Published in final edited form as:

Neurocrit Care. 2010 April ; 12(2): 244–251. doi:10.1007/s12028-009-9308-7.

CSF Neutrophils Are Implicated in the Development of Vasospasm in Subarachnoid Hemorrhage

J. J. Provencio,

NB3, Neuroinflammation Research Center, Lerner Research Institute, Cleveland Clinic, 9500 Euclid Ave, Cleveland, OH 44195, USA; Cerebrovascular Center, Cleveland Clinic, 9500 Euclid Ave, Cleveland, OH 44195, USA

X. Fu,

Department of Cell Biology, Lerner Research Institute, Cleveland Clinic, 9500 Euclid Ave, Cleveland, OH 44195, USA

A. Siu,

NB3, Neuroinflammation Research Center, Lerner Research Institute, Cleveland Clinic, 9500 Euclid Ave, Cleveland, OH 44195, USA

P. A. Rasmussen,

Cerebrovascular Center, Cleveland Clinic, 9500 Euclid Ave, Cleveland, OH 44195, USA

S. L. Hazen, and

Department of Cell Biology, Lerner Research Institute, Cleveland Clinic, 9500 Euclid Ave, Cleveland, OH 44195, USA; Cardiovascular Medicine and Center for Cardiovascular Diagnostics & Prevention, Cleveland Clinic, 9500 Euclid Ave, Cleveland, OH 44195, USA

R. M. Ransohoff

NB3, Neuroinflammation Research Center, Lerner Research Institute, Cleveland Clinic, 9500 Euclid Ave, Cleveland, OH 44195, USA

J. J. Provencio: provenj@ccf.org

Abstract

Background—Cerebral vasospasm is a significant cause of morbidity in patients after aneurysmal subarachnoid hemorrhage (aSAH). There are few effective treatments. The search for new treatments has focused predominantly on dilating cerebral blood vessels. Growing evidence supports a role for inflammation in its pathogenesis but no potential target for intervention has emerged.

Methods—CSF and clinical information from patients with aSAH were collected. Additionally, tyrosine modifications by stable isotope dilution HPLC with online tandem mass spectrometry were quantified in CSF samples.

Results—We report an association between neutrophil accumulation in the cerebrospinal fluid of patients with aSAH and the development of vasospasm. In particular, CSF neutrophil content of >62% on the third day after aSAH is an independent predictor of the later development of vasospasm (OR 6.8, 95% CI 2.0–23.3, $P = 0.002$). Further, activity of myeloperoxidase and NADPH oxidase is elevated in aSAH suggesting a role for modification of CSF proteins by reactive oxidant species.

Conclusions—Neutrophil percentage is an independent predictor of vasospasm in aSAH patients, days prior to its onset suggesting a role of neutrophils in vasospasm. The activity of neutrophil enzymes is also increased suggesting a mechanism for blood vessel damage. Inflammation mediated

by neutrophils is a potential target for therapies in vasospasm. More study is necessary to determine the mechanism by which neutrophils damage cerebral blood vessels.

Keywords

Subarachnoid hemorrhage; Cerebral vasospasm; Neutrophil; Inflammation; Reactive oxidant species

Introduction

Delayed cerebral ischemia secondary to the development of cerebral vasospasm is a major complication of aneurysmal subarachnoid hemorrhage (aSAH). The hallmark of vasospasm is an arteriopathy seen by angiography (angiographic vasospasm) which may lead to acute neurological deficits (symptomatic vasospasm) when severe. The onset of vasospasm is typically 4-14 days after the onset of aSAH [1].

There are currently few treatments for vasospasm and the available standard of care is focused on dilating blood vessels either pharmacologically or mechanically. The search for effective treatments has so far yielded few potential targets for intervention. A better understanding of the pathophysiology of cerebral vasospasm could lead to targets for intervention or prevention.

The precise mechanism by which bleeding from cerebral aneurysms causes vasospasm remains unclear but several lines of evidence implicate inflammation as a major factor in the pathogenesis [2]. Cytokines that signal neutrophils have been implicated in vasospasm in animal models [3–5]. Reactive oxidant species developed by neutrophils and the neutrophil enzyme myeloperoxidase (MPO) have been implicated in blood vessel wall pathology in other vascular diseases [6,7]. Moreover, MPO can catalytically consume nitric oxide (NO) as a substrate (a known contributor to vasospasm), directly promoting endothelial dysfunction and artery spasm [8–10]. To test the hypothesis that neutrophils contribute to the development of vasospasm, we investigated whether neutrophil accumulation in the CSF is associated with vasospasm in aSAH patients.

Methods

This study was done under the supervision of the Cleveland Clinic Institutional Review Board (IRB).

CSF Values in SAH Patients

The study represents an analysis of CSF samples in aSAH patients in a prospectively collected registry from 2000 to 2006 (patients from 2003 were not included due to study personnel changes). Risk factors for vasospasm including age, gender, whether the aneurysm was secured surgically or by endovascular technique, Hunt and Hess clinical grade and Fisher CT score were abstracted at the time of hospitalization. Modified Fisher scores were not used prior to 2006 and were therefore not included in this study [11]. CSF RBC, WBC (and their ratio), leukocyte subtypes, and vasospasm were abstracted after the hospital stay.

For evaluation of CSF cells, laboratory results of samples from patients with external ventricular devices (EVDs) were included. EVDs are placed in SAH patients for CSF diversion due to hydrocephalus commonly caused by clotting of blood in the CSF in the perimesencephalic cistern or ventricular system. The standard protocol for CSF sampling for patients with EVDs is to have CSF samples collected daily during the period of time that the EVD is in place. We used samples from the first 3 days for cellular analysis of study variables. Later samples were not utilized because neutrophil percentages begin to rise in all patients after the third day for unclear reasons. We hypothesize that increases in neutrophils could be due to

a foreign body reaction to the EVD (unpublished observation). The samples were drawn from the anti-reflux burette in the collecting system (Medtronic, Minneapolis, MN). Ten milliliters of CSF were collected and samples were transported immediately to the laboratory for analysis. All laboratory studies were performed by laboratory personnel blinded to patient clinical histories and outcomes. Total CSF leukocyte count was determined by direct visualization in a hemocytometer. Lineage percentages were tabulated from a centrifuged, stained preparation.

The absolute number of WBC and neutrophils were not used in the analysis. As a result of variable clot dissolution, RBC and WBC counts varied greatly from day to day. In addition, samples with fewer than 10 WBC/ μ l were not used in the analysis because percentages of neutrophils in patient samples with fewer than 10 WBC/ μ l were found to be unreliable (a difference in one cell could mean a change in percentage of greater than 20%).

Patients with systemic infections in the first 3 days were excluded from the analysis as indicated by increased systemic blood WBC counts with either positive cultures from blood, urine, or sputum, infiltrates on chest X-ray, and appropriate response to antibiotics.

Patients were monitored for vasospasm on admission and then every other day with transcranial Doppler examinations (TCD). Patients with mean middle cerebral artery (MCA) velocities > 140 cm/s or a mean basilar artery velocity > 90 cm/s by TCD suggested vasoconstriction and prompted further evaluation with CT angiography/perfusion scanning or digital subtraction cerebral angiography. Patients who were reported by the neuroradiologist (in the case of CT angiography) or the neurointerventional surgeon (for conventional angiograms) to have moderate or severe vasospasm were diagnosed with cerebral vasospasm (angiographic vasospasm). Because there is no consensus about which physical exam changes signify symptomatic vasospasm, particularly in patients with Hunt and Hess grade IV and V hemorrhages, we chose not to categorize patients by symptoms. We feel justified in this because we feel that asymptomatic vasospasm has the same pathophysiology as symptomatic disease and therefore should have similar targets for new treatments. CSF in this patient population was compared to CSF in the non-vasospasm patients.

Patient variables and CSF leukocyte percentages were compared using Mann-Whitney test and Fisher's exact test as appropriate. Receiver operator characteristic (ROC) analysis was performed using Graphpad Prism 5.0 (Graphpad Software Inc. San Diego, CA). Univariate analysis of dichotomized variables was performed using Fisher's exact test. A binary logistic regression model using the enter method was performed using variables with a *P* value <0.20 in univariate analysis (SPSS 9.0, Chicago, IL). Variables with a *P* value of <0.05 were considered independent predictors of CV.

Tyrosine and Phenylalanine Modification Assay

Based on an interim analysis of the cellular study that suggested that neutrophils were associated with vasospasm, we got consent from 17 patients in whom appropriate morning samples of CSF could be obtained for tyrosine and phenylalanine modification analysis. Samples were drawn over the first 3 days. The samples were incubated with a cocktail of protease inhibitors and stored for analysis at -20°C .

Multiple reactive oxidant species (ROS) can be generated in biological systems. Many ROS modify tyrosine and phenylalanine residues (oxidized amino acids) in proteins by substitution on the aromatic ring. Distinct substituted amino acid residues result from specific ROS which in turn inform the sources of the ROS (Table 1).

Oxidized amino acids were quantified by stable isotope dilution HPLC with online tandem mass spectrometry (LC/ESI/MS/MS) as previously described [12]. Briefly, the content of

multiple distinct oxidized tyrosine and phenylalanine species and their precursors were quantified in proteins recovered from biological specimens. [13C6] ring labeled, isotopically labeled synthetic oxidized tyrosine species, universally labeled tyrosine and phenylalanine (i.e., all carbons 13C, all nitrogen 15N) were added to the delipidated and desalted protein pellet as internal standards before protein hydrolysis. Multiple distinct natural abundance oxidized tyrosine species were identified and quantified by the corresponding [13C6] ring labeled isotopomer, while simultaneously the ions possessing the anticipated m/z transitions for universally labeled oxidized amino acids were monitored as an indicator of the potential artificial generation of oxidation products *ex vivo*. Universally labeled tyrosine and phenylalanine also served as internal standards for quantification of natural abundance tyrosine and phenylalanine, respectively. Under the conditions employed, no artificial generation of oxidation products was detected. Peak values from the first 3 days were compared to controls.

The 17 patients with aSAH were compared to 17 control patients being evaluated for normal pressure hydrocephalus. These patients were admitted to the hospital for CSF diversion to evaluate the need for permanent shunting. Although some patients were not subsequently diagnosed with the disorder, none had inflammatory brain disease.

Finally, to test whether there are differences in the neutrophil-derived tyrosine residues in patients who later develop vasospasm, we compared patients in aSAH group who did and did not develop vasospasm. Statistical analysis was performed using Graphpad 5.0. Medians values were evaluated using the Fisher's exact test.

Results

CSF Neutrophil Percentage is Associated with Vasospasm

We evaluated 236 patients with aSAH. Four patients were disqualified because of systemic infection although none were excluded due to ventriculitis. 116 (50%) of patients did not have EVDs placed. Of the remaining 116 patients, 70 could be included in the study based on their CSF laboratory values in the first 3 days post-hemorrhage.

The mean age for patients in the entire cohort was 57 (range 32–89) and 63% were women. The median Hunt and Hess grade was 3 (mean 3.1) as was the Fisher CT grade (mean 3.0). There were no significant differences between these variables in the patients excluded from the study because they either did not have EVDs or did not have adequate CSF samples. Twenty-five of 70 patients (36%) met the criteria for vasospasm. Patients who developed vasospasm had a significantly lower mean age and a higher admission Hunt and Hess score (Table 2). Gender and aneurysm management [endovascular or open surgery (19/25 vs. 31/45, $P = 0.591$)] were not significantly different between vasospasm and non-vasospasm groups. Fisher scale score was not significantly associated with vasospasm although there was a bias in our population due to the observation that EVD placement for SAH greatly favored Fisher grade 3 and 4 patients affecting the sample size needed to determine a difference.

There were no significant differences between the number of RBCs, WBCs, or their ratio in patients that did or did not develop vasospasm. By contrast, the median percentage of CSF leukocytes represented by neutrophils on the third day post-hemorrhage was significantly different in patients who developed vasospasm compared to patients who did not [76% (63–90) vs. 52% (30–76) (median (interquartile range)), $P = 0.009$] (Fig. 1a). There was a proportionate decrease in the percentage of lymphocytes in the vasospasm group ($P = 0.040$) that represents percentage displacement by neutrophils.

Patients who were excluded from the CSF analysis due to fewer than 10 WBC/ μ l in the CSF had a similar incidence of vasospasm as patients included in the study [12/25 (48%)]. There

was no association between neutrophil percentage and the site of vasospasm. The median day post-hemorrhage for vasospasm diagnosis was 5 days (range 2–9 days) corresponding well with previous reports [13]. Only one patient was diagnosed with vasospasm prior to completion of CSF sample collection on day 3 post-hemorrhage.

Risk of Vasospasm Is Associated with CSF Neutrophil Percentage Above 62% on Day 3 After Hemorrhage

The area under the ROC curve was 0.69 (95% CI 0.56–0.82; $P = 0.008$) suggesting that neutrophil percentage is a predictor of vasospasm. The neutrophil percentage that best discriminated patients who later developed vasospasm was 62% with a sensitivity of 0.8 and specificity of 0.58 (Fig. 1b).

Neutrophil Percentage in the CSF Is an Independent Predictor of Vasospasm

To determine if inflammatory mechanisms may be primary drivers of vasospasm, we developed a model using neutrophil percentage >62% for the development of vasospasm controlling for dichotomized variables for commonly attributed risk factors. Table 3 shows the results of the univariate analysis. A binary logistic regression model was constructed and the following were found to be independent predictors of developing vasospasm: CSF neutrophil percentage >62% (OR 6.8, 95% CI 2–23.3, $P = 0.002$) and Hunt and Hess grade 3–5 (OR 4.0, 95% CI 1.02–15.7, $P = 0.046$).

CSF Oxidized Amino Acids Implicates the Neutrophil Enzymes MPO and NADPH Oxidase, and Heme-Mediated Catalysis in the Pathogenesis of SAH

CSF samples taken from 17 aSAH patients were compared to 17 control patients. Comparing patients with aSAH and control, protein content of the MPO-generated chlorotyrosine and bromotyrosine were increased in aSAH patients. Chlorotyrosine levels were 23.1 (11.6–39.0) $\mu\text{mol/mol}$ tyrosine in aSAH patients compared to 15.8 (0–29.5) [median (interquartile range)] ($P = 0.04$), in controls (Fig. 2a). Bromotyrosine levels were also elevated in aSAH patients [44.8 (34.4–56.6) $\mu\text{mol/mol}$ tyrosine vs. 27.0 (12.9–31.9) (median (interquartile range)); $P = 0.002$] (Fig. 2b). These findings suggest elevated MPO activity in the subarachnoid space of patients with aSAH. Although bromide is also a substrate for a related eosinophil enzyme eosinophil peroxidase (EPO), eosinophils were uncommon in the CSF of our patients.

Of the 17 patients with aSAH, four had vasospasm. Chlorotyrosine levels were greater in patients with vasospasm compared to those without vasospasm [17.1 (0.0–145.6) $\mu\text{mol/mol}$ tyrosine (median) (range) vs. 6.3 (0.0–61.4)]. Bromotyrosine levels were also increased in patients with vasospasm compared to those without [59.1 (27.9–108.9) $\mu\text{mol/mol}$ tyrosine (median) (range) vs. 29.4 (0.0–54.3)]. Because only 4 patients had vasospasm, the standard deviation was high and reliable statistical analysis was not possible.

The levels of ortho-tyrosine, a non-physiological isomer of tyrosine formed by hydroxyl radical mediated oxidation of protein phenylalanine residues, were also markedly increased (Fig. 2c). Peak levels of protein-bound nitrotyrosine, a molecular marker NO-derived oxidants, were also significantly elevated in aSAH patients (Fig. 2d). Di-tyrosine, a protein cross-link formed by tyrosyl radical, was even more strikingly elevated within the first 3 days post-hemorrhage, a reaction potentially mediated as a result of both free heme reaction of O_2^- or H_2O_2 , peroxynitrite (an NO-derived oxidant) or MPO catalyzed oxidation of protein tyrosine residues [12] (Fig. 2e).

Discussion

In aSAH patients, inflammation mediated by neutrophils is a potential target for treatment or prevention of cerebral vasospasm. Neutrophil levels in the CSF observed 2 days before the period of vasospasm are an independent predictor of developing vasospasm. The development of the neutrophilia prior to vasospasm suggests that neutrophils may be an effector of vasospasm as opposed to a reaction to vasospasm. In addition, elevated levels of chloro- and bromotyrosine suggests a role for the neutrophil enzymes MPO in the development of a pro-oxidant state that may contribute to protein damage and, possibly, cerebral vasospasm.

Inflammation has been implicated in vasospasm in a number of studies. The earliest studies found a link between blood leukocyte counts, CSF leukocytes and poor outcome in aSAH [13–16]. By determining that neutrophils and, particularly neutrophils in the CSF, are associated with vasospasm, we have identified a WBC component that accumulates in the CSF and has the cellular processes capable of causing many of the vascular findings in vasospasm.

Other studies of inflammation in aSAH support our findings. Elevated levels of soluble E-selectin, ICAM-1, IL-1, IL-6 and soluble vascular cell adhesion molecule-1 were found in CSF of patients with aSAH [17–19]. Recently, the systemic inflammatory response syndrome (SIRS) on admission to the hospital has been associated with vasospasm [20]. In animal models, blocking E-selectin (a mediator of leukocyte entry into the central nervous system) [21], and administering antibodies against the neutrophil/macrophage adhesion molecule CD11/CD18 decreased the severity of vasospasm [5].

It is interesting that neutrophils, which in infection are mediators of acute inflammation, may play a role in a delayed process such as vasospasm. In non-infectious environments, neutrophils can effect change in a delayed fashion. There are examples of delayed effects of neutrophils in coronary artery plaque formation and in transplant rejection [10,22–24].

There is a report that is contradictory to our findings. Trabold et al. [25] reported CSF neutrophils in aSAH have a diminished capacity to generate oxidative bursts. This finding is contradictory to what our tyrosine modification results suggest in SAH patients. Further studies of neutrophil oxidative burst are needed to link the production of ROS by neutrophils and the increase in modified halide tyrosine residues found in our study.

There are a number of limitations to the present study. This project was designed to find potentially treatable targets for intervention in vasospasm. Due to this aim we limited samples to those that were ethically feasible and reliably provided interpretable data. As such, patients were excluded if they did not have EVDs for easy collection of CSF or had <10 WBC/μl in CSF. We felt that it was unethical to subject patients to lumbar puncture for this initial study since EVD samples were readily available. Although this approach limits the ability to generalize the data as a method of predicting vasospasm in all aSAH patients, we feel that it serves the goal of identifying potential targets for future therapies.

Another limitation of the study is that the ventricular fluid is distant from the probable site of the most intense inflammation. The fluid in the ventricles, in the perimesencephalic cisterns and in the lumbar space is derived from the same source and normally flows freely. There are differences in the concentration of protein between ventricular space and the lumbar space. The cellular content of ventricular and lumbar CSF spaces are not different [26,27].

In addition, neutrophils were significantly associated with vasospasm only on day 3. There are a number of reasons that neutrophils percentage on day 3 is a significant predictor of vasospasm when it fails as a predictor on other days. The development of neutrophil inflammation in a non-infectious environment takes time to develop which likely accounts for the lack of

discrimination in the first 2 days. Later, neutrophils percentages begin to rise in all patients due presumably to a foreign body reaction to the EVD (unpublished observation). These constraints establish a small window during which neutrophils are predictive of vasospasm. It will be evident that it was not possible to perform a kinetic evaluation to determine at what time point neutrophil percentage first began to correlate with later onset of vasospasm.

There are a number of variables that could not be controlled for due to the nature of the study. We did not collect detailed information about medications administered to the patients. Although patients taking immunosuppressive medicines were not included in the study, many medicines have immunomodulatory effects. Since no commonly used medicines in the ICU are known to be associated with vasospasm, we felt the risk of not finding a result where one exists was higher than finding an association where none exists. Given the goals of the present study to investigate potentially treatable targets for vasospasm, we felt this was acceptable.

The small sample size in the tyrosine modification study limits our ability to extrapolate the findings. Chlorotyrosine and bromotyrosine levels are elevated suggesting that neutrophil enzymes are active in the CSF of patients with SAH. The number of patients with vasospasm in our cohort was small (4/17 or 24%) due to random patient selection. Although the trend favors our hypothesis, the small sample size unfortunately limits our conclusions. Nitrotyrosine levels compared to control patients were increased suggesting increased NO which seems counter to previous theories of vasospasm [28]. It is important to note that our tyrosine modification data shows the effect of NO on tyrosine during the first 3 days and not during vasospasm when NO should be decreased.

Our study suggests that inflammation may be an important modifiable factor in the development of vasospasm. Importantly, the timing of neutrophil increase reported here offers a window of opportunity between day 3 when CSF neutrophils become elevated and day 5 to 12 when patients generally develop symptoms of vasospasm for prevention.

Current therapies for vasospasm are limited. Control of neutrophil action in the CSF of patients with aSAH in the first 3 days after the ictus is a feasible goal. Further work will be necessary to better understand the role of neutrophils in the pathogenesis of vasospasm. Targeted interventions designed to interrupt PMN-specific pathways or the development of neutrophil-derived reactive oxidant species may prove beneficial.

Acknowledgments

The authors would like to thank Michael Chow, MD, MPH, University of Alberta for his help with the 2000–2002 database; Rishi Gupta, MD for statistical help and review of the manuscript; and Edward Manno, MD and Saksith Smithason, MD for review of the manuscript. This work was supported by National Institutes of Health Grants K08 NS051350 (to J.J.P.), and P01 HL076491 (to S.L.H.), and K24 NS51400 (to R.M.R.).

References

1. van Gijn J, Rinkel GJ. Subarachnoid haemorrhage: diagnosis, causes and management. *Brain* 2001;124:249–78. [PubMed: 11157554]
2. Provencio JJ, Vora N. Subarachnoid hemorrhage and inflammation: bench to bedside and back. *Semin Neurol* 2005;25:435–44. [PubMed: 16341999]
3. Clatterbuck RE, Oshiro EM, Hoffman PA, Dietsch GN, Pardoll DM, Tamargo RJ. Inhibition of vasospasm with lymphocyte function-associated antigen-1 monoclonal antibody in a femoral artery model in rats. *J Neurosurg* 2002;97:676–82. [PubMed: 12296653]
4. Chaichana KL, Levy AP, Miller-Lotan R, Shakur S, Tamargo RJ. Haptoglobin 2–2 genotype determines chronic vasospasm after experimental subarachnoid hemorrhage. *Stroke* 2007;38:3266–71. [PubMed: 17962599]

5. Pradilla G, Wang PP, Legnani FG, Ogata L, Dietsch GN, Tamargo RJ. Prevention of vasospasm by anti-CD11/CD18 monoclonal antibody therapy following subarachnoid hemorrhage in rabbits. *J Neurosurg* 2004;101:88–92. [PubMed: 15255256]
6. Naruko T, Ueda M, Haze K, et al. Neutrophil infiltration of culprit lesions in acute coronary syndromes. *Circulation* 2002;106:2894–900. [PubMed: 12460868]
7. Brennan ML, Hazen SL. Emerging role of myeloperoxidase and oxidant stress markers in cardiovascular risk assessment. *Curr Opin Lipidol* 2003;14:353–9. [PubMed: 12865732]
8. Abu-Soud HM, Hazen SL. Nitric oxide is a physiological substrate for mammalian peroxidases. *J Biol Chem* 2000;275:37524–32. [PubMed: 11090610]
9. Eiserich JP, Baldus S, Brennan ML, et al. Myeloperoxidase, a leukocyte-derived vascular NO oxidase. *Science* 2002;296:2391–4. [PubMed: 12089442]
10. Vita JA, Brennan ML, Gokce N, et al. Serum myeloperoxidase levels independently predict endothelial dysfunction in humans. *Circulation* 2004;110:1134–9. [PubMed: 15326065]
11. Frontera JA, Claassen J, Schmidt JM, et al. Prediction of symptomatic vasospasm after subarachnoid hemorrhage: the modified fisher scale. *Neurosurgery* 2006;59:21–7. discussion 7. [PubMed: 16823296]
12. Brennan ML, Wu W, Fu X, et al. A tale of two controversies: defining both the role of peroxidases in nitrotyrosine formation in vivo using eosinophil peroxidase and myeloperoxidase-deficient mice, and the nature of peroxidase-generated reactive nitrogen species. *J Biol Chem* 2002;277:17415–27. [PubMed: 11877405]
13. Weir B, Grace M, Hansen J, Rothberg C. Time course of vasospasm in man. *J Neurosurg* 1978;48:173–8. [PubMed: 624965]
14. Neil-Dwyer G, Cruickshank J. The blood leucocyte count and its prognostic significance in subarachnoid haemorrhage. *Brain* 1974;97:79–86. [PubMed: 4434173]
15. Niikawa S, Hara S, Ohe N, Miwa Y, Ohkuma A. Correlation between blood parameters and symptomatic vasospasm in subarachnoid hemorrhage patients. *Neurol Med Chir (Tokyo)* 1997;37:881–4. discussion 4–5. [PubMed: 9465585]
16. Maiuri F, Gallicchio B, Donati P, Carandente M. The blood leukocyte count and its prognostic significance in subarachnoid hemorrhage. *J Neurosurg Sci* 1987;31:45–8. [PubMed: 3668657]
17. Fassbender K, Hodapp B, Rossol S, et al. Inflammatory cytokines in subarachnoid haemorrhage: association with abnormal blood flow velocities in basal cerebral arteries. *J Neurol Neurosurg Psychiatry* 2001;70:534–7. [PubMed: 11254783]
18. Takizawa T, Tada T, Kitazawa K, et al. Inflammatory cytokine cascade released by leukocytes in cerebrospinal fluid after subarachnoid hemorrhage. *Neurol Res* 2001;23:724–30. [PubMed: 11680512]
19. Polin RS, Bavbek M, Shaffrey ME, et al. Detection of soluble E-selectin, ICAM-1, VCAM-1, and L-selectin in the cerebrospinal fluid of patients after subarachnoid hemorrhage. *J Neurosurg* 1998;89:559–67. [PubMed: 9761049]
20. Dhar R, Diringer MN. The burden of the systemic inflammatory response predicts vasospasm and outcome after subarachnoid hemorrhage. *Neurocrit Care* 2008;8:404–12. [PubMed: 18196475]
21. Lin CL, Dumont AS, Calisaneller T, Kwan AL, Hwang SL, Lee KS. Monoclonal antibody against E selectin attenuates subarachnoid hemorrhage-induced cerebral vasospasm. *Surg Neurol* 2005;64:201–5. discussion 5–6. [PubMed: 16099244]
22. Lebkowska U, Malyszko J, Lebkowska A, et al. Neutrophil gelatinase-associated lipocalin and cystatin C could predict renal outcome in patients undergoing kidney allograft transplantation: a prospective study. *Transplant Proc* 2009;41:154–7. [PubMed: 19249501]
23. Turunen AJ, Lindgren L, Salmela KT, et al. Association of graft neutrophil sequestration with delayed graft function in clinical renal transplantation. *Transplantation* 2004;77:1821–6. [PubMed: 15223898]
24. Devouassoux G, Drouet C, Pin I, et al. Alveolar neutrophilia is a predictor for the bronchiolitis obliterans syndrome, and increases with degree of severity. *Transpl Immunol* 2002;10:303–10. [PubMed: 12507402]

25. Trabold B, Rothoerl R, Wittmann S, Woertgen C, Frohlich D. Cerebrospinal fluid and neutrophil respiratory burst after subarachnoid hemorrhage. *Neuroimmunomodulation* 2005;12:152–6. [PubMed: 15905623]
26. Provencio JJ, Kivisakk P, Tucky BH, Luciano MG, Ransohoff RM. Comparison of ventricular and lumbar cerebrospinal fluid T cells in non-inflammatory neurological disorder (NIND) patients. *J Neuroimmunol* 2005;163:179–84. [PubMed: 15885320]
27. Sommer JB, Gaul C, Heckmann J, Neundorfer B, Erbguth FJ. Does lumbar cerebrospinal fluid reflect ventricular cerebrospinal fluid? A prospective study in patients with external ventricular drainage. *Eur Neurol* 2002;47:224–32. [PubMed: 12037437]
28. Fukuda S, Hashimoto N, Naritomi H, et al. Prevention of rat cerebral aneurysm formation by inhibition of nitric oxide synthase. *Circulation* 2000;101:2532–8. [PubMed: 10831529]

Abbreviations

HOCl	Hypochlorous acid
HOBr	Hypobromous acid
LC/ESI/MS/MS	Liquid chromatography electrospray, ionization tandem mass spectrometry
EPO	Eosinophil peroxidase
MPO	Myeloperoxidase

Fig. 1.

CSF neutrophil percentage is associated with vasospasm after SAH. **a** In a cohort of 70 patients, there is an increased median neutrophil percentage in CSF 3 days after aSAH in patients who later develop vasospasm. *Boxes* denote interquartile range; *whiskers* are 90–10% intervals. **b** The receiver operator characteristic (ROC) identifies a cutoff of 62% neutrophils in the CSF as having the best predictive value for vasospasm (sensitivity 0.8, specificity 0.58)

Fig. 2.

Levels of modifications to tyrosine residues that predict the source of ROS in CSF of patients with SAH ($n = 17$) and controls ($n = 17$). **a, b** Chlorotyrosine and bromotyrosine levels are significantly greater in patients with SAH supporting the action of the neutrophil enzyme myeloperoxidase in the production of ROS in SAH. **c, d** Orthotyrosine and nitrotyrosine level elevation suggests the specific actions of the neutrophil enzyme NADPH oxidase and myeloid cell iNOS. **e** Di-tyrosine levels were greatly elevated in SAH compared to controls although the levels varied greatly in SAH patients. Di-tyrosine levels may reflect the action of MPO and NADPH oxidase but also reflects the reactive oxidant milieu of the CSF after SAH. *Boxes* denote interquartile range; *whiskers* are 90–10% intervals

Table 1

Reactive oxidant species in SAH and their sources

Inflammatory cell	Enzyme	Reactive oxidant species	Measurable tyrosine modification
Monocytes, macrophages, neutrophils	NADPH oxidase	$\cdot$ Tyrosyl	Di-tyrosine
		$\cdot$ OH	Orthotyrosine
Neutrophils	Myeloperoxidase (MPO)	$\cdot$ Tyrosyl	Di-tyrosine
		HOCl	Chlorotyrosine
		HOBr	Bromotyrosine
		$\cdot$ NO ₂	Nitrotyrosine
Macrophage/endothelium	iNOS/eNOS	OONO ⁻	Nitrotyrosine

Table 2

Comparison of demographic data in SAH patients with and without vasospasm

	No vasospasm (<i>n</i> = 45)	Vasospasm (<i>n</i> = 25)	<i>P</i> value
Age [mean (range)]	60 (32–89)	51.7 (32–70)	0.009*
Female gender (%)	29 (64%)	18 (72%)	0.602
Hunt and Hess grade [median (mean)]	3 (3.12)	4 (3.4)	0.005*
Fisher CT grade [median (mean)]	3 (3.2)	3 (3.2)	0.983
Endovascular treatment (%)	19 (76)	31 (69)	0.591

* Statistical significance

Table 3Univariate analysis of five predictors of vasospasm after SAH ($n = 70$)

Variable	Odds ratio	95% CI	P value
PMN > 62%	6.8	1.90–18.90	0.002
Gender (female)	1.4	0.49–4.12	0.602
Coiling	0.7	0.23–2.12	0.591
Hunt Hess grade 3–5	3.2	0.93–10.88	0.064
Age < 50 years	0.5	0.18–1.34	0.201
Fisher scale score of 3 or 4	6.9	0.37–130.73	0.152