Monocyte chemoattractant protein–1 predicts outcome and vasospasm following aneurysmal subarachnoid hemorrhage

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Object. Despite efforts to elucidate both the molecular mechanism and the clinical predictors of vasospasm after aneurysmal subarachnoid hemorrhage (ASAH), its pathogenesis remains unclear. Monocyte chemoattractant protein–1 (MCP-1) is a chemokine that has been firmly implicated in the pathophysiology of vasospasm and in neural tissue injury following focal ischemia in both animal models and human studies. The authors hypothesized that MCP-1 would be found in increased concentrations in the blood and cerebrospinal fluid (CSF) of patients with ASAH and would correlate with both outcome and the occurrence of vasospasm.

Methods. Seventy-seven patients who presented with ASAH were prospectively enrolled in this study between July 2001 and May 2002. Using an enzyme-linked immunosorbent assay, MCP-1 levels were measured in serum daily and in CSF when available. The mean serum and CSF MCP-1 concentrations were calculated for each patient throughout the entire hospital stay. Neurological outcome was evaluated at discharge or 14 days posthemorrhage using the modified Rankin Scale. Vasospasm was evaluated on angiography.

Results. The serum MCP-1 concentrations correlated with negative outcome such that a 10% increase in concentration predicted a 25% increase in the probability of a poor outcome, whereas the serum MCP-1 levels did not correlate with vasospasm. Concentrations of MCP-1 in the CSF, however, proved to be significantly higher in patients with angiographically demonstrated vasospasm.

Conclusions. These findings suggest a role for MCP-1 in neurological injury and imply that it may act as a biomarker of poor outcome in the serum and of vasospasm in the CSF. (DOI: 10.3171/JNS/2008/109/7/0038)

Key Words • monocyte chemoattractant protein–1 • subarachnoid hemorrhage • vasospasm

Aneurysmal subarachnoid hemorrhage is a major cause of neurological morbidity and death, occurring annually at a rate of 1 in 10,000 individuals in the US.13 The mortality rate is ~ 45% in the first 30 days, and long-term outcome is poor with 30% of survivors suffering moderate to severe disability at 1 year posttreatment2,14. Over recent decades, numerous attempts have been made in this patient population to predict outcome with clinical signs, radiographic imaging, and more recently biochemical markers. Research into these markers may provide physicians with a tool both to predict outcome in patients with ASAH and to offer insight into the pathogenesis of the injurious processes following ASAH.

Research into the mechanism of neurological injury following ASAH has strongly implicated the inflammatory pathway in the occurrence of vasospasm and the extent of the subsequent ischemic damage.2,21 Authors of previous studies have correlated a host of proinflammatory cytokines and chemokines with ASAH outcome and/or the occurrence of vasospasm, including intercellular adhesion molecule–1, vascular cell adhesion molecule–1, E-selectin, S100ß, MCP-1, IL-1ß, IL-6, IL-8, matrix metalloproteinase–9, and cyclooxygenase-2 as well as others.2,5,7,17,30 Although the bulk of research on inflammation in ASAH has been focused on leukocyte recruitment and infiltration, some evidence points to a significant role for monocytes as well.

Monocyte chemoattractant protein–1 is a CC chemokine specific for monocytes that is produced by monocytes, smooth-muscle cells, fibroblasts, and vascular endothelial cells in response to various stimuli such as IL-1, TNF-α, interferon-γ, and lipopolysaccharide6,32,36. In the central nervous system, MCP-1 has been shown to be involved in the damaging inflammatory processes associated with stroke, infection, neoplasia, vasospasm, and others.

Monocyte chemoattractant protein–1 is known to play a large part in neurological injury. A murine MCP-1 knock-
Monocyte chemoattractant protein–1 (MCP-1) has also been implicated in the pathogenesis of arterial injury and more specifically vasospasm. Research into the pathogenesis of atherosclerosis has revealed a direct interaction with monocytes and endothelial cells that leads to inflammatory activation of arterial endothelium.\(^2\) Specific to vasospasm, a gene expression array study showed that MCP-1 is upregulated in the major cerebral arteries in a canine model of vasospasm.\(^3\) Another study demonstrated that monocytes are found in high concentrations around the major cerebral arteries in a rat model of vasospasm.\(^4\)

Based on this evidence, it is likely that MCP-1 plays a role in both the pathogenesis of vasospasm and the exacerbation of subsequent ischemic injury. We therefore hypothesized that MCP-1 would be found at an increased concentration in the serum and CSF in patients with aneurysmal subarachnoid hemorrhage (ASAH) and that the MCP-1 concentration would correlate with both the occurrence of vasospasm and clinical outcome.

### Methods

Seventy-seven of the 95 patients who had presented with aneurysmal SAH to Columbia University Medical Center between July 2001 and May 2002 consented to participate in this institutional review board–approved study and had adequate blood sampling (at least 2 samples) and outcome measures for MCP-1 analysis. Serum and CSF were collected on a daily basis when possible until SAH Day 14 or discharge from the hospital, whichever came first. Cerebrospinal fluid was collected from an external ventricular drain, and serum was collected from an arterial line. Fifty-one patients had adequate CSF sampling (at least 1 sample). Fourteen patients undergoing clipping of unruptured aneurysms during the same time period were enrolled as controls. In these patients, a single sample of serum and CSF was obtained in the same manner as the samples obtained in the ASAH group.

**Sample Collection and Processing**

Blood was collected from each patient on a daily basis. When possible, CSF was collected intraoperatively via an external ventricular drain or lumbar puncture. All samples were allowed to clot for 20–30 minutes, centrifuged at 5000 rpm for 10 minutes, and immediately stored at −80°C. Monocyte chemoattractant protein–1 concentrations were determined for each sample by using a commercially available enzyme-linked immunosorbent assay (R&D Systems).

### Stratification and Outcome Measures

Hunt and Hess grade was determined according to the clinical status on admission. Grades I and II were considered good, III was intermediate, and IV and V were poor.\(^5\) Neurological outcome was evaluated based on the mRS and was scored on SAH Day 14 or discharge by a neurologist independent of the study. On average, the mRS score was determined on the 12th day after SAH. An mRS score of 0–3 was considered favorable, whereas a score of 4–6 was considered unfavorable. Vasospasm was defined based on angiography data and diagnosed by a neuroradiologist not involved in the study. In the vasospasm analysis, 5 patients were excluded because either no angiographic data were available in their cases or they died within 3 days of admission, before the vasospasm period.

### Statistical Analysis

The distribution of both serum and CSF MCP-1 mean concentrations were skewed right, necessitating a logarithmic transformation to normalize the data for statistical analysis. A logistic regression analysis was performed to predict an unfavorable outcome according to the mean serum and CSF MCP-1 concentrations. Patients were stratified by admission Hunt and Hess grade, and a 1-way analysis of variance test was conducted and followed by a Dunnett post hoc analysis. A second logistic regression was performed to predict an unfavorable outcome based on the mean serum and CSF levels after Hunt and Hess stratification. Finally, a Student t-test was used to compare the mean serum and CSF MCP-1 concentrations between patients with vasospasm and those without. Data are presented as the means ± standard errors of the mean unless otherwise noted, and a probability value < 0.05 was interpreted as significant.

### Results

Possible confounders were analyzed by using the Student t-test in a comparison of MCP-1 levels in patients with and without the confounder. Table 1 shows that age, sex, a history of hypertension, coronary artery disease, diabetes mellitus, and a history of smoking have no effect on serum or CSF MCP-1 levels. Similarly, there were no differences in the rates of these potential confounders between the ASAH group and controls (Table 2). There was no statistically significant difference between the average serum or CSF levels of MCP-1 in patients who underwent aneurysm clipping rather than coiling (p > 0.05).

#### Elevation of Serum and CSF Levels of MCP-1 in Patients With ASAH

Monocyte chemoattractant protein–1 was elevated in both the serum and CSF of patients with ASAH compared with controls (p < 0.05; Figs. 1 and 2). As a group, patients with ASAH had a mean serum concentration of 2.28 ± 0.05 log ng/ml and a CSF concentration of 3.37 ± 0.09 log ng/ml compared with 2.14 ± 0.05 log ng/ml (p < 0.05) and 2.33 ± 0.09 log ng/ml (p < 0.05), respectively, in con-
controls. When stratified by admission Hunt and Hess grades, all groups showed statistically higher serum MCP-1 levels compared with controls except in the good-grade patients (Hunt and Hess I–II: 2.69 ± 0.05 log ng/ml, p = NS; Hunt and Hess III: 2.22 ± 0.09 log ng/ml, p < 0.05; and Hunt and Hess IV–V: 2.54 ± 0.08 log ng/ml, p < 0.05). With regard to CSF MCP-1, concentrations increased as the Hunt and Hess grade increased, and all subgroups had significantly higher concentrations compared with controls (Hunt and Hess I and II: 3.23 ± 0.14 log ng/ml, p < 0.05; Hunt and Hess III: 3.56 ± 0.10 log ng/ml, p < 0.05; and Hunt and Hess IV and V: 3.79 ± 0.09 log ng/ml, p < 0.05).

Outcome Prediction Based on Serum MCP-1 Levels

Among all the patients with ASAH, MCP-1 levels in the serum were predictive of the neurological outcome on discharge such that a 10% increase in MCP-1 levels led to a 25% increase in the risk for a worse outcome (OR = 1.25, 95% confidence interval = 1.09–1.44, p < 0.01; Fig. 3). The levels of MCP-1 in the CSF of patients were not predictive of outcome (p = NS).

When patients were stratified by admission Hunt and Hess grades, serum MCP-1 concentrations continued to predict outcome in patients with good and medium grades. Patients with poor grades were not included in our analysis because there were no poor-grade patients among those with a favorable outcome on SAH Day 14. In patients with good and medium Hunt and Hess grades, serum MCP-1 concentrations predicted an unfavorable outcome at discharge such that a 10% increase in MCP-1 levels led to a 16% increase in the risk for an unfavorable outcome (OR = 1.16, 95% confidence interval = 1.00–1.34, p < 0.05).

Prediction of Vasospasm Based on CSF MCP-1 Levels

The levels of MCP-1 in patients with angiographically demonstrated vasospasm were compared with those in patients without vasospasm. All patients with ASAH and vasospasm showed a statistical trend toward MCP-1 elevation compared with levels in patients without vasospasm (3.63 ± 0.10 log ng/ml vs 3.39 ± 0.10 log ng/ml, respectively, p < 0.10; Fig. 4). The levels of MCP-1 in the CSF were higher in medium-grade patients with vasospasm than in those without (3.66 ± 0.12 log ng/ml vs 3.24 ± 0.16 log ng/ml, respectively, p < 0.05). Poor-grade patients with vasospasm showed a trend toward significantly different CSF MCP-1 levels (4.01 ± 0.16 log ng/ml vs 3.61 ± 0.12 log ng/ml, respectively, p < 0.10); however, good-grade patients with vasospasm did not have MCP-1 levels that were statistically significantly different from those without vasospasm (3.23 ± 0.15 log ng/ml vs 3.22 ± 0.24 log ng/ml, respectively). Serum levels of MCP-1 did not show any correlation with vasospasm (p = NS, for all comparisons).

Discussion

Our research showed that MCP-1 is upregulated in patients with ASAH and can predict a poor outcome. In addition, CSF levels of MCP-1 correlated with an angiographically demonstrated vasospasm. Much recent laboratory work has been focused on characterizing the molecular cas-
cades after various forms of brain injury and ischemia. Efforts from a clinical perspective have centered on identifying key molecular markers in patients suffering neurological injury, such as ASAH and stroke. In ASAH specifically, numerous inflammatory markers have correlated with a poor outcome and/or vasospasm, such as TNF-α, IL-1, IL-6, IL-8, S100β, intercellular adhesion molecule–1, C5a, and C5a, indicating important roles for leukocyte infiltration and complement activation.

Elevated MCP-1 Levels in the Serum and CSF

Monocyte chemoattractant protein–1 has been shown to be upregulated in the CSF of patients with ASAH, but no authors have commented on the relationship between MCP-1 and outcome or vasospasm. We found elevated MCP-1 concentrations in the CSF of patients in each of the Hunt and Hess subgroups and in the serum of patients in the medium- and poor-grade groups. The finding that MCP-1 was not elevated in the serum of good-grade patients may be explained by the decreased systemic inflammatory response in less severe cases of SAH. Good-grade patients are also less likely to have elevated serum C-reactive protein levels and fever. However, the CSF levels of MCP-1 may be a more sensitive marker of neurological damage and therefore elevated compared with controls.

Although it is likely that ASAH causes increased MCP-1 levels, we cannot rule out the possibility that an increase in inflammatory mediators preceded aneurysm rupture. Previous research comparing the pathophysiology of ruptured and unruptured aneurysms at surgery showed macrophage infiltration in the endothelium of ruptured aneurysms. One hypothesis for the pathogenesis of aneurysm rupture asserts that gradual thinning of the endothelial wall leads to subendothelial exposure, which results in rapid thrombosis and inflammation that leads to rupture. Further study is necessary to characterize the inflammatory milieu leading to aneurysm rupture, which is separate from the inflammatory response to ASAH.

Cerebrospinal Fluid MCP-1 Levels as a Marker of Vasospasm

We found that CSF levels of MCP-1 correlate with vasospasm, whereas serum levels do not. One explanation for this finding may be that the CSF levels of MCP-1 are a more specific indicator of cytokine and chemokine levels in the subarachnoid space, whereas serum levels are dampened by the blood–brain barrier and/or overshadowed by a generalized systemic inflammatory response. Serum levels of MCP-1 may be more significantly affected by systemic causes of inflammation, such as urinary tract infection or pneumonia, making them imperturbable to alterations in CSF MCP-1 levels. Therefore, it is likely that serum MCP-1 levels are not specific enough to predict vasospasm.

Past research has also shown that CSF levels, but not serum levels, of MCP-1 are increased in stroke. Moreover, research on ischemia in cardiac myocytes has firmly implicated MCP-1 in apoptotic pathways that lead to increased cell death and poor cardiac outcome. The mechanism behind this process is thought to be caused by MCP-1 binding to its receptor chemokine (C-C motif) receptor 2, which induces the transcription factor MCP-induced protein, which in turn activates caspase 3–mediated apoptosis. Thus, there is the possibility that MCP-1 is involved in the ischemic damage subsequent to vasospasm as well as in the process of vasospasm itself. In cerebral ischemia, as previously mentioned, blocking MCP-1 in a murine stroke model decreases the infarct volume and improves neurological
outcome. In response to MCP-1 elevation, microglia migrate to areas of neuronal injury \cite{1, 27} and are thought to contribute to neuronal cell death \cite{2, 26}.

Our finding of increased MCP-1 in the CSF of patients with vasospasm indicates a possible role for monocytes in the pathogenesis of vasospasm. Notably, the good-grade patients with and without vasospasm showed no difference in CSF MCP-1 levels, potentially because of less severe vasospasm in this subgroup. Previous work has linked admission Hunt and Hess grades to the severity of spasms. \cite{21} The involvement of MCP-1 in vasospasm is supported by data showing increased MCP-1 (CCL2) expression in a canine model of vasospasm as well as increased monocyte numbers surrounding the major cerebral arteries in a rat model of vasospasm. \cite{19, 28} Monocytes are thought to play a role in vasospasm both directly and through the propagation of the inflammatory response by the secretion of cytokines such as IL-1β, IL-6, and TNF-α. A mechanistic hypothesis relating monocyte activation and vasospasm was recently proposed by Fassbender et al. \cite{8} who showed that endothelin-1 is produced and released by monocytes in ASAH along with other acute-phase reactants. Endothelin-1 has been implicated as a prime suspect in the pathophysiology of vasospasm because of its upregulation in patients with ASAH and its potency as a vasoconstrictor. \cite{27}

### Levels of MCP-1 as a Serum Marker of Poor Outcome

Authors of many previous studies have attempted to identify markers of poor outcome in patients with ASAH. \cite{19, 23, 25, 34} We found that serum levels of MCP-1 predicted outcome independently of the Hunt and Hess grade in ASAH. Elevated levels may reflect a systemic stress response that correlates with outcome, a capacity beyond the Hunt and Hess scale. Although serum MCP-1 levels correlated directly with poor outcome, they did not correlate with vasospasm, implying that serum MCP-1 levels more broadly predict poor outcome from factors beyond vasospasm. Thus, MCP-1 may serve as a biomarker of poor outcome. We were surprised to find that MCP-1 levels correlated with outcome in the serum, but not in the CSF. We propose that this difference is attributable to the greatly elevated levels of MCP-1 in the CSF of patients with ASAH so that this protein is no longer a sensitive marker for differences in outcome. Unfortunately, our study is limited by the absence of long-term outcome data, and additional studies are necessary to determine the relationship between MCP-1 levels and long-term outcome.

Our data showed a strong correlation among MCP-1 levels, outcome, and vasospasm and characterized the differences in MCP-1 expression in the CSF and serum in patients with ASAH. Prior studies pertaining to the role of inflammation in ASAH have been focused on the involvement of leukocytes and related cytokines, whereas our work suggests that there may be a larger role for monocytes than previously thought. It has been shown that many inflammatory mediators and varieties of immune cells are involved in the processes of both ischemic neuronal injury and vasospasm, and we suggest that MCP-1, and therefore monocytes, plays a large part in these pathological processes. Monocytes have been shown to play an important role in both the pathophysiology of vasospasm and the subsequent neurological damage. Monocyte suppression following ASAH may therefore prove to be a valuable therapeutic strategy.

### Conclusions

We showed that MCP-1 levels are elevated in the serum and CSF of patients with ASAH and that these higher levels in the serum of good- and medium-grade patients with ASAH serve as strong predictors of a poor outcome. In addition, we found that CSF MCP-1 levels correlate with angiographically demonstrated vasospasm in a subset of patients. Further studies are necessary to evaluate the role of MCP-1 in acute neurological injury and the pathogenesis or subsequent damage related to vasospasm after SAH.

### References

Predicting outcome and vasospasm in aneurysmal SAH


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