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Evaluation of GAPDH and HSP7C Levels in Cerebral Microdialysis: Potential Predictors of Vasospasm in SAH Patients

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A. Study Proposal and Rationale

Subarachnoid hemorrhage (SAH) accounts for only 1-7% of all strokes, but accounts for 22-25% of all cerebrovascular disease deaths.[1] Moreover, because it affects a relatively young population and confers dismal outcomes, its impact is on productive life years is similar to that of cerebral infarction.[2, 3] Cerebral aneurysms underlie most cases; in the United States, approximately 1-12 million people are affected by these arterial defects and approximately 30,000 of these patients develop SAH annually.[1] After diagnosis of SAH, usually based on an emergency computed tomography (CT) scan, patients are transferred to tertiary care centers and monitored in a neurointensive care unit. Medical therapies that control blood pressure, volume status, serum electrolytes, and temperature are the standard of care. Aneurysm coiling and aneurysm ablation are the mainstay endovascular and surgical interventions. Death or dependence (poor outcome) occurs in 70% of aneurysmal SAH (aSAH) patients and delayed cerebral ischemia (DCI) is the cause in about one-third of patients.[4]

Vasospasm is the most dreaded acute complication after SAH. 70% of all SAH patients and 17-40% of patients with neurological complication show evidence of vasospasm.[5, 6] Greenberg defines it as “the delayed occurrence of narrowing of large capacity arteries at the base of the brain after subarachnoid hemorrhage, often associated with diminished perfusion in the territory distal to the affected vessel.”[7] Risk factors for SAH vasospasm include age, hypertension, cigarette smoking, cocaine use, and certain findings on CT.[8] The peak incidence for vasospasm is 4 to 10 days after the initial aSAH bleed. This results in DCI and neurologic deficit. Less often, hyperacute vasospasm leads to “ictal” infarction.[9] The pathogenesis of vasospasm is an abnormal state of inflammatory vasoconstriction triggered by subarachnoid hemoglobin and the superoxide free radical. Both disrupt the action of nitrous oxide in the vessel wall leading to levels of activated protein kinase C, endothelin-1 and other circulating macromolecules that favor vasoconstriction. This environment promotes intimal hyperplasia, subendothelial fibrosis, and leukocyte and platelet aggregation. Microcirculatory dysfunction and diffuse cerebral edema may be a result of the same derangement. [10, 11]

The treatment of vasospasm is diverse and rapidly expanding. Calcium-channel blockers, specifically nimodipine, have been shown to prevent vasospasm in controlled trials and are standard treatment.[12] “Triple-H” therapy, another commonly employed treatment, includes hypervolemia, hypertension, and hemodilution. It improves cerebral blood flow (CBF) in vasospastic patients. Less-established medical therapies include magnesium and fasudil hydrochloride, calcium antagonists, and tirilazad mesylate, which blocks free radical scavenging.[13] Endovascular interventions, such as balloon angioplasty for larger vessels and intra-arterial administration of vasodilators, e.g. papaverine, for microcirculatory compromise are used in cases of vasospasm unresponsive to more conservative treatment.[14]

Detection of vasospasm includes many modalities; some are currently used clinically while others are in the developmental stage. Transcranial Doppler (TCD) is an important, non-invasive measurement of blood velocity. Blood velocity ratios are the basis of identification of vasospasm with TCD. Radiographic means can be used to document vasospasm. Perfusion CT and magnetic resonance imaging, along with proton magnetic resonance spectroscopy are both used, but limited because of the risks inherent in SAH patients leaving the neurointensive care unit. Direct visualization via cerebral angiography is the gold

standard for diagnosing vasospasm. Vessel caliber is accurately measured and interventions (see above) are performed, but the risks of iatrogenic stroke and vessel dissection or rupture limit its use.[14] More invasive methods of vasospasm detection include cerebral microdialysis devices (MD), which are coupled to intracranial pressure monitors. In current clinical practice, bedside cerebral MD provides continuous, *in vivo* measurements of the metabolites, glucose, lactate, pyruvate, and occasionally glutamate. These reflect the local extracellular environment and cellular function, which in turn can suggest vasospasm. Despite the diversity of modalities to detect vasospasm, no molecular risk factors that predict outcome or vasospasm have been identified.

Recently, several research groups around the world have begun to evaluate macromolecules, instead of small metabolites, using the cerebral MD technique. In 2002, Winter et al. published one of the first reports that demonstrated the recovery of cytokines and proteins via cerebral MD. Simply, cerebral MD consists of a concentric catheters with a total diameter less than 1mm. Infusate runs through the inner compartment and fluid is extracted from the outer compartment.[15]

Reports in the literature over the last five years have identified proteins in microdialysate fluid that are candidate markers for brain injury, neurological outcome, and, most interestingly, vasospasm. In January 2007 a paper authored by Maurer et al. demonstrated detection of elevated levels of glyceraldehydes-3-phosphate dehydrogenase (GAPDH) and decreased levels of heat-shock cognate 71 kDa protein (HSP7C) in the microdialysate samples of five aSAH patients relative to controls up to four days prior to vasospastic events.[16] Because these results are from a proteome-wide screen, they must be interpreted with caution.

Based on this preliminary data, we hypothesize that microdialysate levels of GAPDH and HSP7C are indeed predictors of cerebral vasospasm in aSAH patients.

B. Study Design and Statistical Analysis

We propose a prospective, observational ,nested case-control study investigating the predictive role of two candidate proteins in cerebral vasospasm. “Controls” will be patients who present with aSAH and do not develop symptomatic vasospasm and “cases” will be aSAH patients who do develop symptomatic vasospasm. The study will consist of 25 patients who present to the Columbia-Presbyterian Neurological Intensive Care Unit (NICU). About one in every five aSAH patients will experience vasospasm, thus the study will include 20 controls and five cases. The total study number, 25, was derived from the fact that about 40 aSAH patients per year will be monitored with cerebral MD, and we expect 65% will consent to the this study. Randomization and cross-over techniques do not apply to this study. Primary statistical analysis will be performed on levels of GAPDH and HSP7C from the 5 cases and the 5 best matched-controls. The selection of the matched controls will attempt to minimize differences in patient age, sex, hypertension history, cigarette smoking history, history of cocaine use, aneurysm characteristics, Glasgow Coma Scale rating, radiologic Fischer scale rating at presentation, and time from onset of symptoms to aneurysm ablation surgery and placement of MD probe. These measures will be taken to obviate the confounding effects of known vasospasm predictors. Secondary statistical analysis will include all 20 control participants. Specific outcome variables will be chosen subsequent to visualizing the general trend of the protein concentration profile. These may include mean, maximum, rate of change, and area under the curve. Preliminary power analysis: Using a Student’s t-test on the outcome variable we will, conservatively, be able to detect a difference of greater than 2.39 standard deviations in our primary statistical analysis and a difference of greater than 1.89 standard deviations in our secondary analysis (α=0.025, power = 0.80). The probability of type 1 error is set to 0.05, but Bonferroni’s correction mandates use of α=0.025.

C. Study Procedure and Data Collection

Eligible patients will be monitored in the Columbia-Presbyterian NICU post-operatively. To clarify, only patients who have a clinical indication for cerebral MD catheter insertion as determined by the neurology critical care and neurosurgery attendings will undergo cerebral MD probe placement. Following surgery, the operating neurosurgeon will place the cerebral MD catheter into the brain parenchyma, near the newly repaired vessel. Cerebral MD sample collection will commence immediately. The initial 30 min of microdialysate fluid will be discarded and subsequent samples will be collected hourly as is routinely performed. 18 microliters are produced each hour and five microliters are needed for clinical purposes. Thus, the remaining 13 microliters will be frozen and stored at -80 degrees Fahrenheit. This will be repeated for 14 days or until the MD catheter is removed.

Participants will not experience any additional pain, discomfort, or inconvenience.

D. Study Drugs

There are no medications being investigated in this study. Neurology and neurosurgery teams will administer drugs as clinically indicated.

E. Medical Device

Flexible microdialysis probes (CMA 70 custom probes, CMA Microdialysis, Solna, Sweden) have been part of clinical care in tertiary-care NICUs for more than ten years; their use is safe and standardized.[17] Risks include bleeding, infection, and damage to cerebral neurons, although these are rare. As stated above (section C), the presence of a clinical indication for MD probe insertion will be based on the judgment of neurology intensivist attending and neurological surgery attending as is the case for patients not enrolled in this study. The rationale for its use is measurement of the ratios of various metabolites (see above section A), which reflect cell function (i.e. anaerobic vs. aerobic metabolism) and estimate local blood flow.

F. Study Questionnaires

Participants will not be asked to complete study questionnaires.

G. Study Subjects

Inclusion criteria:

- aSAH confirmed by CT scan
- Aneurysm ablation by surgical clipping and insertion of cerebral microdialysis probe
- Absence of vasospasm before the insertion of a cerebral microdialysis probe

Exclusion criteria:

- Patients younger than 21 years of age

Definition of vasospasm:

"Cases" or "vasospasm" subjects will be patients who meet the definition criteria for the diagnosis of clinical vasospasm: [18]

- Delayed onset or persisting neurological deficit
- Onset 4-20 days after the onset of SAH
- Deficit appropriate to involved arteries
- Other causes of deterioration ruled out
    - Rebleeding
    - Hydrocephalus
    - Cerebral edema

    - Seizure
    - Metabolic disturbances, e.g. hypernatremia
    - Hypoxia
    - Sepsis
- Confirmatory ancillary test
    - Transcranial Doppler
    - CBF studies (imaging)
    - Cerebral angiography

Conscious patients will be consented before surgical intervention. If a patient is unconscious, we will collect and store their samples until they regain decision-making capacity or we speak to their next-of-kin. At this time, we will explain the study and ask for their consent. If they do not desire to participate in the study, we will destroy and discard the collected samples.

H. Recruitment of subjects

Patients presenting to the Columbia-Presbyterian Emergency Department and the Neurological Intensive Care Unit will be screened for the aforementioned criteria.

I. Confidentiality of Study

We will ensure confidentiality by assigning study numbers to each patient, which will be used in lieu of names and medical record numbers. Identifiers and data will be stored in a secure location, only accessible to the study investigators.

J. Potential Conflict of Interest

None of the investigators or Columbia University has a proprietary interest in a drug, device, or procedure that will be evaluated in this study, nor does any member from either group stand to benefit financially from the results of this investigation.

K. Location of the Study

This study will take place in the Milstein Hospital neuro-intensive care unit. Samples will be stored and analyzed in Dr. Connolly's bench laboratory, on the fifth floor of the College of Physicians and Surgeons building.

L. Potential Risks

As outlined above, no additive medical risks exist because the microdialysate samples are merely the volume remaining after clinical use, and would be discarded outside of this study. The breach of confidentiality of medical information risk is minute.

M. Potential Benefits

There is no potential benefit to the study participants. However, the potential benefits to society include a new method of vasospasm prediction in aSAH patients, which would enable neurologists and neurosurgeons to take preemptive measures to prevent and minimize the effects of imminent and often devastating vasospasm.

N. Alternative Therapies

Not applicable because no experiment therapies are under investigation.

O. Compensation to Subjects
No compensation will be provided.

P. Costs to Subjects
Subjects will not incur any additional costs as a result of participating in the study.

Q. Minors as Research Subjects
Minors are not eligible for this study (See exclusion criteria)

R. Radiation or Radioactive Substances
This study does not propose an evaluation of radiation modalities or radioactive substances.

References

1. Dumont AS, D.F., Chow MM, Lin CL, Calisaneller T, Ley DF, Kassell NF, Lee KS, *Cerebral vasospasm after subarachnoid hemorrhage: putatie role of inflammation.* Neurosurgery, 2003. **53**: p. 123-33.
2. Feignin VL, L.C., Bennnett DA, Anderson CS., *Stroke epidemiology: a review of populatio-based studies of incidence, prevalence, and case-fatality in the late 20th century.* Lancet Neurology, 2003. **2**: p. 43-53.
3. Johnston SC, S.S., Gress DR, *The burden, trends, and demographics of mortality from subarachnoid hemorrhage.* Neurology, 1998. **50**: p. 1413-1418.
4. Feigin VL, F., M, *Advances in subarchnoid hemorrhage.* Stroke, 2006. **37**: p. 305-308.
5. Condette-Auliac S, B.S., Anxionnat R, et al., *Vasospasm after SAH: interest in diffusion-weight MR imaging.* Stroke, 2001. **32**: p. 1818-1824.
6. King WA, M.N., *Critical care of patients with saubarachnid hemorrhage.* Neurosurg Clin North Am, 1994. **5**: p. 767-787.
7. Greenberg, M., *Handbook of Neurosurgery.* New York: Thieme Medical Publishers, 2000.
8. Qureshi AL, S.F., Yahia AM, et al., *Risk factors for cerebral vasospasms after aneurysmal subarachnoid hemorrhage.* Stroke, 2001. **32**(607-612).
9. Deitrich HH, D.R., *Molecular keys to the problems of cerebral vasospasm.* Neurosurgery, 2000. **46**: p. 517-530.
10. Macdonald, R., *Pathophysiology and molecular genetics of vasospasm.* Acta Neurochir Suppl, 2001. **77**: p. 7-11.
11. Treggiaria-Venzi MM, S.P., Roman J, *Review of medical prevention of vasospasm after aneurysmal subarachnoid hemorrhage: a problem of neurointensive care.* Neurosurgery, 2001. **48**: p. 249-262.
12. Allen GS, A.H., Preziosi TJ, et al., *Cerebral arterial spasm- a controlled trial of nimodipine in patients with subarachnoid hemorrhage.* New England Journal of Medicine, 1983. **308**: p. 619-624.
13. Dorsch NW, K.N., Sinkula MS, et al., *Metaanalysis of trials of tirilazad mesylate in aneurysmal SAH.* Acta Neurochir Suppl, 2001. **77**: p. 233-235.
14. Janjua N, M.S., *Cerebral vasospasm after subarachnoid hemorrhage.* Current Opinion in Critical Care, 2003. **9**: p. 113-119.
15. Winter CD, I.F., Pringle AK, Trikkas C, Claugh G, Church M, *A microdialysis method for the recovery of IL-1beta, IL-6 and nerve growth factor from human brain in vivo.* Journal of Neuroscience Methods, 2002. **119**: p. 45-50.
16. Maurer MH, H.D., Sakowitz OW, Unterberg AW, Kuschinsky W, *Identification of early markers for symptomatic vasospasm in human cerebral microdialysate after subarchnoid hemorrhge: Preliminary results of a proteome-wide screeing.* Journal of Cerebral Blood Flow and Metabolism, 2007: p. 1-9.
17. Hillered L, V.P., Hovda D, *Translational Neurochemical REsearch in Acute Human Brain INjury: The Current Status and Potential Future for Cerebral Microdialysis.* Journal of Neurotrauma, 2005. **22**(1): p. 3-41.
18. Greenberg, M., *Handbook of Neurosurgery*. 6th ed. 2006, Lakeland, FL: Thieme Medical Publishers.