A midline vertical neck incision occurred to collect an internal jugular blood draw. Glass rods were used to separate the large muscle pad and the sternocleidomastoid muscle (SCM). Retractors were placed to pull musculature of the SCM and skin tightly away from the targeted area. Dissection of the omohyoid muscle, fat, and connective tissues were made to isolate and expose the right common carotid artery from the vagus nerve. Blunt dissection with glass rods occurred around the internal carotid artery (ICA)

with removal of lymph nodes and fat surrounding the internal/external cerebral artery (ICA/ECA) bifurcation to expose the pterygopalatine/middle cerebral artery (MCA) bifurcation. The first clamp was placed on the ICA prior to the pterygopalatine/MCA bifurcation, while the second clamp was placed on the common carotid artery (CCA). The placement of the second clamp further in the posterior direction allows more room for manipulation during the embolus insertion. The ECA was isolated, and used to access the arterial system. A 40mm nylon monofilament was fed distally into the ECA and advanced through to the ICA at approximately 25mm until it reached the ICA clamp. The monofilament was loosely sutured in place, the first clamp was removed, and the embolus was advanced then sutured in place to obtain permanent occlusion of the middle cerebral artery (MCA), M1 segment. The second clamp was removed with careful observation of minimal blood, then the removal of the retractors. Before the neck incision closure, a post-MCAO blood draw occurred from the internal jugular vein.

Post-Surgical Fluid Management and Pain Control

Immediately post-operatively the animals received 2ml of sterile saline (0.9%) subcutaneous. An additional 1ml of saline was given if extra blood loss occurred during surgery. The animals were injected with sterile filtered PBS pH 7.4 at 6, 24, 48, and 72 hours post-MCAO. The animals were weighed every morning post-MCAO to determine dehydration. Hydration status was checked by pinching up or "tenting" the skin over the nape of the neck. The skin should immediately relax into its normal position. If the skin remains tented longer than normal, the rat was deemed dehydrated and saline was given. Per DLAR guidelines, rats can receive up to 10ml at a time and no more than 2ml at any one location per 6 hours. If warranted, additional saline (1-2ml) will be given in addition

to 6, 24, 48, and 72 hours. Also, we added an additional water bottle in each cage to allow more availability to free water and moist food to encourage feeding and additional water intake.

Post-surgical pain control was managed with carprofen, which is based on weight of the animal. Animal weights are taken prior to surgery (pMCAO) and daily until animals are euthanized at 72 hours (post MRI). The animals received a dosage of carprofen 5mg/kg prior to surgery and every 24 hours for three days post-pMCAO until 72 hours when they were euthanized (post MRI).

Venous Blood Gas Collection

Blood gas samples were collected and analyzed pre- and post-MCAO, and prior to euthanization at 72 hours. The venous blood sample was analyzed using iSTAT Portable Clinical Analyzer (Abbott Laboratories, Abbott Park, IL) for pH, carbon dioxide (pCO_2), oxygen (pO_2), bicarbonate (HCO_3^-), base excess of extracellular fluid (Beecf), glucose, hematocrit, hemoglobin, and serum electrolytes, including ionized calcium (iCa^{2+}), potassium (K^+), and sodium (Na^+). Individual readings errors and these errors were excluded from statistical tests. An internal jugular line was inserted, prior to MCAO monofilament placement and an approximately 100µl of venous blood sample was collected (representing the pre-MCAO sample, Figure 3.1). The venous circulation drains into the confluence of the sinuses and then splits into right and left internal jugular veins before recirculation through the heart [14]. Venous blood samples were collected from the affected MCAO side of the animal. However, in certain cases venous blood samples were collected from the contralateral side (unaffected MCAO side). The pre-pMCAO blood sample takes approximately 30 minutes to obtain given the details of the procedure

and anesthesia induction (represented by the shaded box for the variable time points, Figure 3.1). Post-MCAO (approximately taken within seven minutes of occlusion), rats remained anesthetized on the operating table and venous blood sample was again collected (representing the post-MCAO sample). The third venous blood sample was collected from the right atrium (Figure 3.1) in animals that survived to 72 hours.

Magnetic Resonance Imaging

MRI images were acquired on a 7T Bruker Clinscan horizontal bore system (7.0T, 30cm, 300Hz) equipped with a triple-axis gradient system (630 mT/m and 6300 T/m/s) with a closed cycle. Diffusion Tensor Imaging (DTI) images were acquired coronally with a fat saturated, double refocused echo planar sequence: 0.297 x 0.297 x 0.7 mm^3, TR/TE 2200/34, 128 x 128 matrix, 3 av, 12 slices, four b=0 volumes, 256 directions with b=800, in 28:23 minutes (Figure 3.2.A). T2 weighted images were acquired coronally with a fat saturated, turbo spin echo sequence: 0.125 x 0.125 x 0.4 mm^3 resolution, TR/TE 6000/27, 192 x 192 matrix, 44 slices, is 9:03 (Figure 3.2.C).

Male rats were anesthetized with an average of 2.25% isoflurane in oxygen, while female rats were anesthetized with an average of 1.75% isoflurane in oxygen using an MRI compatible CWE Inc. equipment (Ardmore, PA). They were held in place on a Bruker scanning bed with a tooth bar, ear bars, and tape. Body temperature, heart rate, and respiratory rates were continuously monitored throughout the MRI scans (SA Instruments, Inc., Stony Brook, NY). The animal's body temperature were maintained at 37°C with a water heating system built into the scanning bed. The scanning procedure took approximately 40 minutes per animal.

MRI Processing for Infarct Volume and Edema Volume

The DTI and T2 MR images were analyzed by a blinded neuroradiologist who identified infarct volume and edema volume. These volumes were counted and the number referenced to the number of images counted to provide a per section count. The volume of brain parenchyma demonstrating restricted diffusion (infarct volume) visibly affected were calculated by manual segmentation using ITK-SNAP software (www.itksnap.org, version 3.6, Figure 3.2.B) [15]. The volume of brain parenchyma visibly affected by cerebral edema (edema volume) were calculated in a similar fashion (Figure 3.2.D). The data are given as absolute volume in cubic millimeters. The calculation was based on all slices from each MR sequence.

Statistical Analysis

Data analysis began with a descriptive examination of all variables, including frequency distribution, means, and standard deviations. The power analysis was completed using G*Power software [16, 17]. Given a desired power of 0.8 and a significance level for between-group comparisons set at $p < 0.05$, the required sample size was calculated to be 14 animals (i.e., $n = 7$ per group). All variables in the study were transformed to meet assumptions of normality. The transformation procedures began with Shapiro-Wilks and for measures with $p < 0.05$, natural log with a constant of 10 added to measure. Data from the aged male and female rats were analyzed using SPSS, version 24 software (IBM, Armonk, NY).

A repeated-measures analysis of variance (ANOVA) was used to examine between-group differences of blood gas parameters of the sexes at three time points, pre-MCAO, post-MCAO, and at 72 hours-MCAO. Paired samples t-test were used to

compare venous blood gas mean values between pre- and post-MCAO for the animals that died prior to 72 hours, in addition to an independent t-test to compare venous blood gas mean values between pre-MCAO animals that survived versus died prior to 72 hours. A multiple linear regression with backward variable entry was performed with magnitude of change data from aged male and female rats to determine whether venous acid/base and electrolyte changes predicted infarct volume and/or edema volume. The variables were entered into the regression model in one step. The adjusted R^2 was used to determine the variance explained in infarct volume and/or edema by each variable. An assessment of multicollinearity was performed by inspecting correlation coefficients and tolerance/variance inflation factor values. Inspecting the coefficients table and if tolerance values are less than 0.10 or VIF is above 10, collinearity is present and was not found in final model. The P-to-P plot was used to assess the changes in pH, iCa^{2+}, and Na^{+} data sets for normal distribution. A Kaplan-Meier curve using Mantel-Haenszel log-rank test analysis was performed to compare survival curves between aged male and female rats. A Cox regression curve was used to assess the relationship of event-free survival in aged male and female rats using predictors of acid/base and electrolyte concentration parameters. A p-value of 0.05 was set a priori to determine statistical significance.

Sample Size

The total sample size from the permanent MCAO model were 31. These animals are furthered categorized into those that survived to 72 hours (n = 16; males = 7 and females = 9), and those that died prior to 72 hours (n = 15; males = 8 and females = 7).

Results

Acid/Base and Electrolyte Values for Survival

Venous Blood Gas Differences Between Aged Male and Female Rats

A repeated-measures analysis of variance (ANOVA) was used to examine between-group differences of blood gas parameters of the sexes at three time points, pre-pMCAO, post-MCAO, and at 72 hours-MCAO (Table 3.1). Mauchly's test indicated that the assumption of sphericity had been violated for each blood gas parameter therefore degrees of freedom were corrected with Greenhouse-Geisser estimates of sphericity (Table 3.2). There was not a significant interaction of time by group effect between the three time points of blood gas parameters and sex (Table 3.3).

Acid/Base and Electrolyte Values for Death

Because there were no baseline differences between males and females rats that died prior to 72 hours their data were collapsed into a single group. There were significant differences in blood gas values seen from pre- to post-MCAO in animals that died prior to 72 hours (n = 15, Table 3.4). pH ($p = 0.026$) and Na^+ (0.039) decreased from pre- to post-MCAO, while pCO_2 ($p = 0.015$), HCO^{3-} ($p = 0.001$) and glucose ($p = 0.014$) increased. There were no significant differences seen in baseline (pre-MCAO) values from those animals that survived and those that died prior to 72 hours (Supplemental Table 3.1).

Predictors of Infarct Volume and Edema Volume

Blood gas and electrolyte changes from male and female rats were combined for multiple linear regression analyses. Multiple regression analysis (n = 15) showed that the changes in pH from pre- to post-MCAO and in pH from post to 72 hours-MCAO were

predictors of infarct volume (F (2,14) = 14.77, p = 0.030). These variables explained 81% of the total variance in the model (Table 3.5).

Multiple regression analysis (n = 16) revealed that the changes in iCa^{2+} from post to 72 hours-MCAO and Na^{+} post to 72 hours-MCAO were predictors of edema volume (F (2,15) = 12.32, p = 0.015). These variables explained 75% of the total variance in the model (Table 3.6). The mean infarct size is 203.64 ± 61.41, while the mean edema volume is 124.04 ± 41.39. There was no significant difference (p = 0.524) in infarct size between aged male (n = 6) and female rats (n = 9) or in edema volume (p = 0.730) between aged male (n = 7) and female rats (n = 9).

Relationship Between MCAO and Mortality

A Kaplan-Meier curve with Mantel-Haenszel log-rank test was used to analyze event-free survival between aged male and female rats. There was no difference between male and female rats based on mortality (p = 0.321, data not shown). In the Cox regression, there a 3.25 times (or 325%) greater risk for mortality for rats within the -2.00 to -7.00 cut-off range for HCO^{3-} (pre- to post-MCAO), after controlling for change in Na^{+} and sex (p = 0.025) (Figure 3.3).

Discussion

Previously, we have reported that changes in venous blood pH and ionized calcium (pre- to post-MCAO) predicted infarct volume in young male rats [12]. We now extend our findings to aged male and female rats after pMCAO. The venous blood gas profile after pMCAO significantly differed between the young and aged. Our aged rats (18 months equivalent to approximately 55-65 years old in humans [18]) are a better age match for human stroke patients. A major issue in stroke research is the lack of

translating findings from the rodent to the human patient. The almost uniform use of young male rats in stroke research is one of the contributing factors for this problem. Our expectations were that data acquired from aged rats should be more translatable to human stroke. While we did not find any differences in the venous blood between the sexes, we use both sexes to better reflect the human population. Gender-specific responses to stroke therapies are well-documented, and most recently exemplified in the experience with the clinical trial showing that uric acid is effective with females but not male stroke patients [19].

These changes in pH taken together are predictors that affect infarct volume. The importance of this finding occurring in the post-MCAO to 72-hour mark is important for translational studies, because this period would be the most applicable as a prognostic tool for the human patient. The pH is tightly regulated in the blood and other tissues to maintain homeostasis. The accumulation of lactic acid and CO_2 in ischemic brain disrupts the normal pH balance [9]. Our study showed significant changes of pH as a predictor of infarct volume, but interestingly, a predictor that varied depending on the time point pH was measured. At the first timepoint (from pre- to post- MCAO), our results demonstrate that as changes in pH increased, infarct volume increased. At the second timepoint (from post-MCAO to 72 hours), as changes in pH increased, infarct volume decreased.

Carbon dioxide and water are catalyzed by carbonic anhydrase, which results in the generation of bicarbonate and H^+ [20]. The H^+ proton facilitates transport of Na^+ across membranes. Other electrolytes and water follow the transport of Na^+ during ischemia, causing malignant cerebral edema. Bicarbonate production is low in blood under alkaline conditions, and is high in acidic conditions found in ischemia. Its purpose

is to regulate pH homeostasis, and to facilitate anion/water transport. In heart ischemia reperfusion, the presence of bicarbonate causes increased oxidative damage [21], which leads to increased inflammation. While ELVO causes significant injury to the affected brain, the associated malignant edema injures surrounding otherwise unaffected brain, caused herniation and death [22]. These early changes in bicarbonate predict mortality of aged rats of both sexes and provides a potential biomarker to be explored in human stroke patients.

We have also identified predictors related to edema, which can be a lethal consequence of cerebral infarction. Changes in ionized calcium and sodium post-MCAO to 72 hours predicted for edema volume. We found an inverse relationship, as changes in ionized calcium and sodium (post-MCAO to 72 hours) to edema volume, as ionized calcium and sodium increased, edema volume decreased. To our knowledge neither ionized calcium, nor sodium were previously reported to affect edema volume as a stroke outcome. They are, however, discussed in connection with infarct volume, patient functional outcomes, and mortality. In human stroke patients, severe clinical presentation was seen after stroke onset, as was worsening of functional outcomes, and the potential for hemorrhagic conversion post-thrombectomy; these were associated with lower total serum calcium concentrations in peripheral venous blood [23-25]. Conversely, better functional outcomes and decreased infarcts were associated with higher venous total serum calcium levels [26-28]. These findings link positive stroke outcomes to increased serum calcium levels. Yet, also seen in human stroke patients were inconsistent results of associations between serum Na^+ levels and stroke severity. Patients with increased stroke severity and mortality exhibited associations with hyponatremia or lower Na^+ levels [29,

30]. While others found associations between increase stroke incidence and neurological worsening with higher venous serum Na^+ concentrations [31-33]. However, these studies relied on univariate analyses so multivariate analyses appear to add more understanding of the associations of biomarkers with functional or neurological outcomes.

The Cox regression showed changes in bicarbonate levels were also an important predictor for increased risk for mortality. Bicarbonate is important for pH, electrolyte, and water balance, which is disturbed during ischemic stroke. Bicarbonate levels changed rapidly after inserting the embolus into the internal carotid artery to block the middle cerebral artery, approximately within 7 minutes. Our data show that the physiological buffering response of the rats is impaired post-pMCAO; they are unable to adjust their blood homeostasis, leading to mortality. To our knowledge, this is the earliest change in bicarbonate measurement after experimental stroke to be reported. Moreover, this finding shows that there are two rat subpopulations. One is able to regulate bicarbonate and survive and other unregulated bicarbonate levels increased probability of death by 328%. If translated to humans, this would suggest that early bicarbonate levels could inform treatment for the patient after stroke.

There is, however, some distinction between the pMCAO model in young and aged rats in acid/base balance and electrolyte concentrations as predictors of infarct volume. In our previous paper, we discovered as changes in pH and ionized calcium (from pre- to post-MCAO) increased, infarct volume decreased in young male rats [12]. This finding is inverse from results related to aged rats, which show that as changes in pH (from pre- to post-MCAO) increased, infarct volume increased. We also previously found that as changes in ionized calcium (from pre- to post- MCAO) increased, infarct volume

decreased. These changes were not found in aged rats, rather there was an association with changes in ionized calcium at a different time point (post-MCAO to 72 hours) with an increase in edema volume. The differences between young and aged rats demonstrate that some blood gas and electrolyte parameters are age dependent, while others are not. Translation of findings from rodent to human has been problematic in stroke research with the almost exclusive use of young male rats. As demonstrated by this study, differences in the response to stroke arise between young and aged rats. These aged rats are a closer age match to human stroke patients and could be a better model for translation to humans.

The physiological significant of this study is that the response to ischemia is rapid within minutes. Water movement is associated with transport of electrolytes, primarily with sodium. Our data indicate that if the brain is able to maintain the electrolytes, ionized calcium and sodium, within the blood then edema is reduced. This study suggests infarct volume and death may occur if buffering in the blood is unable to maintain blood homeostasis. The maintenance of pH is essential as acidosis will result in neural cell death to expand the infarct volume [15, 34, 35]. Bicarbonate, as discussed above, plays a critical role in maintaining blood homeostasis and the dysregulation of bicarbonate. In fact, serum bicarbonate is associated with heart failure and mortality [36].

Conclusion

In conclusion, we showed shows that blood chemistry in the systemic circulation responds rapidly to an ischemic event in the brain. Of the three time points used, the post-MCAO and 72-hour time points would be most applicable to the human stroke patients since they receive treatment hours after having a stroke. Finally, bicarbonate stands out as

the most promising indicator from our study, providing us with a predictive range for mortality but must be examined in the context of the human patient. Overall, these studies will provide acid/base and electrolyte concentrations as potential predictive biomarkers for stroke outcomes. These studies need be verified in human patients an at appropriate time points in patient care. However, the use of multivariate analyses appears to be more appropriate approach in determining predictive biomarkers.

Table 3.1: Venous blood gas analyses at pre-, post-, and 72 hours pMCAO in aged male and female Sprague Dawley rats.

Parameters	All Rats Mean ± SD	Male Mean ± SD	Female Mean ± SD	P-value
pH				
pre-MCAO	7.30 ± 0.04	7.32 ± 0.04	7.29 ± 0.03	0.125
post-MCAO	7.23 ± 0.07	7.27 ± 0.04	7.20 ± 0.06	
72 hours	7.24 ± 0.04	7.26 ± 0.03	7.23 ± 0.04	
pCO_2 (mmHg)				
pre-MCAO	60.11 ± 6.29	58.38 ± 6.58	61.27 ± 7.23	0.213
post-MCAO	77.08 ± 6.77	71.05 ± 6.94	81.10 ± 6.06	
72 hours	74.68 ± 7.62	73.83 ± 7.66	75.24 ± 7.58	
pO_2 (mmHg)				
pre-MCAO	125.33 ± 13.87	127.00 ± 11.24	123.22 ± 15.56	0.409
post-MCAO	103.33 ± 14.72	102.50 ± 16.67	103.89 ± 12.21	
72 hours	9.93 ± 1.76	9.17 ± 1.48	10.44 ± 1.96	
Beecf (mmol/L)				
pre-MCAO	3.33 ± 0.49	4.50 ± 0.64	3.56 ± 0.35	0.612
post-MCAO	4.80 ± 0.27	5.33 ± 0.36	3.78 ± 0.20	
72 hours	5.00 ± 0.62	6.83 ± 0.81	3.76 ± 0.45	
HCO^{3-} (mmol/L)				
pre-MCAO	29.95 ± 1.59	30.35 ± 1.26	29.69 ± 1.83	0.317
post-MCAO	31.65 ± 2.03	32.27 ± 1.43	31.23 ± 2.34	
72 hours	32.47 ± 2.84	34.18 ± 2.88	31.33 ± 2.80	
Na^+ (mmol/L)				
pre-MCAO	137.60 ± 1.51	137.17 ± 1.72	137.89 ± 1.36	0.774
post-MCAO	136.00 ± 1.56	135.67 ± 1.37	137.22 ± 1.72	
72 hours	144.20 ± 3.35	143.33 ± 3.23	144.78 ± 3.58	
K^+ (mmol/L)				
pre-MCAO	3.73 ± 0.34	3.97 ± 0.21	3.57 ± 0.32	0.871
post-MCAO	3.87 ± 0.30	4.03 ± 0.31	3.77 ± 0.27	
72 hours	4.59 ± 0.48	4.77 ± 0.66	4.45 ± 0.30	
iCa^{2+} (mg/dL)				
pre-MCAO	1.01 ± 0.16	1.01 ± 0.11	1.02 ± 0.19	0.673
post-MCAO	1.03 ± 0.13	1.07 ± 0.15	1.01 ± 0.12	
72 hours	1.22 ± 0.13	1.27 ± 0.10	1.18 ± 0.14	
Glu (mg/dL)				
pre-MCAO	223.67 ± 22.67	237.67 ± 19.52	214.33 ± 24.81	0.712
post-MCAO	346.87 ± 36.26	357.67 ± 33.87	339.67 ± 38.36	
72 hours	156.67 ± 16.19	158.50 ± 18.63	155.44 ± 14.89	

Table 3.1 (continued).

Hct (% PCV)				
pre-MCAO	40.13 ± 2.13	42.33 ± 2.07	38.67 ± 2.24	0.268
post-MCAO	41.23 ± 2.65	42.67 ± 2.80	39.45 ± 2.42	
72 hours	35.27 ± 1.20	37.17 ± 1.31	33.44 ± 1.13	
Hbg (g/dL)				
pre-MCAO	13.65 ± 0.76	14.40 ± 0.71	13.14 ± 0.77	0.158
post-MCAO	13.65 ± 0.68	14.53 ± 0.57	13.07 ± 0.72	
72 hours	11.99 ± 1.48	13.63 ± 1.20	10.90 ± 1.63	

Data are presented as mean ± standard deviations. Total sample size N = 16; n = 7 male rats, n = 9 female rats. P values represent the interaction of 3 time points x gender. Blood gas and electrolyte parameters: pCO2: Partial pressure of carbon dioxide (mmHg); pO2: partial pressure of oxygen (mmHg); HCO3-: bicarbonate (mmol/L); BD: base deficit (mmol/L); BE: base excess (mmol/L); sO2: oxygen saturation (%); Na+: sodium (mmol/L); K+: potassium (mmol/L); iCa^{2+}: ionized calcium (mmol/L); Glu: glucose (mg/dL); Hct: hematocrit (% PCV); and Hbg: hemoglobin (g/dL).

Table 3.2: Mauchly's test indicated that the assumption of sphericity had been violated for each blood gas parameter therefore degrees of freedom were corrected with Greenhouse-Geisser estimates of sphericity.

Variable	Approximate Chi-Square	*df*	Greenhouse-Geisser Estimate of Sphericity	Significance
pH	22.26	2	$\varepsilon = 0.678$	$p < 0.001$
pCO_2	23.49	2	$\varepsilon = 0.791$	$p < 0.001$
pO_2	20.45	2	$\varepsilon = 0.550$	$p < 0.001$
HCO^{3-}	15.61	2	$\varepsilon = 0.579$	$p < 0.001$
Beecf	17.75	2	$\varepsilon = 0.564$	$p < 0.001$
Na^+	13.64	2	$\varepsilon = 0.596$	$p < 0.001$
K^+	11.05	2	$\varepsilon = 0.517$	$p < 0.001$
iCa^{2+}	12.26	2	$\varepsilon = 0.502$	$p < 0.001$
Glu	8.31	2	$\varepsilon = 0.667$	$p = 0.016$
Hct	15.98	2	$\varepsilon = 0.569$	$p < 0.001$
Hbg	17.06	2	$\varepsilon = 0.572$	$p < 0.001$

Blood gas and electrolyte parameters: pCO2: Partial pressure of carbon dioxide (mmHg); pO2: partial pressure of oxygen (mmHg); HCO3-: bicarbonate (mmol/L); BD: base deficit (mmol/L); BE: base excess (mmol/L); sO2: oxygen saturation (%); Na+: sodium (mmol/L); K+: potassium (mmol/L); iCa^{2+}: ionized calcium (mmol/L); Glu: glucose (mg/dL); Hct: hematocrit (% PCV); and Hbg: hemoglobin (g/dL).

Table 3.3: There was not a significant interaction effect between the three time points of blood gas parameters and sex.

Variable	F	*df*	Significance
pH	2.27	2	p = 0.125
pCO_2	1.64	2	p = 0.213
pO_2	1.17	2	p =0.409
HCO^{3-}	1.12	2	p = 0.317
Beecf	0.31	2	p = 0.612
Na^+	0.12	2	p = 0.774
K^+	0.14	2	p = 0.871
iCa^{2+}	0.39	2	p = 0.673
Glu	0.22	2	p =0.712
Hct	1.75	2	p = 0.268
Hbg	1.39	2	p = 0.158

Blood gas and electrolyte parameters: pCO2: Partial pressure of carbon dioxide (mmHg); pO2: partial pressure of oxygen (mmHg); HCO3-: bicarbonate (mmol/L); BD: base deficit (mmol/L); BE: base excess (mmol/L); sO2: oxygen saturation (%); Na+: sodium (mmol/L); K+: potassium (mmol/L); iCa^{2+}: ionized calcium (mmol/L); Glu: glucose (mg/dL); Hct: hematocrit (% PCV); and Hbg: hemoglobin (g/dL).

Table 3.4: Pre- and post-MCAO acid/base and electrolyte values for animals that died prior to 72 hours.

Parameters	*n*	Pre-MCAO Mean ± SD	Post-MCAO Mean ± SD	P-value
pH	15	7.31 ± 0.05	7.19 ± 0.12	**0.026**
pCO_2 (mmHg)	15	58.54 ± 4.80	91.13 ± 6.09	**0.015**
pO_2 (mmHg)	15	121.29 ± 14.41	119.57 ± 13.72	0.686
Beecf (mmol/L)	15	3.50 ± 0.52	3.52 ± 0.55	0.919
HCO^{3-} (mmol/L)	15	29.50 ± 2.24	35.67 ± 2.36	**0.001**
Na^+ (mmol/L)	15	137.19 ± 1.60	134.00 ± 1.67	**0.039**
K^+ (mmol/L)	15	3.81 ± 0.47	4.11 ± 0.43	0.151
iCa^{2+} (mg/dL)	15	1.02 ± 0.11	1.04 ± 0.13	0.657
Glu (mg/dL)	15	259.86 ± 31.99	360.00 ± 34.04	**0.014**
Hct (% PCV)	15	41.84 ± 2.48	42.86 ± 3.19	0.522
Hbg (g/dL)	15	13.99 ± 0.83	13.90 ± 1.10	0.589

Data are presented as mean ± standard deviations. Blood gas and electrolyte parameters: pCO2: Partial pressure of carbon dioxide (mmHg); pO2: partial pressure of oxygen (mmHg); HCO3-: bicarbonate (mmol/L); BD: base deficit (mmol/L); BE: base excess (mmol/L); sO2: oxygen saturation (%); Na+: sodium (mmol/L); K+: potassium (mmol/L); iCa^{2+}: ionized calcium (mmol/L); Glu: glucose (mg/dL); Hct: hematocrit (% PCV); and Hbg: hemoglobin (g/dL).

Supplemental Table 3.1: Baseline acid/base and electrolyte values for combined males and females groups.

		Survived N=16		Died Prior to 72 hours N = 15	
Parameters	*n*	Pre-MCAO Mean ± SD	*n*	Pre-MCAO Mean ± SD	P-value
pH	16	7.30 ± 0.04	15	7.31 ± 0.05	0.525
pCO_2 (mmHg)	16	60.11 ± 6.29	15	58.54 ± 4.80	0.537
pO_2 (mmHg)	16	125.33 ± 13.87	15	121.29 ± 14.41	0.412
Beecf (mmol/L)	16	3.33 ± 0.49	15	3.50 ± 0.52	0.673
HCO^{3-} (mmol/L)	16	29.95 ± 1.59	15	29.50 ± 2.24	0.883
Na^+ (mmol/L)	16	137.60 ± 1.51	15	137.19 ± 1.60	0.792
K^+ (mmol/L)	16	3.73 ± 0.34	15	3.81 ± 0.47	0.463
iCa^{2+} (mg/dL)	16	1.01 ± 0.16	15	1.02 ± 0.11	0.633
Glu (mg/dL)	16	223.67 ± 22.67	15	259.86 ± 31.99	0.179
Hct (% PCV)	16	40.13 ± 2.13	15	41.84 ± 2.48	0.347
Hbg (g/dL)	16	13.65 ± 0.76	15	13.99 ± 0.83	0.266

Data are presented as mean ± standard deviations. Blood gas and electrolyte parameters: pCO2: Partial pressure of carbon dioxide (mmHg); pO2: partial pressure of oxygen (mmHg); HCO3-: bicarbonate (mmol/L); BD: base deficit (mmol/L); BE: base excess (mmol/L); sO2: oxygen saturation (%); Na+: sodium (mmol/L); K+: potassium (mmol/L); iCa^{2+}: ionized calcium (mmol/L); Glu: glucose (mg/dL); Hct: hematocrit (% PCV); and Hbg: hemoglobin (g/dL).

Table 3.5: Predictors of infarct volume in aged rats (n = 15)

Variable	β	P-value
ΔpH (pre- to post-MCAO)	0.667	0.031
ΔpH (post- to 72 hours-MCAO	-0.683	0.034

$R^2 = 0.862$, adjusted $R^2 = 0.813$, $df = 2$, $F = 14.77$, $p = 0.030$, Durbin-Watson = 1.35

Table 3.6: Predictors of edema volume in aged rats (n = 16)

Variable	β	P-value
ΔNa^{+} (post- to 72 hours-MCAO)	-1.355	0.011
ΔiCa^{2+} (post- to 72 hours-MCAO)	-0.973	0.018

$R^2 = 0.813$, adjusted $R^2 = 0.745$, *df* = 2, F = 12.32, p = 0.015, Durbin-Watson = 2.13

Figure 3.1 Permanent Timeline**:** The first venous blood sample collection (represented by 1st iSTAT) to MCAO takes approximately 30 minutes (represented by the shaded box). The post-MCAO sample was taken approximately seven minutes after induction of the MCAO (represented by 2nd iSTAT). The MRI scans and 3rd iSTAT collection were obtained before euthanization at 72 hours.

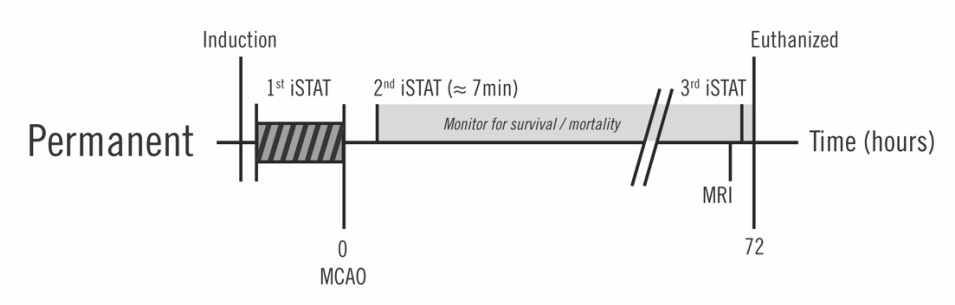
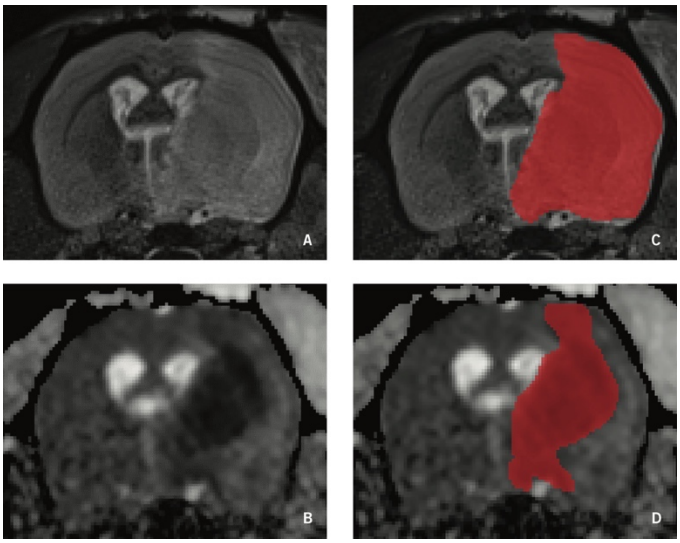
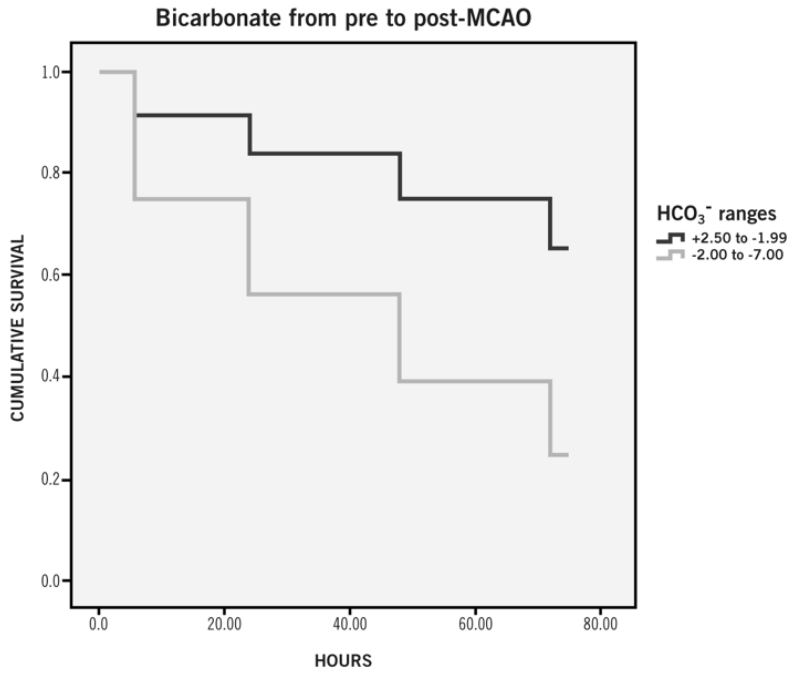


Figure 3.2.A-D MRI Images of Infarct and Edema Volumes



3.2a: Representative permanent-MCAO Diffusion Tensor Imaging (DTI) image of infarct volume. 3.2b: Representative permanent-MCAO T2-weighted image of edema volume. 3.2c: DTI imaging revealed the volume of brain parenchyma demonstrating restricted diffusion of infarct volume visibly affected after pMCAO. Shading indicates the calculation made by manual segmentation. 3.2d: T2 weighted imaging revealed the volume of brain parenchyma demonstrating restricted diffusion of edema volume visibly affected after pMCAO. Shading indicates the calculation made by manual segmentation.

Figure 3.3: Cox Regression and Mortality (n = 31).



CHAPTER 4. EVALUATION OF SEX DIFFERENCES IN ACID/BASE AND ELECTROLYTE CONCENTRATIONS IN ACUTE LARGE VESSEL STROKE

Abstract

Introduction and Purpose: We have developed a protocol to collect and evaluate arterial blood immediately distal and proximal from the removed intracranial thrombus during mechanical thrombectomy. These samples provide a unique resource in evaluating acute changes in acid/base and electrolyte concentrations at the time of ischemic stroke. The purpose of this study was to compare acid/base and electrolytes obtained proximal and distal to the occluded intracranial thrombi between male and female acute ischemic stroke participants at the time of thrombectomy; and to determine whether arterial blood gas values predict outcomes in male and female participants.

Methods: We analyzed the first 49 subjects (age = 67 ± 15.0, 21 males) in the BACTRAC registry. We compared arterial blood gas of blood distal versus proximal to the thrombus during thrombectomy which provided acid/base levels (pH, pCO_2, pO_2, BD, HCO_{3-}) and electrolyte values (iCa^{2+}, K^+, and Na^+). Comparisons were evaluated by one-way repeated measures ANOVA ($p < 0.05$. Moderated multiple regression with an interaction term of sex determined predictors of infarct volume, edema volume, and infarct time.

Results: Our participants revealed compensated metabolic acidosis with an elevated oxygen concentration in both blood samples. Analysis indicated several significant differences in the proximal blood samples between sex (pH, pCO_2, and K^+). Bicarbonate and base deficit were predictors of infarct time in only female participants.

Discussion and Conclusion: Acid/base and electrolyte response to ischemic conditions differ between men and women and these early changes could be used to predict stroke

outcomes in a sex-dependent manner. These findings provide a novel insight into the pathology of large vessel stroke in humans, particularly in men and women.

Introduction

Ischemic stroke is the leading cause of disability in the United States and affects over 800,000 Americans per year [1]. Approximately 87% of strokes are ischemic, resulting from an obstruction of blood flow to the brain [1]. When a cerebral thrombus blocks an artery, tissue damage transpires within a few hours of ischemic stroke onset due to neuronal cell death but with treatment the dying neurons in the penumbra may be salvaged [2, 3]. Acute ischemic stroke triggers an ischemic cascade which causes injury to cerebral tissue; this cascade may persist for many days post stroke. The pathophysiological processes following ischemic stroke are complex, involving acidosis, energy failure, increased intracellular calcium, excitotoxicity, activation of neuronal and glial cells, free radical production, and disruption of the blood-brain barrier (BBB) [4, 5]. Because time is crucial, treatments have focused on removing the thrombus as quickly as possible.

The current standard treatment of acute ischemic stroke focuses on recanalization of the occluded intracranial vessel. Current therapies aim to dissolve or remove the thrombus; this includes the use of tissue plasminogen activator (tPA) and mechanical thrombectomy [6-8]. tPA is a serine enzyme that catalyzes the conversion of plasminogen to plasmin, which is the primary mechanism for thrombolysis and mechanical thrombectomy is a procedure to remove a thrombus and re-establish blood flow for emergent large vessel occlusions (ELVO) [9, 10]. Unfortunately, patients must meet specific criteria to receive either or both of these treatments, and the treatments must be administered within the first few hours from stroke onset to achieve optimal efficacy for better functional outcomes [9-13]. Rates of arterial recanalization are higher due to the

use of tPA and mechanical thrombectomy, yet their effect on patient long-term functional outcomes remains inconclusive [14-16]. It is also unclear whether outcomes after stroke treatment for ELVO differ by sex.

A principal variable that may be affecting stroke outcomes is the biological sex of the patient. Ischemic stroke is the fifth leading cause of death for men in the US, but third leading cause of death for women, occuring in about 55,000 more women than men each year [1]. Women age 45-65 years old are at a higher risk (20%) of having a recurring stroke compared to their male counterparts (10%) [1]. Clinical studies have demonstrated the existance of sex-specific effects of stroke therapies. In a failed clinical trial, uric acid was found as an effective treatment in women, but not men [17]. This finding initially went unnoticed and was detected only after the results were reanalyzed according to sex differences [17]. As such, there is a need to further explore the relationship of sex differences and stroke outcome by an evaluation of males and females neurophysiological changes after stroke.

While mechanical thrombectomy and tPA have both become standards of care, mechanical thrombectomy provides a rare opportunity to examine neurophysiological changes occurring during stroke. In the setting of mechanical thrombectomy for emergent large vessel occlusion (ELVO), we have developed a protocol to collect and evaluate blood immediately distal and proximal from the removed intracranial thrombus [18]. We can analyze these tissues to better understand their neurochemical properties and assist in the development of adjunctive therapies to compliment current stroke treatments, particularly whether those treatments need to be specially tailored to men or women.

Our previous two studies reported changes in venous acid/base and electrolyte concentrations within a few minutes of focal ischemia in young male rats, in addition to aged male and female rats, and these changes predicted infarct and edema volumes and mortality [19]. Our findings in animal models of stroke suggested that changes in early blood chemistry might be used to predict stroke outcomes. Thus, the purpose of this study was to evaluate the acid/base and electrolytes in distal and proximal blood to the occluded intracranial thrombi between male and female acute ischemic stroke participants at the time of thrombectomy; and to determine whether these acid base and electrolyte values predict stroke outcomes (infarct and edema volume and/or infarct time) dependent on sex.

Methods

Study Design

We developed the Blood and Clot Thrombectomy Registry and Collaboration (BACTRAC) protocol: an Institiutional Review Board approved tissue banking strategy for emergent large vessel occlusion (clinicaltrials.gov NCT03153683) [18]. The BACTRAC study is a non-probability, convenience sample of participants (≥ 18 year olds) treated with mechanical thrombectomy for ELVO ischemic stroke at a large academic comprehensive stroke center in Lexington, Kentucky between May 2017 through December 2018. Institiutional Review Board approval was obtained, and all participants or legal authorized representative provided informed consent.

A sample size of 45 was determined by a power analysis calculation based on planned analyses for independent t-tests, one-way repeated measures analysis of variance

(RM-ANOVA), and moderated multiple regression. Independent t-tests were calculated using α level of 0.05, and power of 0.80, and a medium effect size. RM-ANOVA were calculated using α level of 0.05, and power of 0.80, and a large effect size. For moderated multiple regression, sample size were calculated with α level of 0.05, power of 0.80, a large effect size, and appropriate number of predictors.

Sample Population

Participants age 18 and over who were hospitalized with a diagnosis of an ischemic stroke who underwent thrombectomy were screened for study eligibility. Participants were candidates for inclusion if they meet criteria: a) signed and dated informed consent (within 24 hours of thrombectomy completion time), b) adult males and females of all races/ethnicities, c) acute ischemic stroke based on clinical and radiographic evidence as determined by stroke neurology team, d) for intra-arterial thrombectomy as determined by neurointerventional radiologist attending physican, e) have an acute thromboembolus within an intracranial artery in a large cerebral artery (i.e internal carotid, anterior cerebral, middle cerebral) or posterior circulation (vertebral/basilar), f) participants with impaired capacity may be included, since the ischemic stroke might be the cause of their incapacity, and g) to undergo endovascular thrombectomy. Participants were excluded if a) they were pregnant or breastfeeding, b) had disability from a prior stroke due to the inability to control for preexisting neurological impairment, and c) were participating in other studies receiving treatment that may alter their functional outcomes. The study adheres to IRB protocols.

Data Collection

On admission to the study, demographic data were collected from the participant's medical record. Baseline medical history and comorbidity conditions, concomittant medications, vital signs, National Institutes of Health Stroke Scale (NIHSS), computerized tomography (CT) scan to verify large vessel occlusion stroke and thrombectomy eligibility, and time of last known normal (LKN) symptoms (prior to ischemic stroke) were identified.

Once a participant was determined by neurointerventional radiologist (NIR) for thrombectomy they were then enrolled into the study. During the thrombectomy procedure, the NIR obtained approximately 1ml of whole arterial blood from the microcatheter distal to the cerebral thrombus, prior to thrombectomy and approximately 7ml of whole arterial peripheral blood from the internal carotid artery. This was a coordinated effort between the on-call research staff, attending NIR, and procedural nurse for the participant. From the distal and peripheral blood samples collected, 500 μL (0.5 ml) of peripheral blood and distal blood were sent to the hospital's central laboratory for blood gas (pH, partial pressure carbon dioxide, partial pressure oxygen, base deficit, and bicarbonate) and electrolyte values (ionized calcium, potassium, and sodium) separately through aliquots placed into standard hospital blood gas kits. The remaining blood was initially processed and stored in -80 freezer for future analyses. Within 24 hours post-thrombectomy, LKN to thrombectomy completion time (infarct time), thrombolysis in cerebral infarction (TICI) score, magnetic resonance imaging (MRI) and/or computed tomography (CT) scan was performed and radiographic endpoints were collected: infarct and edema volumes, hemorrhagic grade, and CTA collateral score. A final NIHSS was

performed at discharge. These data were entered into the study institution's stroke registry that is maintained using REDCap software.

Data Analysis

Descriptive statistics were used to characterize the participants by male and female. Participant demographics and baseline characteristics were assessed with independent t-tests for continous variables and Chi-square tests of associations based on categorical (ordinal and binary) variables to assess for sex differences. Comparisons of acid/base and electrolyte concentrations from distal to peripheral blood between the sexes were evaluated by one-way repeated measures ANOVA. A moderated multiple regression with interaction term of sex was used to determine predictors of stroke outcomes (infarct and edema volumes, and/or infarct time). An interaction term was created with acid/base and electrolyte variables x sex and were entered into the regression model in two steps with backward entry. Dummy variable of sex was coded as male = 0, female = 1. Age was entered into block 1 as a control variable, the functional outcome variables (infarct volume, edema volume, and infarct time) were dependent variables, while mean changes in acid/base parameters x sex were entered into block 2. The adjusted R^2 determined the variance explained in the functional outcomes by each variable. Multicollinearity was assessed in each regression and P-to-P plot was used to assess the changes in acid/base and electrolyte concentration data for normal distribution. Data from ischemic stroke participants were analyzed using SPSS, version 24 software (IBM, Armonk, NY). An alpha of 0.05 was used to determine statistical significance.

Results

Sample Characteristics

Forty-nine participants were treated with mechanical thrombectomy during the study period. Our participants were primarily female (57%), Caucasians (84%), and aged 67 ± 15 (Table 1). Sample participant characteristics and comparison based on gender are presented in Table 1. There were similarities in BMI, smoking status, NIHSS on admission and discharge, recanalization rate of TICI scores, infarct and edema volumes, and hemorrhagic grade between the sexes. The majority of comorbidities were similar between the sexes, however more females (42.9%) than males (9.5%) were diabetic ($p = 0.004$). More males (47.6%) than females (17.9%) displayed absent collaterals on presentation, over 50% of an M2 territory ($p = 0.027$), while more females (57.1%) than males (42.9%) presented with diminished collaterals, over 50% of M2 territory. The mean infarct time based on symptom onset to thrombectomy completion time demonstrated over a two hour difference between females (8 hours and 53 minutes) compared to males (6 hours and 35 minutes; $p = 0.081$).

Arterial Blood Gas Differences Between Male and Female Participants

Results from the one-way repeated measures analysis of variance (RM-ANOVA) of blood gas parameters of the sexes from distal and proximal arterial blood at the time of thrombectomy are presented in Table 2. There were sex differences in pH (male 7.34 ± 0.04 vs female 7.39 ± 0.06, $p = 0.046$), partial pressure carbon dioxide (male 39.60 ± 4.22 vs female 36.40 ± 3.87, $p = 0.002$), and potassium (male 3.66 ± 0.42 vs female 3.31 ± 0.46, $p = 0.004$) in proximal samples. The other measured blood gas and electrolyte

concentrations did not show significant differences between the sexes. Mauchly's test indicated that the assumption of sphericity had been met since it is based on two levels (distal and proximal). There were significant interaction effects between two of the distal and proximal samples based on sex (partial pressure carbon dioxide: $df = 1$, $F = 8.87$, $p = 0.005$; and potassium: $df = 1$, $F = 1.816$, $p = 0.009$).

Predictors of Stroke Outcomes

To examine the possibility that sex was a moderator, an interaction term of sex was created for acid/base and electrolyte concentrations. Moderated multiple regression analyses (n = 49) were nonsignificant in the changes of acid/base and electrolyte parameters to infarct volume and edema volume ($p > 0.05$) (Tables 4.3 and 4.4). An additional moderated multiple regression analyses (n = 49) with interaction term of sex demonstrated the changes in base deficit (BD) and bicarbonate (HCO_3^-) from distal to proximal arterial blood samples were predictors of infarct time ($F(4, 27) = 3.023$, $p = 0.042$) after controlling for age. This indicates a positive coefficient between BD and HCO_3^- together with the extent of infarct time, as changes in BD and HCO_3^- increased, infarct time increased. These variables explained 12% of the total variance in the model, as predictors of infarct time (Table 4.5).

Discussion

Overall, our results revealed there was relationship between base deficit, bicarbonate, and infarct time, in women but not men. Women had increased infarct time compared to men and on average and had a higher volume of collaterals on presentation to the hospital (57.1% to 42.9%). Additionally, we expected to see acidosis and hypoxia

in the distal blood due to the blockage of the artery from the cerebral thrombus. This study shows that regardless of sex differences there was a compensated metabolic acidosis with an elevated oxygen concentration in our ischemic stroke participants at the time of thrombectomy.

We determined that as changes in base deficit and bicarbonate increased, infarct time increased, but only in female participants. On average our female participants had a two and half hour increase in time from their last known normal symptom to thrombectomy completion time compared to male participants. We are unsure why women's treatment time varied from men's treatment time, but we might speculate from existing research that this is due to the different social experience of men and women. Women experience pre-stroke social isolation and lack of support, which has been found to contribute to poorer functional outcomes after stroke due to poor compliance, depression, and stress compared to men [21]. Framingham Heart Study found women more at risk for stroke incidence, lifetime risk of stroke, age at first stroke, post-stroke disability, and institutionalization rates [22]. Therefore, a delay in treatment for women could increase the damage from an ischemic stroke and decrease their chances of receiving treatments, such as tPA. In an experimental model of stroke, we found venous blood bicarbonate in aged rats to be a predictor for mortality with no sex differences [23]. However, a larger sample size is needed for the variability seen in the ischemic stroke population to uncover associations, such as mortality or other poorer stroke outcomes, especially if sex dependent.

Other sex differences were detected in proximal blood in pH, partial pressure carbon dioxide (pCO^2), and potassium (K^+). These findings demonstrate differences in

the early response in blood to stroke between men and women. A larger sample would give us insights into whether these factors could be used as determinants of prognostic factors for stroke outcomes which may differ between men and women.

To our knowledge, only one other report exists that evaluated distal and proximal arterial blood samples adjacent to the cerebral thrombus, Flores et al. (2011) found significant differences in partial pressure oxygen and oxygen saturation from ischemic stroke patients (n = 16) [24]. Their study was a smaller pilot study of acid/base and electrolyte concentrations at time of thrombectomy and results were not categorized by sex. Our results demonstrated similar values in both blood samples for pH, Na^+ and iCa^{2+}, in addition to proximal pCO_2. However, we found an elevated concentration of oxygen and lower concentrations in both blood samples for HCO^{3-} and K^+, and distal pCO_2 in our results. These differences between the studies could be due to a variety of reasons, such as thrombectomy technique, handling of samples or patient population. More studies examining blood collected during thrombectomy will enable answering the causes of the descrepancies between these first two studies.

Overall, we expected the distal blood to reveal acidosis and hypoxia due to the blockage of the artery by the cerebral thrombus, however, we did not see those changes in our results. This may be due to the participant's cerebrovascular system, collaterals, or the vessel is not fully occluded by the thrombus. More female than males had collaterals on presentation to the hospital. A limitation to the study is we did not collect a comparison CTA collateral score post-thrombectomy to determine the amount of perfusion the brain regained. Researchers recently evaluated collaterals based on sex in young, aged, obese, and hypertensive male and female mice [25]. Female mice

demonstrated smaller infarcts compared to male mice but was not associated with having greater extent of collaterals or remodeling [25]. An increase in sample size and collection of long-term imaging will permit a better understanding of the relationship between collaterals and blood gases during stroke.

Our blood gas results display compensated metabolic acidosis with pH within normal range, decreased values for pCO_2, HCO_{3-}, and base deficit [26, 27]. The majority of our participants were mechanically ventilated or received supplemental oxygen throughout the thrombectomy procedure, which is a possible reason for the elevated oxygen concentration in both the distal and proximal blood samples. Another possibility is over half of our participants having at least 50% of collaterals on presentation to the hospital. This increase of intraluminal partial pressure oxygen concentration did not correlate to a clinical improvement in our participant population showing that other factors could be accounting for maintenance in acid/base balance.

Hypokalemia and hypocalcemia were seen in both blood samples from our population. Hypokalemia may be caused by hyperventilation, which often occurs under anesthesia on a ventilator during mechanical thrombectomy [28]. Another explanation may be excessive urine loss, prolonged diarrhea, or vomiting, which are commonly seen in critically ill patients [29]. Hypocalcemia may result from the dilution of plasma seen in administration of fluid during resuscitative efforts in the ICU [30], in addition to increased mortality rate in ICU patients [31]. Lower venous total serum calcium concentrations were associated with more severe clinical symptoms following stroke onset, worse functional outcomes, and hemorrhagic transformation post-thrombectomy in ischemic stroke patients [32-34]. Higher venous total serum calcium levels detected

within 24 hours of stroke onset were associated with better functional outcomes and reduced cerebral infarcts in acute ischemic patients [35-37].

Limitations

There are several limitations to this study. The sample for this study was small and mostly Caucasian. This was a non-probability sample based on completed mechanical thrombectomy procedures. The standard of care includes baseline imaging of CT and/or MRI upon presentation to the hospital. Based on this imaging, the neuroradiologist calculates infarct and edema volumes, CTA collateral scores, and hemorrhagic grade scores. Follow-up imaging is needed to understand how thrombectomy relates to participant stroke and functional outcomes.

Conclusion

This study is among the first to examine early changes in blood chemistry of acute ischemic stroke participants who underwent mechanical thrombectomy. As demonstrated by previous reports, we found differences in the physiological response to stroke between men and women, which suggests therapeutic approaches may want to take into consideration sex. Thrombectomy is now a standard of care for ischemic stroke and our study population is growing. With an increased sample size, more associations could be elucidated not only based on sex but also co-morbidities and other patient factors relating to the patient population.

Table 4.1: Baseline Demographics and Characteristics for Ischemic Stroke Participants.

	Males (n = 21)	Females (n = 28)	p value
Age (Years)	67 ± 16.54	67 ± 14.11	0.961
Ethnicity			
African-American	1 (4.8)	2 (7.1)	0.835
Caucasian	20 (95.2)	22 (78.6)	0.099
Unknown	0 (0.0)	4 (14.3)	0.071
BMI			
Under/Normal Weight	7 (33.3)	11 (39.3)	0.762
Overweight	10 (42.9)	8 (28.6)	0.241
Obese	0 (0.0)	3 (10.7)	0.131
Morbidly Obese	4 (19.0)	6 (21.4)	0.904
Comorbidities			
Hypertension	16 (76.2)	21 (75.0)	0.433
Atrial Fibrillation	7 (33.3)	11 (39.3)	0.305
Diabetes	2 (9.5)	12 (42.9)	**0.004**
Hyperlipidemia	5 (23.8)	9 (32.1)	0.256
Previous Stroke	4 (19.0)	6 (21.4)	0.611
COPD	4 (19.0)	4 (14.3)	0.746
CAD	5 (23.8)	1 (3.6)	0.076
Smoking Status			
Never	13 (61.9)	17 (60.7)	0.933
Currently	6 (28.6)	6 (21.4)	0.409
Previously (> 6 months)	2 (9.5)	5 (17.9)	0.565
NIHSS on Admission	18 ± 7.57	16 ± 6.41	0.460
Minor Stroke (1-4)	3 (14.3)	3 (10.7)	0.706
Moderate Stroke (5-15)	5 (23.8)	10 (35.7)	0.371
Moderate/Severe (16-20)	5 (23.8)	8 (28.6)	0.709
Severe Stroke (≥ 21)	8 (38.1)	7 (25.0)	0.325
NIHSS at Discharge	12 ± 12.34	11 ± 10.99	0.804
Minor Stroke (1-4)	10 (47.6)	11 (39.3)	0.560
Moderate Stroke (5-15)	5 (23.8)	8 (28.6)	0.709
Moderate/Severe (16-20)	3 (14.3)	5 (17.9)	0.738
Severe Stroke (≥ 21)	3 (14.3)	4 (14.3)	0.654

Table 4.1 (continued).

TICI Score			
2A = < 50% Perfusion	0 (0.0)	3 (10.7)	0.115
2B = > 50% Perfusion	5 (23.8)	11 (39.3)	0.217
3 = Full Perfusion	16 (76.2)	13 (46.4)	0.075
LKN to Thrombectomy Completion Time (minutes)	395.29 ± 285.12	533.07 ± 254.68	0.081
Infarct Volume (cm^3)	81.43 ± 97.27	66.30 ± 94.07	0.632
Edema Volume (cm^3)	70.68 ± 97.4	64.71 ± 86.34	0.822
Hemorrhagic Grade			
None	5 (23.8)	6 (21.4)	0.838
HI1	8 (38.1)	12 (42.9)	0.737
H12	7 (33.3)	7 (25.0)	0.523
PH1	0 (0.0)	2 (7.1)	0.211
PH2	1 (4.8)	1 (3.6)	0.835
CTA Collateral Score			
0	10 (47.6)	5 (17.9)	**0.027**
1	9 (42.9)	16 (57.1)	0.264
2	1 (4.8)	4 (14.3)	0.262
4	0 (0.0)	1 (3.6)	0.375

Values are mean ± SD or (%). Comparisons were performed with independent t-tests, Chi-square tests based on distribution of data.

Table 4.2: RM-ANOVA results of sex and blood gas parameters during thrombectomy.

Parameters	All Participants (N = 49) Mean ± SD	Male (n = 21) Mean ± SD	Female (n = 28) Mean ± SD	P-value
pH				
Distal	7.36 ± 0.06	7.35 ± 0.05	7.38 ± 0.05	0.152
Proximal	7.37 ± 0.06	7.34 ± 0.04	7.39 ± 0.06	**0.046**
pCO_2 (mmHg)				
Distal	32.21 ± 5.11	34.70 ± 4.92	30.34 ± 4.96	0.059
Proximal	37.77 ± 3.87	39.60 ± 4.22	36.40 ± 3.87	**0.002**
pO_2 (mmHg)				
Distal	213.98 ± 59.51	209.86 ± 64.12	217.07 ± 56.80	0.779
Proximal	251.43 ± 67.64	233.82 ± 61.10	264.64 ± 61.87	0.427
BD (mmol/L)				
Distal	-6.28 ± 2.68	-5.83 ± 2.27	-6.65 ± 2.07	0.560
Proximal	-3.85 ± 2.55	-3.89 ± 2.31	-3.82 ± 2.80	0.926
HCO^{3-} (mmol/L)				
Distal	19.17 ± 2.41	19.38 ± 2.18	19.02 ± 5.66	0.508
Proximal	21.72 ± 2.70	21.54 ± 2.20	21.85 ± 3.06	0.357
Na^+ (mmol/L)				
Distal	140.47 ± 4.66	140.19 ± 4.09	140.68 ± 4.11	0.748
Proximal	138.45 ± 3.20	138.62 ± 1.91	138.32 ± 3.94	0.777
K^+ (mmol/L)				
Distal	3.04 ± 0.86	3.24 ± 0.82	2.88 ± 0.87	0.116
Proximal	3.46 ± 0.47	3.66 ± 0.42	3.31 ± 0.46	**0.004**

Table 4.2 (continued).

iCa^{2+} (mg/dL)				
Distal	3.86 ± 0.79	3.94 ± 0.75	3.81 ± 0.83	0.552
Proximal	4.33 ± 0.22	4.32 ± 0.25	4.34 ± 0.20	0.994

Data are presented as mean ± standard deviations. pCO2: Partial pressure of carbon dioxide (mmHg); pO2: partial pressure of oxygen (mmHg); HCO3-: bicarbonate (mmol/L); BD: base deficit (mmol/L); BE: base excess (mmol/L); sO2: oxygen saturation (%); Na+: sodium (mmol/L); K+: potassium (mmol/L); iCa^{2+}: ionized calcium (mmol/L).

Table 4.3: Moderated Multiple Regression Variables Predicting Infarct Volume (n = 49)

Variable	B	SE B	β	P value
Age	-1.56	1.22	-0.245	0.213
ΔK^+ x sex	-78.86	74.20	-0.491	0.296
ΔBD x sex	-14.30	12.30	-0.537	0.254

Abbreviations: K^+: potassium (mmol/L); and BD: base deficit (mmol/L).
These were the remaining variables in the model with VIF < 10.

Table 4.4: Moderated Multiple Regression Variables Predicting Edema Volume (n = 49)

Variable	B	SE B	β	P value
Age	-1.18	1.31	-0.177	0.377
ΔK^+ x sex	-83.33	77.49	-0.502	0.291
ΔBD x sex	-16.11	12.87	-0.584	0.220

Abbreviations: K^+: potassium (mmol/L); and BD: base deficit (mmol/L).
These were the remaining variables in the model with VIF < 10.

Table 4.5: Moderated Multiple Regression Variables Predicting Infarct Time (n = 49)

Variable	B	SE B	β	P value
Age	0.65	2.69	0.38	**0.037**
ΔHCO_3^- x sex	37.81	18.15	0.55	**0.019**
ΔBD x sex	37.50	15.40	0.47	**0.044**

$R^2 = 0.18$, adjusted $R^2 = 0.12$, *df* = 4, F = 3.023, Durbin-Watson = 1.918
Abbreviations: HCO_3^-: bicarbonate (mmol/L); and BD: base deficit (mmol/L).

CHAPTER 5. DISCUSSION AND CONCLUSIONS

Background and Purpose

Given that ischemic stroke is the leading cause of disability in the United States, the need to refine and develop innovative treatments remains a pressing priority for medical researchers. Nurse researchers can contribute to these efforts by bridging the gap between clinical and bench science, and harnessing their knowledge of patient experiences to assist in the creation of multi-disciplinary studies. This dissertation emerges from such a collaboration and contributes to the effort to expand the field of nursing research interest in neuropathology and quantitative research.

The purpose of this dissertation was to examine neurochemical factors associated with the initial pathophysiological reaction to acute large vessel occlusion in rodent models and individuals diagnosed with ischemic stroke. Three studies were conducted focused on acid/base and electrolyte concentrations in these populations: 1) a descriptive correlational analysis of the associations between venous blood gases, infarct volume and mortality in young male (three month old) Sprague Dawley rats; 2) a descriptive correlational analysis of the relationship between venous blood gases to infarct and edema volumes, and mortality in aged (18 months old) male and female Sprague Dawley rats; 3) a descriptive correlational study comparing acid/base and electrolyte concentrations in patients diagnosed with ischemic stroke who underwent mechanical thrombectomy.

This chapter will present a summary of the findings from the three manuscipts. I will then synthesize those findings to explain how the results help further our

understanding of the neurochemical changes that take place during stroke. Recomendations for future research will be addressed, as will limitations both of the current study and existing efforts to study ischemic stroke.

Summary of Findings

In Chapter Two, we examined the differences between acid/base balance and electrolyte concentrations in two focal ischemia models in young male rats. This was a descriptive correlational study evaluating three-month old Sprague-Dawley male rats that underwent permanent (n = 18) or transient (n = 19) middle cerebral artery occlusion (MCAO). Pre- and post-MCAO venous blood samples from permanent and transient models provided pH, carbon dioxide (CO_2), oxygen (O_2) , bicarbonate (HCO_{3-}), glucose, hemoglobin, hematocrit, and electrolyte values of ionized calcium (iCa^{2+}), potassium (K^+) and sodium (Na^+). Mean differences were seen in the blood gas and electrolyte concentrations between pre- to post-MCAO in both models. pH and iCa2+ were predictors of infarct volume in the permanent MCAO model. The transient MCAO model exhibited greater survival to 72 hours compared to the permanent MCAO model ($p = 0.045$).

In Chapter Three, we compared acid/base balance and electrolyte concentrations at three time points between aged male and female rats. We examined venous blood gas samples of aged (18 month old) Sprague Dawley male (n = 15) and female (n = 16) rats 1) pre-, 2) post-, and 3) at 72 hours of permanent MCAO. The repeated measures ANOVA revealed no mean differences in acid/base and electrolyte concentrations from the three time points between the sexes. However, changes in pH (from pre- to post-MCAO and post-MCAO to 72 hours) and changes in Na^+ and iCa^{2+} (from post-MCAO to

72 hours) were predictors of infarct volume and edema volume, respectively. There was a 3.25 times increased risk for mortality in rats based on changes (cut-off range within -2.00 to -7.00) in HCO^{3-} levels (pre- to post-MCAO).

In Chapter Four, we compared acid/base balance and electrolyte concentrations in arterial distal and proximal blood to the cerebral thrombus between male (n = 21) and female (n = 28) ischemic stroke participants who underwent mechanical thrombectomy. On average female participants had a two and half hour increase in time from ischemic stroke symptom onset to vessel recanalization or *infarct time* compared to male counterparts. Changes in HCO_{3-} and base deficit were found to be predictors of infarct time, but only in females.

Implication of Findings

The results from this dissertation have expanded our understanding of neurochemical factors and neuropathology at the time of focal ischemia in rats and patients with ischemic stroke at the time of mechanical thrombectomy in a number of ways:

1) In examining two different rodent models (permanent MCAO and transient MCAO), we were able to compare baseline blood gas values to blood gas values taken immediately after the occlusion. First, because the majority of stroke patients do not receive tPA and/or mechanical thrombectomy, this comparison in the permanent MCAO model is important because it reveals insights about the speed at which neurochemical changes take place. Second, the transient MCAO model resembles recanalization and

reperfusion of the artery and allows us to examine blood chemistry changes that occur in the brain after treatment.

2) In examining young male and aged male and female rats, we have identified predictors of infarct volume, edema volume, and mortality in an animal model. This insight is novel because there is no existing research of acid/base and electrolytes concentrations as predictors of stroke, let alone at this early phase of stroke onset. This gives us an insights about the speed at which neurochemical changes are taking place. This is something we can observe in rodent models but which could aid us in understanding what occurs in patients who have an ischemic stroke.

3) We are among only a few researchers to compare acid/base and electrolytes from blood distal and proximal to the cerebral thrombus in patients after ischemic stroke. The protocol developed for the Blood and Clot Thrombectomy Registry and Collaboration (BACTRAC) study uses an innovative method to obtain blood specimens and allows for a systematic understanding of ischemic stroke neuropathology at the time of mechanical thrombectomy. Our results challenge the current literature by demonstrating that a compensated metabolic acidosis with an elevated oxygen concentration appeared in the distal blood. Future studies should aim to identify the percentage of arterial occlusion that occurred in patients undergoing thrombectomy, ventilator settings during thrombectomy, and the amount of the time the patient was provided supplemental oxygen. In understanding and improving stroke outcomes, investigators should evaluate and compare arterial blood gases not only during thrombectomy but across several points during the patient's hospital stay and the relationship to functional outcomes.

4) By examining sex differences in acid/base and electrolyte concentrations in patients, we discovered significant differences in the proximal blood in patients diagnosed with ischemic stroke. In females, we found that changes in bicarbonate and base deficit were predictors of infarct time. Additionally, in females, there was approximately 2.5 hour increase from symptom onset to thrombectomy completion time compared with males. In our study population, more males than females displayed absent collaterals on presentation to the hospital. Sex differences are generally ignored in clinical trials for stroke therapies and our results suggest a renewed importance in considering how differences in treatment times affect overall outcomes for female patients. Future studies should aim to identify different parameters to determine stroke outcomes based on sex. Investigators should focus on factors like distance and time from LKN to the hospital, marriage status, insurance status, imaging issues, and delay in hospital diagnosis.

Limitations

Emergent large vessel occlusion (ELVO) accounts for 20-40% of ischemic strokes and is the most disabling form of stroke [1-3]. Nevertheless, our understanding of stroke pathophysiology remains limited due to the challenges of studying stroke onset in humans. Animal models, particularly young rodents, have long served as a way for researchers to model stroke pathophysiology, yet stroke research has, on the whole, suffered an inability to provide clinically relevant animal-to-human translation. This is due in part to how animal studies are conducted, which typically follows a uniform practice of selecting young male rodents as its test subjects. The majority of ELVO stroke patients are middle-aged to elderly and are of both sexes, revealing that there is an age

and sex mismatch between ischemic stroke patients and animal models**.** Rethinking of the experimental animal models needs is required, especially in encouraging the use of aged male and female rats with comorbidities to more closely mirror human populations.

A limitation for Chapters Two and Three is venous blood samples were not always obtained pre-, post-, and/or at 72 hours of MCAO procedure and therefore reduced the number of samples in analyses. We have identified several issues with the collection of blood samples, faulty iSTAT cartridges, air bubbles in blood, fragile vasculature of aged animals were fragile and IJ collapsed, and/or insufficient amount of blood drawn. Working with aged animals is challenging. Post-MCAO we had approximately 50% mortality rate decreasing our sample size, especially in aged female rats. We believe this is due to the aging vasculature of the animals.

Another limitation with our human study is the nature and complexity of Blood and Clot Thrombectomy Registry and Collaboration (BACTRAC) study. BACTRAC is a multidisciplinary team operating in a clinical environment, and therefore, the potential data collection and specimen processing errors occurred. Additionally, the potential for sampling bias was present since the sampling strategy was purposeful, non-probability sample, and based on number of thrombectomy procedures completed. The current patient population used in this study is largely Caucasian and limited to the hospital's location in Central Kentucky. A larger and more diverse sample size is needed. An additional limitation is that we could not control for all comorbidities present in our sample population of ischemic stroke participants.

The BACTRAC study data are limited to the patient's hospitalization, limiting our ability to study long-term impact on functional outcomes. The two blood samples

(proximal and distal) were drawn at approximately the same time during mechanical thrombectomy. Thus, it was not possible to compare across time points and longitudinal data is needed for a systematic understanding of changes. Additionally, imaging for the infarct and edema volumes were based on CT and/or MRI that was completed prior to thrombectomy. Based on this initial imaging, CTA scores and hemorrhagic grade scores were assigned. However, even though this is the standard of care, there is no long-term follow-up or re-evaluation to understand how the treatment relates to these outcomes.

Recommendations for Future Research

Future studies are needed to identify serum biomarkers to predict the risk of worsened long-term outcomes and/or increased risk for mortality. Mechanical thrombectomy provides a unique opportunity for researchers to further this work by expanding the collection and analysis of biospecimens (distal blood, proximal blood, and cerebral thrombus). To understand the complexity of stroke, researchers can analyze these tissues for different molecular targets (such as proteomics and genomics) that occur in response to ischemic stroke. Associations may be found between patient characteristics (based on comorbidities, age, and sex) and functional recovery. This information may aid in the reduction of symptom burden for individuals diagnosed with ischemic stroke. Investigators should also focus on data from ischemic stroke patients and attempt to discover target molecules and then in animals to establish mechanism, this will aid in the development of new stroke therapies, keeping in mind that sex differences may play a role in treatment success. Finally, researchers must begin to explore how patients' respond to thrombectomy through long-term studies with ischemic stroke survivors and evaluation of their functional outcomes.

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3. Campbell, B.C., et al., *Endovascular stent thrombectomy: the new standard of care for large vessel ischaemic stroke.* Lancet Neurol, 2015. 14(8): p. 846-54.

VITA

Education

Institution	Degree	Date Conferred	Field(s) of Study
Portland State University	BS	12/2009	Biology
University of Kentucky	BSN	05/2013	Nursing

Professional Experience

Clinical

09/2014 – 08/2015	Staff Nurse, Neurosurgical Intensive Care Unit, MedStar Georgetown University Hospital, Washington, DC
06/2013 – 08/2014	Staff Nurse, Neurosurgical Intensive Care Unit, University of Kentucky Chandler Medical Center, Lexington, KY

Research

05/2017 - Present	Graduate Research Laboratory Assistant, University of Kentucky, College of Medicine, Departments of Neurology and Neurosurgery, Lexington, KY
08/2015 – 05/2016	Graduate Research Laboratory Assistant, University of Kentucky, College of Nursing, Lexington, KY
08/2010 – 08/2012	Undergraduate Research Laboratory Assistant, University of Kentucky, College of Nursing, Lexington, KY
01/2009 – 06/2010	Laboratory Technician, University of Oregon, Institute of Ecology and Evolution, Eugene, OR
08/2006 – 12/2008	Undergraduate Research Laboratory Assistant, Portland State University, Department of Biology, Portland, OR

Teaching

11/2018, 04/2019	Guest Lecturer on Genetics and Genomics, NUR 403, Public Health Nursing, University of Kentucky, College of Nursing, Lexington, KY
10/2018, 04/2018, 11/2017, 04/2017, 11/2016	Guest Lecturer on Nursing Genetic Research, NUR 310-002, Research for Evidence-Based Nursing Practice, University of Kentucky, College of Nursing, Lexington, KY

02/2018	Guest Lecturer on Biological Nursing Research, BIO 350, Animal Physiology, University of Kentucky, Department of Biology, Lexington, KY
08/2016 – 05/2017	Graduate Teaching Assistant, NUR 310-002, Research for Evidence-Based Nursing Practice, University of Kentucky, College of Nursing, Lexington, KY

Publications

Martha, S. R., Collier, L. A., Davis, S. M., Alhajeri, A., Grupke, S., Pennypacker, K. R., and Fraser, J. F. (2019). Evaluation of sex differences in acid/base and electrolyte alterations in acute large vessel stroke in humans. Manuscript in preparation for Journal of NeuroInterventional Surgery (JNIS).

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Suzanne Estes, Anna L. Coleman-Hulbert, Kiley A. Nusbaum, Gene de Haan, Dana K. Howe, Sarah R. Martha, Jeremiah B. Knapp, Samson W. Smith, Kevin C. Stein, and Dee R. Denver, (2011). Natural variation in life history and aging phenotypes is associated with mitochondrial DNA deletion frequency in Caenorhabditis briggsae. BMC Evolutionary Biology 11(11):1-8.

Professional Honors

06/2018	National Institute of Nursing Research (NINR), Summer Genetics Institute Training Fellowship, National Institutes of Health (NIH), Bethesda, MD
05/2017 – Present	University of Kentucky, Department of Neurosurgery Fellowship
08/2016 – 05/2017	University of Kentucky, Graduate School Academic Year Fellowship (GSAY Award)
08/2010 – 12/2011	University of Kentucky, Healthcare Nursing Educational Award