Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** The Pennsylvania State University

2. **Reporting Period (start and end date of grant award period):** 1/1/2009 – 12/31/2012

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** John Anthony, MPA

4. **Grant Contact Person's Telephone Number:** 814-935-1081

5. **Grant SAP Number:** # 4100047645

6. **Project Number and Title of Research Project:** 22 - The Use of Biomarkers to Predict the Onset of Vasospasm in Aneurysmal Subarachnoid Hemorrhage

7. **Start and End Date of Research Project:** 7/8/2009 – 12/31/2011

8. **Name of Principal Investigator for the Research Project:** Kevin M. Cockroft, MD, MSc, FACS

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 61,835

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project	Cost
None			

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project
Cockroft	PI	2
Connor	Co-PI	1
Patel	Investigator	5
Mitchell	MD/PhD Student	5
Weston	MD/PhD Student	1
Lehman	Statistical Analyst	2

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
None		

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No_____x____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes_____ No_____x_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If

you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
None	☐NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)		$	$

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes____X_____ No_____

If yes, please describe your plans:

The results from these studies will likely be combined with other work to generate an application for further grant funding for the study of delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage.

12. Future of Research Project. What are the future plans for this research project?

We will perform an alternative analysis looking at rate of biomarker change over time to determine if this has a relationship with DCI or clinical outcome in our patient population. Manuscript writing based on our current results is underway for future publication in a peer-reviewed journal. The results from these studies will likely be combined with other work to generate an application for further grant funding for the study of delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes_____ No_____X____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male				
Female				
Unknown				
Total				

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic				
Unknown				
Total				

	Undergraduate	Masters	Pre-doc	Post-doc
White				
Black				
Asian				
Other				
Unknown				
Total				

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No____x_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes_____ No____x_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes_____ No____x_____

If yes, please describe the collaborations:

16(B) Did the research project result in commercial development of any research products?

Yes_____ No____x_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No____x_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure

symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Project goals, Objectives and Specific Aims:

Stroke is the third leading cause of death and the primary cause of disability in the United States [1]. Aneurysmal subarachnoid hemorrhage (aSAH), a form of hemorrhagic stroke, is a particularly devastating disease. The thirty-day mortality rate after aneurysmal SAH is approximately 50% with the majority of survivors left disabled and unable to return to work [2]. Of those patients that survive the initial aneurysm rupture and/or re-rupture, the primary cause of death and further disability is delayed cerebral ischemia (DCI) [3]. Cerebral vasospasm, is becoming rarer terminology for the early brain injury that follows the rupture of an intracranial aneurysm and is an inaccurate surrogate for DCI. There is a correlation between arterial/arteriolar constriction and presence of neurological symptoms in only half of all cases. The dissociation between angiographic vasospasm and outcome after SAH was noted in the double blind randomized control trials with the endothelin receptor antagonist clazosentan [4, 5]. Despite its unequivocal clinical significance, the complete pathogenesis of post-hemorrhagic DCI remains poorly understood. Currently, only one drug (nimodipine) is FDA approved for the treatment of cerebral vasospasm, thought to be the causative factor of DCI [6]. Physicians in North America typically employ hyperdynamic therapy or "triple H"; specifically induced hypervolemia, hemodilution and hypertension. Unfortunately, there are a number of severe complications including pulmonary edema, myocardial ischemia, hyponatremia and cerebral edema, which raises questions the efficacy of such treatment. In the last decade a number of theories have been put forth for the pathogenesis of DCI. Investigators have examined inflammatory mechanisms, volume contraction, autonomic dysregulation, local oxygen free radicals, nitric oxide (NO) production, endothelin-1 and microthrombosis [7-14]. Yet given the myriad of signaling cascades the picture still remains obscure. What is known is that there exists a specific microenvironment during subarachnoid hemorrhage and the presence of a clot is enough to set off a number of molecular and cellular processes [7]. It is controversial whether vasospasm of the principal arteries of the circle of Willis is the major determinant of cortical neuronal ischemia [15]. Both angiographic spasm and trans-cranial Doppler spasm have poor predictive value for the development of DCI. Recent evidence points to cerebral blood flow changes directed by small diameter vasculature or micro-circuitry [11]. However this explanation is controversial and the mechanism as to how this occurs is uncertain.

The role of inflammation during cerebral ischemia has been impractical to study in the general population and would require very large epidemiological trials. Patient's with aSAH represent a unique subset to study the association between plasma inflammatory markers and the subsequent development of ischemia. Although there are clinical and experimental data that suggest an inflammatory mechanism may be one possible etiologic factor in post-hemorrhagic vasospasm, the degree to which inflammation plays a role in cerebral vasospasm is a contended point. Few studies have shown that plasma markers in SAH are consistent with DCI and cerebral vasospasm. Elevated C-reactive protein and leukocytosis may predict poor outcome following

SAH [16, 17]. Some authors have argued that leukocyte-endothelial cell interactions in particular lead to the development of vasospasm.

Immune system regulators, such as inflammatory cytokines, have been implicated in a variety of brain injury scenarios, including traumatic brain injury and aneurysmal rupture. However, the literature is very heterogeneous with regards to the magnitude and relative effect of each particular inflammatory protein or immune cellular interaction. Only one study thus far has demonstrated a correlation between a specific serum biomarker and cerebral vasospasm/DCI. On the contrary, the vast majority of research has focused on indirect indices of DCI. Though the preliminary data is promising, further investigation has failed to validate the usefulness of individual biomarkers. There would be huge potential benefit to examine more than a dozen inflammatory cytokines simultaneously over an extended period of time in human subjects.

Hypothesis:
It is hypothesized that the levels of inflammatory biomarkers will match the time course of vasospasm in aneurysmal SAH. We expect this correlation to be bimodal, as suggested by the natural history of the disease, and reflect the severity of vasospasm based on clinical data. We hope to develop a biomarker signature to act as a predictor of disease severity and outcome.

Objective:
Our objective was to determine a molecular profile based on examination of inflammatory mediators in the serum during the course of aneurysmal SAH. A multiplex immunoassay enabled us to evaluate a panel of inflammatory and trophic proteins that were analyzed in a regression analysis to determine whether inflammatory biomarkers could be used to predict onset of a vasospasm as well as be correlated to outcome variables.

Specific Aim:
To discover the relationship between inflammatory biomarkers and the occurrence of DCI/ cerebral vasospasm and clinical outcome in the setting of aneurysmal subarachnoid hemorrhage (SAH)
The pathogenesis of vasospasm is not known. There are over twenty cellular and molecular factors that have been implicated. No one has been able to satisfactorily predict which patients will undergo cerebral vasospasm/DCI based on the presence of hemorrhage in the subarachnoid space. The mismatch between laboratory findings and clinical evidence is in part due to the multifactorial nature of the disease. Our study will allow us to examine a wide selection of interleukins and growth factors during a patient's hospital stay and identify markers for DCI and clinical outcome based on clinical findings that correlate with laboratory values.

Methods:
Clinical component:
An institutional review board (IRB) proposal was completed and approved prior to the onset of investigation (Hershey Medical Center and Penn State College of Medicine IRB 30616). Between July 1st 2009 and April 30th 2010, 34 patients were enrolled into this study. Patient selection and suitability was based on the presence of subarachnoid hemorrhage due to aneurysmal rupture only. Subarachnoid hemorrhage in this setting was confirmed via radiographic data in all cases. All patients were consented within 48 hours of the onset of their

clinical symptoms. All patients were admitted to the Neurosurgical Service at the Penn State Hershey Medical Center. Demographic and radiographic data were collected at the time of admission. This included age, sex, location and size of aneurysm, Hunt-Hess grade, Fisher grade, World Federation of Neurologic Surgeons grade, cigarette smoking status and placement of external ventricular drain.

Venous blood was obtained on a daily basis for each patient during their hospitalization. If a patient required an external ventricular drain (EVD), cerebrospinal fluid (CSF) was also obtained daily while access was available. Samples were obtained between 7am and 2pm, to limit variations of circadian rhythm. Once samples were obtained they were immediately hand couriered to the neurosurgical laboratory on ice.

All patients were subject to daily Transcranial Doppler (TCD) imaging. TCD provided the velocity of blood flow in the anterior cerebral artery, the middle cerebral artery, the internal carotid artery and the posterior cerebral artery of both hemispheres. As vessel diameter narrows, velocity increases in order to maintain constant flow, hence vasospasm is correlated with increasing TCD velocity. The highest mean velocity, in any arterial territory was used to categorize the patient's degree of TCD vasospasm. If the mean velocity was less than 80 cm/sec it was considered that the vessel was not spastic or narrowed. Similarly, velocities between 80-120 cm/sec denoted mild spasm, 120-200 cm/sec, moderate spasm, and greater than 200 cm/sec, severe vasospasm. In addition, patients were monitored closely for signs of delayed cerebral ischemia (DCI), a.k.a. clinical vasospasm. Further imaging was performed with either MRI or CT if clinically warranted.

Laboratory components

Once the samples were received in the lab, they are spun down in a centrifuge at 1000G for 5 minutes. The supernatant was extracted as to leave all particulate matter behind. Protease inhibitor cocktail (Sigma-Aldrich; St. Louis, MO) was then added 1:100. The samples were then stored at minus 80 degrees Fahrenheit in 200uL aliquots. When it was possible to analyze the samples, they were appropriately thawed.

Multiplex cytokine bead assay

We performed multiplex analysis on undiluted blood and CSF supernatants using the Bio-Plex Human 27-plex panel of cytokines and growth factors (Bio-Rad; Hercules, CA). The analytes tested are listed in Table 1. One percent bovine serum albumin (BSA, Sigma-Aldrich; St. Louis, MO) was added to 200uL of each of the supernatants. The standards were reconstituted in PBS with 1% BSA. Fifty uL of each sample or standard was added in duplicate to a 96 well plate and mixed with 50uL of antibody-conjugated beads for 1 hour at room temperature. Wells were then washed and 25uL of detection antibody was added to each well. After a 30-minute incubation, wells were washed and 50uL of streptavidin-PE was added to each well for a second 10-minute incubation. A final wash cycle was then completed and 125uL of assay buffer was added to each well. The plate was then analyzed using a Bio-Plex 200 workstation (Bio-Rad). Analyte concentration was calculated based on the standard on the respective standard curve for each cytokine. Analyte concentration was measured in picograms per milliliter. For direct comparison and to measure trends between analytes, the concentration values were normalized. This

calculation was based on the analyte concentration on day 1 of patient admission. All data collected from day 1 onwards reflects a change from an arbitrary baseline across all analytes.

Statistical Analysis

Descriptive statistics were computed for variables of interest using means for continuous variables and percentages for categorical variables. Measurements were taken anywhere from 1 to 30 days post-bleed. Because of the randomness of the measurement times, we applied a random coefficients model (JD Singer & JB Willett, Applied Longitudinal Data Analysis, 2003) that used time as a random factor in the model. The model included a group effect not related to time, the time effect not related to the group, and the interaction between the group and time effects. Using this model, we compared the group means for the outcome variables at clinically significant time points at day 1, day 7, day 14, and day 21 post-bleed. Model assumptions and fit were assessed with histograms and residual plots. We performed logistic regression analysis for early (days 1-3) and late (days 7-10) in the hospital course. Adjustments were made for age, gender and smoking. Statistical significance was determined as $p < 0.01$ due to the multiple comparisons made, and all statistical analyses were performed using SAS Software Version 9.2 (SAS Institute, 2008).

Results:

Patient Characteristics and Presentation

All patients enrolled were treated solely at Hershey Medical Center and also received their follow-up care at on campus clinic sites (Table 2). Thirteen women and seven men participated in the study and their average age was 57.6 years. Ten patients were documented to have been smokers prior to hospitalization. Seventeen patients had their aneurysms treated by endovascular coil embolization while the remaining three underwent craniotomy and microsurgical clipping. The median Hunt-Hess grade on admission was 2 and the median WFNS grade was also 2. Thirteen patients required external ventricular drain placement for treatment of increased intracranial pressures. Of these thirteen, two required placement of a permanent venticulo-peritoneal shunt either during hospitalization or at follow-up. Eight patients were noted to have TCD values greater than 200cm/s during their hospitalization. Of these, six were noted to have symptoms indicative of delayed cerebral ischemia or required endovascular treatment for angiographic vasospasm. Two patients had temporal bones too thick for ultrasonographic (TCD) interpretation. Two patients required intracranial endovascular angioplasty for treatment of clinically symptomatic vasospasm that was refractory to noninvasive ICU management.

Hospital Course

Various biomarkers varied with clinical parameters (Table 3). For those patients who presented with poor (defined as 4-5) World Federation of Neurological Surgeons (WFNS) or Hunt-Hess scores, levels of: IL-2, IL-6, and IL-10 were significantly elevated. Conversely, elevated levels of MIP-1beta and TNF-alpha were associated with good scores (defined as 1-3). A subset of patients required temporary cerebrospinal shunting (in the form of an external ventricular drain) or permanent shunting (in the form of a ventricular-peritoneal shunt). Levels of IL-10, B-FGF, and IFN-gamma levels were found to be significantly elevated in these patients. The appearance of cerebral vasospasm was assessed with transcranial Doppler, development of delayed cerebral ischemia and cerebral angiography. TCD velocities above 200cm/s were correlated with IL-2 and Eotaxin levels. Three patients required intracranial angioplasty for acute treatment of

delayed cerebral ischemia. These patients had significantly elevated levels of IL-7 and significantly low levels of MIP-1beta.

Outcome Measures

A modified Rankin Score (mRS) was obtained for each patient on discharge from the hospital and at 6 months. A good score was defined as 0-2 and a poor score was defined as 3-6. Poor scores at discharge were associated with elevated IL-1beta, IL-2, IL-6, IL-10, IL-17, G-CSF, GM-CSF, IFN-gamma and TNF-alpha. Poor scores at 6 month follow-up were associated with elevated IL-7, IL-10, IL-12 and G-CSF.

Predictive Ability

We performed a logistic regression analysis for days 1-3 and days 7-10 to see if the biomarkers were of predictive value to clinical status (Table 4). We were not able to find any significance with this methodology for outcome variables: clinical vasospasm (DCI), modified Rankin at discharge, and modified Rankin at 6 month follow-up. Next, we utilized a simple linear fixed effects model for the average of days 1-3 for the biomarkers to mirror the logistic regression analysis. Unfortunately, this did not yield a significant interaction either. In both analyses $p<0.01$ was considered significant given the multiple comparisons that were made.

Discussion:

Instantaneously following cerebral aneurysm rupture there is a spillage of arterial blood into the subarachnoid space. There is experimental evidence that erythrocytes, platelets and plasma proteins can precipitate inflammatory cascades in the cerebrospinal fluid [7, 18, 19]. Other groups studying SAH pathophysiology have asserted that this inflammatory response is the putative mechanism for delayed cerebral ischemia (DCI), a.k.a. cerebral vasospasm [2, 8, 20-22]. There are myriad experimental animal models that demonstrate an association between vessel caliber and degree of inflammation [21, 23-27]. Human data is lacking however, and there still has not been any significant trial data to support the laboratory findings [28]. Part of the problem is due to the vast array of signaling molecules implicated in vasospasm and thus the added complexity of arranging the evidence, to form a coherent canonical pathway. The literature on this topic suggests that researchers are independently focusing on separate molecules without much consideration for the other possible ‘confounding’ elements and this may lead to inaccurate conclusions.

A frequently overlooked aspect of cerebral vasospasm is the location at which this phenomenon is occurring. There is evidence that there is a chronic effect on both macroscopic vasculature and microcirculation [29]. Tissue breakdown and foreign particulates in the subarachnoid space elicits a global inflammatory reaction [30]. The evidence available that inflammation directly reflects neurological outcome is lacking. The few reports in the literature are conflicting, though none argue against the initial irritative effect of the ictal hemorrhage.

We attempted to discover a relationship between a broad range of inflammatory cytokines and growth factors and clinical evidence of DCI as well as overall clinical outcome. This investigational paradigm has never been attempted/ applied before. While we found some overall associations between elevated overall levels of certain biomarkers across an entire

hospitalization, we were not able to find any predictive value in early measurement of biomarker levels. When measured early (within 1-3 days of admission or even when measure later (7 -10 days, peak incidence of vasopasm/DCI), we found no statistically significant links between the biomarker serum concentrations and the specified outcomes. A few potential trends were seen, but given the number of comparisons analyzed there is a reasonable likelihood that these may have been encountered by chance alone.

The major weakness of this study, possibly introducing type II error, is that we have a small patient cohort. Although our results do not support the theory that inflammation directly impacts a patient's clinical status, we have shown that there may be a few molecules that warrant further investigation in a larger group of patients. Due to subject and funding limitations, our study did not assess cerebrospinal fluid (CSF) concentrations of inflammatory biomarker. CSF may prove to be a much more sensitive location for biomarker assessment as compared to blood/serum monitoring alone.

There is a paucity of adequate and reliable animal models of aneurysmal subarachnoid hemorrhage [31]. Some models rely on ex vivo analysis of tissue or cannot reproduce certain characteristics of the disease, such as its chronicity and associated brain edema. Thus early experiments, utilizing these models, may have inadvertently led the field astray. Alternatively, as mentioned, the small sample size of the present study and the sensitivity issues associated with serum biomarker measurement as opposed to CSF markers may have contributed to the negative results. Recently published work has also suggested that the rate of change seen in the inflammatory markers may actually be more important than the actual level [32]. We plan to analyze our data to see if we can confirm such a finding.

Conclusion:

Inflammation seems a very attractive target to link subarachnoid hemorrhage, cerebral vasospasm and DCI, and numerous preclinical studies exist to corroborate this hypothesis. Nevertheless, interventions aimed at reducing inflammation have not resulted in a reduction in patient mortality or morbidity. Indeed, a number of other theories have arisen to complement our understanding of this phenomena, such as micro-thrombosis, micro-vessel spasm, spreading depolarizations and spreading ischemia. We conducted the present study to examine a host of inflammatory biomarkers that may predict clinical outcome akin to a biologic "finger print". No other study has examined this broad a range of specific inflammatory biomarkers. Though previous studies have linked generalized systemic inflammation to DCI and cerebral vasospasm, our study found that early measurement of serum levels of biomarker were not predictive of DCI or overall clinical outcome. Our study adds to the growing body of literature that inflammation during aneurysmal subarachnoid hemorrhage may be an epiphenomenon that is not directly causative, and that other mechanisms may contribute to the complex pathophysiology associated with this disease process.

Table 1: Collection of Analytes (Inflammatory Biomarkers) Simultaneously Tested

IL-1 beta	**IL-7**	**IL-15**	**Eotaxin**
IL-1ra	**IL-8**	**IL-17**	**Granulocyte-monocyte colony stimulating factor**
IL-2	**IL-9**	**VEGF**	**IFN-gamma**
IL-4	**IL-10**	**Fibroblast growth factor basic**	**Monocyte chemoattract protein-1**
IL-5	**IL-12**	**TNF-alpha**	**Macrophage inflammatory-1-alpha**
IL-6	**IL-13**	**PDGF bb**	**Macrophage inflammatory-1-beta**

Table 2: Demographic Data Collected

Gender	Tobacco Use	HHS	FS	WFNS	EVD placed	VPS placed	mRS on dc	mRS @ 6mos
F	No	2	2	1	y	n	1	1
F	Yes	2	3	1	n	n	1	0
F	Yes	2	4	2	y	n	1	1
F	No	4	3	5	y	y	5	4
M	No	2	3	1	y	y	1	1
M	No	1	4	2	n	n	2	1
F	Yes	3	3	2	y	y	4	2
F	Yes	2	4	2	y	n	1	1
F	No	1	2	1	n	n	1	1
M	Yes	1	4	1	y	y	5	3
F	Yes	1	3	1	y	y	3	2
F	No	2	2	2	y	y	4	3
F	No	5	4	5	y	n	5	3
F	Yes	2	2	1	y	n	2	1
M	Yes	3	4	2	n	n	2	1
F	No	4	4	4	n	n	6	
M	Yes	3	4	2	n	n	4	3
M	Yes	4	4	4	y	n	4	2
M	No	2	3	2	y	n	6	
F	No	4	4	4	y	y	3	1

Key: M: male, F: female, HHS: Hunt Hess Score, FS: Fisher Scale, WFNS: World Federation of Neurological Surgeons Score, EVD: external ventricular drain, VPS: ventriculoperitoneal shunt, mRS: modified Rankin score, dc: discharge

Table 3: Association Between Biomarker Levels and Clinical Parameters using Random Coefficients Models.

Biomarker	**Poor Grade SAH**	**EVD**	**VPS**	**Vasospasm**		**Good Outcome at Discharge**	**Good Outcome at 6 months**
	Hunt-Hess or WFNS 4,5	**Placed**	**Placed**	**DCI/Angiographic**	**TCDs>200**	**mRS 0-2**	**mRS 0-2**
IL-1b	0.036	0.036	0.047	0.026	0.075	**<0.0001**	0.803
IL-1a	0.122	0.610	0.114	0.206	0.751	0.121	0.453
IL-2	**<0.0001**	0.162	0.483	0.909	**<0.0001**	**<0.0001**	0.618
IL-4	0.362	0.131	0.164	0.180	0.442	0.003	0.039
IL-5	0.836	0.794	0.318	0.866	0.085	0.799	0.056
IL-6	**<0.0001**	0.066	0.179	0.623	0.071	**<0.0001**	0.078
IL-7	0.036	0.581	0.730	**0.001**	0.276	0.026	**0.008**
IL-8	0.204	0.017	0.213	0.188	0.502	0.019	0.150
IL-9	0.198	0.345	0.026	0.448	0.062	0.586	0.900
IL-10	**<0.0001**	**<0.0001**	0.145	0.221	0.026	**<0.0001**	**0.002**
IL-12	0.505	0.311	0.325	0.086	0.706	0.002	**0.009**
IL-13	0.874	0.319	0.490	0.922	0.143	0.566	0.628
IL-15	0.115	0.317	0.860	0.643	1.000	0.179	0.054
IL-17	0.107	*	0.017	*	*	**<0.0001**	0.045
Eotaxin	0.163	0.655	0.016	0.023	**0.003**	0.582	0.799
B FGF	0.225	0.443	**<0.0001**	0.433	0.513	0.094	0.510
G-CSF	0.040	0.210	0.018	0.664	0.881	**0.009**	**0.001**
GM-CSF	0.017	0.014	0.179	0.599	0.092	**0.001**	0.640
IFN-g	0.098	0.036	**0.007**	0.494	0.013	**0.003**	0.092
IP-10	0.019	0.152	0.329	0.547	0.230	0.110	0.777
MCAF	0.011	0.310	0.248	0.087	0.151	0.306	0.343
MIP 1a	0.683	0.060	0.853	0.312	0.966	0.349	0.578
MIP 1b	0.037	0.236	0.153	0.035	0.398	0.391	0.217
TNF a	0.931	0.037	0.016	0.145	0.122	**0.009**	0.249
VEGF	0.147	0.018	0.151	0.268	0.416	0.014	0.176

Results are adjusted for age, gender and smoking. Values in **bold** indicate statistical significance; a threshold of $p < 0.01$ was used because of the multiple comparisons. A* indicates that results not measurable due to failure of the experimental assay.
SAH: subarachnoid hemorrhage, WFNS: World Federation of Neurological Surgeons Score, EVD: external ventricular drain, VPS: ventriculoperitoneal shunt, mRS: modified Rankin score, DCI: delayed cerebral ischemia, TCD: transcranial Doppler.

Table 4: Association Between Early (days 1-3) and Late (days 7-10) Biomarker Levels with Clinical Outcome Measures using Logistic Regression

Biomarker	**Good Outcome at Discharge**		**Good Outcome at 6 months**		**Clinical Vasospasm (DCI)**	
	mRS 0-2		**mRS 0-2**			
	day 1-3	**day 7-10**	**day 1-3**	**day 7-10**	**day 1-3**	**day 7-10**
IL-1b	0.185	0.167	0.261	0.776	0.691	0.336
IL-1a	0.974	0.366	0.341	0.633	0.244	0.277
IL-2	0.621	0.172	0.620	0.360	0.474	0.271
IL-4	0.296	0.144	0.809	0.397	0.245	0.232
IL-5	0.712	0.617	0.988	0.660	0.152	0.283
IL-6	0.405	0.243	0.346	0.677	0.557	0.340
IL-7	0.626	0.540	0.968	0.996	0.459	0.693
IL-8	0.516	0.221	0.367	0.180	0.207	0.851
IL-9	0.783	0.618	0.416	0.891	0.243	0.380
IL-10	0.192	**0.099**	0.834	0.779	0.312	0.235
IL-12	0.292	0.241	0.788	0.641	0.636	0.371
IL-13	0.420	0.796	0.191	0.380	0.196	0.205
IL-15	0.151	**0.079**	0.101	0.537	0.640	0.336
IL-17	0.303	0.635	0.487	0.588	0.216	0.858
Eotaxin	0.874	0.237	0.204	0.582	0.978	0.331
B FGF	0.286	0.997	0.149	0.194	0.434	0.904
G-CSF	0.729	0.783	0.765	0.445	0.205	0.335
GM-CSF	**0.091**	0.178	0.126	0.489	0.331	0.499
IFN-g	0.327	0.228	0.568	0.981	0.423	0.397
IP-10	0.705	0.713	0.951	0.426	0.484	0.648
MCAF	0.615	0.914	0.126	0.557	0.271	0.331
MIP 1a	0.655	0.767	0.266	0.473	0.183	0.455
MIP 1b	0.377	0.111	0.822	0.172	0.213	0.349
TNF a	0.396	0.102	0.552	0.229	0.408	0.189
VEGF	0.517	0.494	0.338	0.783	0.809	0.141

Results are adjusted for age, gender and smoking. Values in **bold** may represent a trend; a threshold of $p < 0.01$ was used for statistical significance because of the multiple comparisons. mRS: modified Rankin score, DCI: delayed cerebral ischemia.

References

1. Suarez, J.I., R.W. Tarr, and W.R. Selman, *Aneurysmal subarachnoid hemorrhage.* N Engl J Med, 2006. **354**(4): p. 387-96.
2. van Gijn, J., R.S. Kerr, and G.J. Rinkel, *Subarachnoid haemorrhage.* Lancet, 2007. **369**(9558): p. 306-18.
3. Dankbaar, J.W., et al., *Relationship between vasospasm, cerebral perfusion, and delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage.* Neuroradiology, 2009. **51**(12): p. 813-9.
4. Macdonald, R.L., et al., *Clazosentan, an endothelin receptor antagonist, in patients with aneurysmal subarachnoid haemorrhage undergoing surgical clipping: a randomised, double-blind, placebo-controlled phase 3 trial (CONSCIOUS-2).* Lancet Neurol, 2011. **10**(7): p. 618-25.
5. Macdonald, R.L., et al., *Clazosentan to overcome neurological ischemia and infarction occurring after subarachnoid hemorrhage (CONSCIOUS-1): randomized, double-blind, placebo-controlled phase 2 dose-finding trial.* Stroke, 2008. **39**(11): p. 3015-21.
6. Allen, G.S., et al., *Cerebral arterial spasm--a controlled trial of nimodipine in patients with subarachnoid hemorrhage.* N Engl J Med, 1983. **308**(11): p. 619-24.
7. Macdonald, R.L. and B.K. Weir, *A review of hemoglobin and the pathogenesis of cerebral vasospasm.* Stroke, 1991. **22**(8): p. 971-82.
8. Jayaraman, T., et al., *Tumor necrosis factor alpha is a key modulator of inflammation in cerebral aneurysms.* Neurosurgery, 2005. **57**(3): p. 558-64; discussion 558-64.
9. Tsai, P.S., et al., *Correlations of neuronal and microvascular densities in murine cortex revealed by direct counting and colocalization of nuclei and vessels.* J Neurosci, 2009. **29**(46): p. 14553-70.
10. Ducruet, A.F., et al., *Genetic determinants of cerebral vasospasm, delayed cerebral ischemia, and outcome after aneurysmal subarachnoid hemorrhage.* J Cereb Blood Flow Metab, 2010. **30**(4): p. 676-88.
11. Dreier, J.P., et al., *Delayed ischaemic neurological deficits after subarachnoid haemorrhage are associated with clusters of spreading depolarizations.* Brain, 2006. **129**(Pt 12): p. 3224-37.
12. Sehba, F.A., et al., *Acute alterations in microvascular basal lamina after subarachnoid hemorrhage.* J Neurosurg, 2004. **101**(4): p. 633-40.
13. Khurana, V.G., et al., *Protective vasomotor effects of in vivo recombinant endothelial nitric oxide synthase gene expression in a canine model of cerebral vasospasm.* Stroke, 2002. **33**(3): p. 782-9.
14. Dumont, A.S., et al., *Cerebral vasospasm after subarachnoid hemorrhage: putative role of inflammation.* Neurosurgery, 2003. **53**(1): p. 123-33; discussion 133-5.
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17. McGirt, M.J., et al., *Leukocytosis as an independent risk factor for cerebral vasospasm following aneurysmal subarachnoid hemorrhage.* J Neurosurg, 2003. **98**(6): p. 1222-6.
18. Dietrich, H.H. and R.G. Dacey, Jr., *Molecular keys to the problems of cerebral vasospasm.* Neurosurgery, 2000. **46**(3): p. 517-30.

19. Frontera, J.A., et al., *Early platelet activation, inflammation and acute brain injury after a subarachnoid hemorrhage: a pilot study.* J Thromb Haemost, 2012. **10**(4): p. 711-3.
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21. Bavbek, M., et al., *Monoclonal antibodies against ICAM-1 and CD18 attenuate cerebral vasospasm after experimental subarachnoid hemorrhage in rabbits.* Stroke, 1998. **29**(9): p. 1930-5; discussion 1935-6.
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25. Pluta, R.M., et al., *Nitrite infusions to prevent delayed cerebral vasospasm in a primate model of subarachnoid hemorrhage.* JAMA, 2005. **293**(12): p. 1477-84.
26. Chyatte, D., *Prevention of chronic cerebral vasospasm in dogs with ibuprofen and high-dose methylprednisolone.* Stroke, 1989. **20**(8): p. 1021-6.
27. Bowman, G., et al., *A novel inhibitor of inflammatory cytokine production (CNI-1493) reduces rodent post-hemorrhagic vasospasm.* Neurocrit Care, 2006. **5**(3): p. 222-9.
28. Connolly, E.S., Jr., et al., *Guidelines for the management of aneurysmal subarachnoid hemorrhage: a guideline for healthcare professionals from the American Heart Association/american Stroke Association.* Stroke, 2012. **43**(6): p. 1711-37.
29. Friedrich, B., et al., *Experimental subarachnoid hemorrhage causes early and long-lasting microarterial constriction and microthrombosis: an in-vivo microscopy study.* J Cereb Blood Flow Metab, 2012. **32**(3): p. 447-55.
30. Ostergaard, J.R., et al., *Immune complexes and complement activation following rupture of intracranial saccular aneurysms.* J Neurosurg, 1987. **66**(6): p. 891-7.
31. Pluta, R.M., et al., *Cerebral vasospasm following subarachnoid hemorrhage: time for a new world of thought.* Neurol Res, 2009. **31**(2): p. 151-8.
32. McMahon, C.J., et al., *Inflammation as a predictor for delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage.* J NeuroIntervent Surg, 2012. **0**: p. 1-6.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____ Yes
___X_ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania

Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. None				☐Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes___X_____ No_____

If yes, please describe your plans:

A manuscript summarizing the salient results of this project is in preparation for submission to a peer-reviewed journal.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None – This was an essentially negative study. There may be implications going forward for the role of inflammation in delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage, which may alter potential treatment pathways. However, further confirmatory work will need to be completed.

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None.

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No____X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No____X____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Kevin M. Cockroft, MD, MSc, FACS, FAHA	Associate Professor Director, Cerebrovascular and Endovascular Surgery
eRA COMMONS USER NAME KCOCKROFT	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Johns Hopkins University, Baltimore, MD	BA	1983-1987	Behavioral Biology
Weill-Cornell Medical College, New York, NY	MD	1987-1991	Medicine
Pennsylvania State University, Hershey, PA	MSc	2000-2002	Health Evaluation Sciences

A. PERSONAL STATEMENT

I currently serve as Director of Cerebrovascular and Endovascular Neurosurgery in the Department of Neurosurgery at the Penn State Milton S. Hershey Medical Center and Pennsylvania State University College of Medicine. I am also co-Director of the Penn State Hershey Stroke Center and Director of the Neurosurgery Department's Neuroendovascular Fellowship Training Program. I have joint appointments in the departments of Neurosurgery, Radiology and Public Health Sciences. My clinical and research focus is on the management of cerebrovascular disease. I am or have been the principal investigator on several clinical trials and I have published numerous scientific articles and book chapters dealing with cerebrovascular disease. Our stroke research group has a dedicated research coordinator with years of experience in managing multicenter clinical trials. In addition, we enjoy close collaboration with our stroke neurology colleagues in the evaluation and recruitment of patients into acute interventional stroke trials.

B. POSITIONS AND HONORS

Positions and Employment

1991-1997	Resident, Neurological Surgery, New York Presbyterian Hospital-Weill Cornell Medical Center, NY
1997-1998	Fellow, Neurovascular Surgery, Stanford University Medical Center, CA
1998	Instructor, Department of Neurosurgery, Stanford University Medical Center, CA
1998-2005	Assistant Professor, Department of Neurosurgery, Pennsylvania State University-M.S. Hershey Medical Center, PA
2003-2004	Fellow (on sabbatical), Endovascular Neurosurgery, Thomas Jefferson Hospital for Neuroscience, PA
2005-Present	Associate Professor, Department of Neurosurgery, Pennsylvania State University-M.S. Hershey Medical Center, PA
2005-Present	Director, Cerebrovascular and Endovascular Neurosurgery, Department of Neurosurgery, Pennsylvania State University-M.S. Hershey Medical Center, PA
2005-Present	Co-Director, Penn State Hershey Medical Center Stroke Pennsylvania State University-M.S. Hershey Medical Center Medical Center, PA

Honors

2007 - 2010	Best Doctors in America: 2007 – 2008
2007 - 2009	Consumers' Research Council of America: Guide to America's Top Surgeons
2004 - 2009	Who's Who in America
2004 - 2009	Consumers' Research Council of America: Guide to America's Top Physicians
1988	Cornell University Medical College-Barr Summer Fellowship
1987	The Johns Hopkins University-General Honors
1986/87	The Johns Hopkins University-Dean's List
1985/86	The Johns Hopkins University-Dean's List

C. FIFTEEN SELECTED PEER-REVIEWED PUBLICATIONS (in chronological order)

1. Zimmerman GA, Meistrell III M, Bloom O, Cockroft KM, Bianchi M, Risucci D, Broome J, Farmer P, Cerami A, Vlassara H, Tracey KJ. Neurotoxicity of advanced glycation endproducts during focal stroke, and neuroprotective effects of aminoguanidine. *Proc. Natl. Acad. Sci. (USA)* 92:3744-3748, 1995.
2. Cockroft KM, Meistrell III M, Zimmerman GA, Risucci D, Bloom O, Cerami A, Tracey KJ. Cerebroprotective effects of aminoguanidine in rodent stroke. *Stroke* 27:1393-1398, 1996.
3. Meistrell III M, Botchkina GI, Wang H, DiSanto E, Cockroft KM, Bloom O, Vishnubhakat JM, Ghezzi P, Tracey KJ. TNF is a brain damaging cytokine in stroke. *Shock* 8(5):341-348, 1997.
4. Cockroft KM, Marks MP, Steinberg GK. Planned Direct Dual-Modality Treatment of Complex Broad-Necked Intracranial Aneurysms: A Report of Four Cases. *Neurosurgery*, 46:226-231, 2000.
5. Cockroft KM, Carew JF, Trost D, Fraser RAR. Delayed epistaxis secondary to external carotid artery injury requiring embolization: a rare complication of transsphenoidal surgery. *Neurosurgery*, 47:236-239, 2000.
6. Krishnamurthy S, Tong D, McNamara K, Steinberg GK, Cockroft KM. Early carotid endarterectomy after ischemic stroke improves diffusion/perfusion mismatch on MRI. *Neurosurgery*, 52:238-242, 2003.
7. Liu AY, Lopez JR, Do HM, Steinberg GK, Cockroft KM, Marks MP. Neurophysiologic monitoring in the endovascular therapy of aneurysms. *AJNR Am J Neuroradiol*, 24:1520-7, 2003.
8. Bowman G, Dixit S, Bonneau RH, Chinchilli VM, Cockroft KM. Neutralizing antibody against IL-6 attenuates post-hemorrhagic vasospasm in the rat femoral artery model. *Neurosurgery*, 54:719-25, 2004.
9. Steinberg GK, Ogilvy CS, Shuer LM, Connolly ES, Solomon RA, Lam A, Kassell NF, Baker CJ, Giannotta SL, Cockroft KM, Bell-Stephens TE, Allgren RL. Comparison of endovascular and surface cooling during unruptured cerebral aneurysm repair. *Neurosurgery*, 55:307-315, 2004.
10. Cheung A, Telaghani CK, Wang J, Yang Q, Mosher TJ, Reichwein RK, Cockroft KM. MD Functional Recovery after Decompressive Craniectomy as Assessed by Functional MRI: A Report of Three Patients. *Neurocrit Care*, 03:216-223, 2005.
11. Birknes JK, Hwang SK, Pandey AS, Cockroft KM, Dyer, AM, Benitez R, Veznedaroglu E, Rosenwasser RH. Feasibility and Limitations of Endovascular Coil Embolization of Anterior Communicating Artery Aneurysms: Morphological Considerations. *Neurosurgery,* 59:43-52, 2006.
12. Bowman G, Bonneau RH, Chinchilli VM. Tracey KJ, Cockroft KM. A novel inhibitor of inflammatory cytokine production (CNI-1493) reduces rodent post-hemorrhagic vasospasm. *Neurocrit Care,* 5:222-229*,* 2006.
13. Krishnamurthy S, Kelleher JP, Lehman EB, Cockroft KM. Effects Of Tobacco Dose And Length Of Exposure On Vasospasm And Clinical Outcome After Aneurysmal Subarachnoid Hemorrhage. *Neurosurgery*, 61(3):475-481, 2007.
14. Douds GL, Kalapos P, Cockroft KM. Temporary Test Occlusion of Distal MCA Using GDC Coil: Technical Case Report. *J Neuroimaging,* 20(2):183-186, 2010. (Epub January 2009)*.*
15. Rizk EB, Patel AS, Stetter CM, Chinchilli VM, **Cockroft KM**. Influence of Tracheostomy Timing on Outcome in Severely Head Injured Patients Requiring Mechanical Ventilation: A Retrospective Analysis Of 3,104 Patients. *Neurocritical Care*, 15(3):481-9, 2011.

BIOGRAPHICAL SKETCH

NAME James R. Connor, Ph.D.	POSITION TITLE Distinguished Professor
eRA COMMONS USER NAME James_R_Connor	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Thomas More College, Ft. Mitchell, KY	B.A.	05/75	Psychology
Wright State University, Dayton, OH	M.S.	05/78	Physiology
University of California, Berkeley, CA	Ph.D.	06/81	Anatomy
Boston University Medical Center, Boston, MA	Postdoc	06/81-07/83	Neuroscience

A. Positions and Honors

Professional Positions:

1983-1987 Research Biologist, Veteran's Administration Medical Center, Washington, DC
1983-1987 Assistant Research Professor, Department of Physiology, The George Washington University School of Medicine
1987-1990 Assistant Professor, Department of Anatomy Pennsylvania State University College of Medicine (PSUCOM) Neuroscience & Anatomy, (PSUCOM)
1990-1996 Associate Professor, Department of Neuroscience & Anatomy, (PSUCOM)
1996- Professor, Department of Neuroscience & Anatomy, (PSUCOM)
1999-2002 Vice Chair Department of Neuroscience & Anatomy, (PSUCOM)
2002-2003 Interim Chair Department of Neuroscience & Anatomy, (PSUCOM)
2004- Professor Neurosurgery, (PSUCOM)
2006 Co-Director, Neuro-oncology Experimental Therapeutic Group (PSU Cancer Institute)
2007 University Distinguished Professor (PSUCOM)

Grant Review Experience:
NIH, NIEHS, Veterans Administration, Department of Defense USDA, NSF, MRC, Wellcome Trust, Israel Academy of Science, Alzheimer's Association, Hong Kong Research Council, American Federation for Aging Research, American Heart Association

Honors and Awards:

Samuel Hinkle Society Outstanding Young Investigator Award, Pennsylvania State University College of Medicine (1990);
Mentored two graduate students awarded Marion Kies Award by the American Society for Neurochemistry for outstanding doctoral thesis (1999, 2010);
Elected Chair of the East Coast Iron Club (2000-2002);
Dean's Lecturer Pennsylvania State University College of Medicine/M.S. Hershey Medical Center (2003);
Distinguished Alumni Award, Thomas More College (2007);
Named Distinguished Professor Penn State University (2007)
Honorary Professor, Department of Neurology, Tianjin First Center Hospital, Tianjin (2008-)

Editorial Boards:
Journal of Neuroscience Research (1994-); GLIA (2001-); Fluids and Barriers of the CNS (2004-); Current Neurovascular Research (2004-); CNS Drug Discovery (2005-); Recent Patents on Biotechnology (2008-); Journal of Neurochemistry (Handling Editor, 2011-); PlosOne (2012-).

B. Selected Peer-reviewed Publications

1. Wang X, Lee S, Simmons Z, Boyer P, Scott K, Liu W, **Connor J**. Increased incidence of the Hfe mutation in amyotrophic lateral sclerosis and related cellular consequences. *Journal of Neurological Sciences* 227(1) 27-33, 2004.
2. Lee SY, Patton SM, Henderson RJ, **Connor JR.** Consequences of expressing mutants of the hemochromatosis gene (JFE) into a human neuronal cell line lacking endogenous HFE. *The FASEB Journal* 21(2):564-576, 2007.
3. Mitchell RM, Freeman WM, Randazzo WT, Stephens HE, Beard JL, Simmons Z, **Connor JR**. A CSF biomarker panel for identification of patients with Amyotrophic Lateral Sclerosis. *Neurology* 72:14-19, 2009.
4. Mitchell RM, Lee SY, Simmons Z, **Connor JR**: HFE polymorphisms affect cellular glutamate regulation. *Neurobiology of Aging* 6(6):1-12, 2009.
5. Mitchell RM, Lee SY, Randazoo WT, Simmons Z, **Connor JR**: Influence of HFE variants and cellular iron regulation on monocyte chemoattractant protein-1. *J Neuroinflamm* 6:6, 1-12 Feb, 2009. PMCID: 2656486
6. Hall EC, Lee SY, Simmons Z, Neely EB, Nandar W, **Connor JR**: Prolyl-peptidyl isomerase, Pin1, phosphorylation is compromised in association with the expression of the HFE polymorphic allele, H63D. *Biochimica et Biophysica Acta* 1802:389-395, 2010.
7. Mitchell RM, Beard JL, Stephens HE, Simmons Z, Connor JR. Plasma Biomarkers Associated with ALS and Their Relationship to Iron Homeostasis. *Muscle and Nerve* 42:95-103, 2010.
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