Serum and cerebrospinal fluid C-reactive protein levels as predictors of vasospasm in aneurysmal subarachnoid hemorrhage

Clinical article

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Object. Cerebral vasospasm is a common and potentially devastating complication of aneurysmal subarachnoid hemorrhage (aSAH). Inflammatory processes seem to play a major role in the pathogenesis of vasospasm. The C-reactive protein (CRP) constitutes a highly sensitive inflammatory marker. The association of elevated systemic CRP and coronary vasospasm has been well established. Additionally, elevation of the serum CRP levels has been demonstrated in patients with aSAH. The purpose of the current study was to evaluate the possible relationship between elevated CRP levels in the serum and CSF and the development of vasospasm in patients with aSAH.

Methods. A total of 41 adult patients in whom aSAH was diagnosed were included in the study. Their demographics, the admitting Glasgow Coma Scale (GCS) score, Hunt and Hess grade, Fisher grade, CT scans, digital subtraction angiography studies, and daily neurological examinations were recorded. Serial serum and CSF CRP measurements were obtained on Days 0, 1, 2, 3, 5, 7, and 9. All patients underwent either surgical or endovascular treatment within 48 hours of their admission. The outcome was evaluated using the Glasgow Outcome Scale and the modified Rankin Scale.

Results. The CRP levels in serum and CSF peaked on the 3rd postadmission day, and the CRP levels in CSF were always higher than the serum levels. Patients with lower admission GCS scores and higher Hunt and Hess and Fisher grades had statistically significantly higher levels of CRP in serum and CSF. Patients with angiographic vasospasm had higher CRP measurements in serum and CSF, in a statistically significant fashion (p < 0.0001). Additionally, patients with higher CRP levels in serum and CSF had less favorable outcome in this cohort.

Conclusions. Patients with aSAH who had high Hunt and Hess and Fisher grades and low GCS scores showed elevated CRP levels in their CSF and serum. Furthermore, patients developing angiographically proven vasospasm demonstrated significantly elevated CRP levels in serum and CSF, and increased CRP measurements were strongly associated with poor clinical outcome in this cohort. (DOI: 10.3171/2009.2.FOCUS08311)

KEY WORDS • aneurysm • C-reactive protein • digital subtraction angiography • inflammation • subarachnoid hemorrhage • vasospasm

Aneurysmal subarachnoid hemorrhage constitutes a devastating and complicated clinical entity. Despite the recent advances in its early detection, diagnosis, and its proper treatment, the overall outcome of patients with aSAH remains poor.11,58 Approximately 50% of patients suffering aSAH will die, 15% of them will become severely disabled, and only 20–35% will return to normal life and activities.11,15,43,58

Cerebral vasospasm remains the most troublesome complication of aSAH. It is associated with high morbidity and mortality rates, even after successfully treating the ruptured aneurysm. The occurrence of cerebral vasospasm varies significantly. It has been demonstrated to be as high as 70% based on angiographic findings, and
Several theories have been proposed in an attempt to explain the underlying pathophysiological mechanisms of cerebral vasospasm. However, none of the proposed theories has been experimentally proven, and the underlying mechanism causing this complex problem remains unknown. Among these theories, a relatively recent one postulates that an inflammation-based pathogenetic mechanism is implicated in the development of coronary vasospasm. Previous experimental and clinical studies have demonstrated that inflammatory changes occur in spastic coronary arteries. It has been demonstrated that eosinophilic cell counts and plasma fibrinogen levels could predict the severity of vasospastic angina pectoris. Additionally, previous clinical studies have shown that elevated levels of high-sensitivity CRP could predict the development of coronary vasospasm. The measurement of sensitive inflammatory markers such as CRP significantly increases our ability to predict with accuracy and possibly to prevent or appropriately treat coronary thrombotic events. Furthermore, elevated CRP is associated with increased risk of a subsequent myocardial infarction.

A similar inflammatory response affecting the cerebral vasculature is elicited in cases of aSAH via the release of various cytokines such as IL-6, IL-1, and tumor necrosis factor; increased leukocyte trafficking; and macrophage and complement cascade activation. Additionally, it has been demonstrated that CRP, an acute-phase protein, which is a highly sensitive indicator of inflammatory response, is produced by hepatocytes in response to increased production of IL-6. Because IL-6 has been associated with the development of cerebral vasospasm, increased levels of CRP at the time of the patients’ admissions and early in their postictal courses might have some predictive value in the early detection and proper management of vasospasm.

In our current study, we present our results from measuring the CRP levels in the serum and CSF of patients suffering aSAH, and we explore the relationship of systemic and CSF CRP levels and the development of cerebral vasospasm.

### Methods

Our prospective clinical study was approved by the institutional review boards of the participating institutions, and the collection and analysis of all data were performed according to the current Health Insurance Portability and Accountability Act regulations. A detailed written consent form was obtained from all participants or from their legal representatives or next of kin in those cases in which the patient was unable to consent. The inclusion criteria of our study were as follows: 1) diagnosis of aSAH established by a CT scan and a subsequent 4-vessel DS angiography study; 2) patient age > 18 years; 3) patient admission to our institutions within the first 24 hours postictus; 4) patient’s surgical or endovascular treatment within 48 hours from admission to our hospitals; 5) pre- and post-treatment DS angiography for vasospasm documentation purposes; and 6) external ventriculostomy insertion. Patients with concomitant or recent acute myocardial infarction, history of recent (≤ 30 days) surgery prior to the ictal event, and/or clinical or laboratory evidence of chronic systemic infection were excluded from our study. In addition, patients who received no external ventriculostomy or who died before completing 10 days of treatment were excluded from our current study.

The study covered a 4-year period (2004–2007). A total of 286 patients was admitted during this period with an established diagnosis of aSAH. However, only 41 patients met our inclusion criteria and participated in our study. There were 25 men and 16 women, with a mean age of 51.8 years and ages ranging between 34 and 72 years. A total of 46 aneurysms were identified in our cohort.

<table>
<thead>
<tr>
<th>Location of Lesion</th>
<th>No. of Aneurysms</th>
</tr>
</thead>
<tbody>
<tr>
<td>posterior communicating artery</td>
<td>14</td>
</tr>
<tr>
<td>anterior communicating artery</td>
<td>11</td>
</tr>
<tr>
<td>middle cerebral artery</td>
<td>7</td>
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<tr>
<td>internal carotid artery</td>
<td>5</td>
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<tr>
<td>basilar artery</td>
<td>5</td>
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<tr>
<td>anterior choroidal artery</td>
<td>2</td>
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<tr>
<td>posterior inferior cerebellar artery</td>
<td>2</td>
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<table>
<thead>
<tr>
<th>Max Diameter of Aneurysmal Dome</th>
<th>No. of Aneurysms</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10 mm</td>
<td>9</td>
</tr>
<tr>
<td>10–25 mm</td>
<td>31</td>
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<tr>
<td>&gt;25 mm</td>
<td>6</td>
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* The dome’s largest diameter was measured on the preoperative DS angiography study.
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and morphological features of the aneurysm, the presence of multiple aneurysms, the presence of mass effect caused by the aneurysm and/or an associated hematoma, the patient’s neurological and general medical condition, and the patient’s preference. It has to be emphasized that either surgical or endovascular treatment was performed within 48 hours of patient admission.

The CRP level in serum and CSF was measured in each patient on Days 0 (on admission to our centers), 1, 2, 3, 5, 7, and 9, and the measurements obtained were recorded. Blood serum specimens were collected from an inserted venous line, whereas the CSF specimens were collected through an external ventriculostomy. The CRP levels were measured using a nephelometric methodology. All the CRP measurements were expressed as milligrams/liter. The mean serum and CSF values were calculated for each participant.

The patients’ clinical outcome was evaluated using the GOS and mRS at discharge from our institutions, and all participants’ scores were recorded.

Results

The anatomical location of the lesions and the preoperatively measured maximum diameter of the aneurysmal dome in our cohort are summarized in Tables 1 and 2, respectively. The admission GCS scores ranged between 4 and 15 (mean 11.7). The Hunt and Hess scores on admission ranged between I and V (mean 2.5), and the Fisher grades in our patients ranged between 1 and 4 (mean 1.9). The GOS scores on discharge ranged between 2 and 5 (mean 4.3), and the range of mRS scores was 0–5 (mean 1.1). Analytical data obtained in our patients are summarized in Table 3.

In regard to the measured CRP levels, there was a progressive increase in the CRP levels from the admission to the 3rd postadmission day (3rd or 4th postictal day), which was followed by a slow decrease toward the 9th postadmission day (Fig. 1). There was a parallel course of increase in the CRP levels in CSF and serum. The observed CRP levels in the CSF were significantly higher than the levels of CRP in the serum throughout the entire postictal period (paired t-test methodology; t = 6.547, p < 0.0001) (Fig. 1).

Angiographic vasospasm was detected in 15 (36.6%) of our 41 patients in the DS angiography studies obtained posttreatment. Analysis of our angiographic data demonstrated that there was a regional pattern of vasospasm, affecting mainly the parent and adjacent vessels in 9 (21.9%)
of our 41 patients, whereas in 6 (14.6%) of 41, global vasospasm was evident. Interestingly, clinical symptoms caused by the observed cerebral vasospasm developed in 8 (19.5%) of 41 patients in our cohort. These symptoms were managed solely medically in 6 cases, whereas in the remaining 2 cases intravascular balloon angioplasty was necessary.

Statistical analysis of our data showed that patients developing angiographic vasospasm in our cohort had higher CRP levels in serum (Fig. 2 upper). This differ-
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ence reached the level of statistical significance (unpaired t-test methodology; df = 39, t = -4.678, p < 0.0001). Similarly, patients with angiographically proven vasospasm demonstrated higher CRP levels in CSF, in a statistically significant fashion (unpaired t-test methodology; df = 39, t = -4.201, p < 0.0001) (Fig. 2 lower). On the contrary, the difference in occurrence of angiographic vasospasm between patients undergoing surgical clipping and those undergoing endovascular coil insertion was not statistically significant (chi-square statistical methodology; $\chi^2 = 0.004$, df = 1, p = 0.95). The strength of our last statistical analysis is very limited, however, due to the small number of participants in the endovascular group.

In regard to the admission GCS score and the serum CRP levels, we found that patients with lower GCS scores had increased CRP measurements in serum (correlation coefficient methodology; z = -8.139, p < 0.0001, r = -0.87) (Fig. 3 upper) and CSF (z = -6.443, p < 0.0001, r = -0.78) (Fig. 3 lower). Low admission GCS scores were significantly inversely correlated with high CRP values in serum and CSF. Likewise, patients with higher Hunt and Hess grades on admission developed significantly higher CRP levels in serum (correlation coefficient methodology; z = -5.861, p < 0.0001, r = -0.74) (Fig. 5 upper) and CSF (z = -5.091, p < 0.0001, r = -0.68) (Fig. 5 lower) had less favorable outcomes. A statistically significant inverse correlation was established in our series between CRP levels (in serum and CSF) and GOS scores. A similar statistically strong relationship was found between mRS scores on discharge and CRP measurements in serum (correlation coefficient methodology; z = 6.062, p < 0.0001, r = 0.76) and CSF (z = 5.304, p < 0.0001, r = 0.70).

Discussion

The role of inflammation in the development and maintenance of cerebral vasospasm has been previously demonstrated. Several animal studies and clinical series have shown that inflammatory processes contribute to the pathogenesis of cerebral vasospasm. It is well known that leukocyte trafficking increases in SAH due to a breakdown of the blood-brain barrier. Increased levels of various soluble adhesion molecules

Fig. 4. Upper: Graph showing CRP measurements in serum in surgically versus endovascularly treated patients in our cohort. Lower: Graph showing CRP measurements in CSF in surgically versus endovascularly treated patients in our study.

Fig. 5. Upper: Schematic representation of CRP measurements in serum in patients with GOS score ≥ 4 versus patients with GOS score < 4 at discharge. Lower: Schematic representation of CRP levels in CSF in patients with GOS score ≥ 4 versus patients with GOS score < 4 at discharge.
(such as E-selectin, intercellular adhesion molecule-1, and vascular adhesion molecule-1) and cytokines (such as IL-6 and IL-1) have been noted in the plasma and CSF of patients with SAH.\textsuperscript{31,12,16,22,30,54,57,60} Kubo et al.\textsuperscript{39} demonstrated that serum concentrations of intercellular adhesion molecule-1 and vascular adhesion molecule-1 were significantly higher among patients suffering aSAH, and that their increased serum levels of these molecules were associated with increased incidence of DIND.\textsuperscript{4} An increased concentration of platelet-activating factors was found in the jugular venous blood samples of patients with SAH.\textsuperscript{32} and tumor necrosis factor–α levels increased after SAH, and this increased concentration was correlated in time and extent with increased blood flow velocities of the basal cerebral arteries as measured by transcranial Doppler ultrasonography.\textsuperscript{11,12} Furthermore, increased levels of immunoglobulins and complement factors have been found in the serum and cerebral vessel walls during vasospasm.\textsuperscript{11,24,30,48,50,55} Kubota et al.\textsuperscript{35} in an animal model of SAH, found that activated macrophages and T-cell counts were elevated in such circumstances, with peak levels occurring 2 days after the ictal event. In addition, it has been demonstrated that endothelin-1, which is produced by activated leukocytes accompanying an inflammatory response, is involved in the development of cerebral vasospasm.\textsuperscript{11} Furthermore, recent series of animal models of SAH have shown changes in the gene expression of inflammation-related products.\textsuperscript{1,11,16,21,31,32,36,38–42,45–47,49,59,62,67} Cyclooxygenase (also called COX-2), which is known to be an important component in many inflammatory responses, is upregulated after induced SAH in canine and rabbit basilar arteries.\textsuperscript{49,64} Macdonald and Weir,\textsuperscript{38} in their SAH primate model studies, have demonstrated the up-regulation of certain inflammation-related genes.

It is apparent that multiple inflammatory mechanisms are directly involved in the pathogenesis of cerebral vasospasm. It is also well established that CRP is a sensitive inflammatory marker. Interleukin-6 constitutes a strong stimulus for CRP synthesis by hepatocytes.\textsuperscript{44,63} Additionally, IL-1, which has been implicated in the pathogenesis of cerebral vasospasm, also represents a strong stimulus for CRP synthesis.\textsuperscript{60} Therefore, elevated concentrations of CRP may well be associated with an increased possibility of developing cerebral vasospasm and subsequently a DIND.\textsuperscript{34}

Our results showed that elevated CRP levels in serum and CSF were associated with increased incidence of angiographic vasospasm, based on the DS angiography findings in our cohort. Additionally, there was a strong inverse correlation between admitting GCS scores and CRP levels in serum and CSF (r = −0.87 and r = −0.78, respectively). The admission Hunt and Hess and Fisher grades were also correlated in a statistically significant fashion with the CRP measurements in serum and CSF in our cohort. Furthermore, the elevated CSF and serum CRP levels that we observed were associated with worse clinical outcome, as expressed in GOS and mRS scores in our cohort. It is noteworthy that the CRP levels were always higher in CSF than in serum in all patients throughout the entire postictal period. This may be explained by the fact that the underlying aSAH caused a disruption of the blood-brain barrier, which allowed massive intrathecal CRP transportation as an immediate systemic inflammatory response to the presence of blood and blood clots in the subarachnoid space. We found no statistically significant difference in the occurrence of angiographic vasospasm between patients undergoing surgical treatment and those undergoing endovascular coil occlusion. Interestingly, the surgically treated group demonstrated higher levels of CRP in serum and CSF compared with those who were endovascularly treated; however, this difference did not reach levels of statistical significance. It has to be emphasized that the extraction of any powerful conclusions from these last comparisons is impossible due to the very limited number of endovascularly treated patients in our study.

Our current findings are in agreement with the observations of other clinical investigators.\textsuperscript{2,28,34,56,63} Bengzon et al. examined the serum CRP levels of patients undergoing standard neurosurgical procedures in a prospective clinical study. They found that all their patients demonstrated elevated CRP levels postoperatively, and the magnitude of the CRP elevation was associated with the extent of the surgical trauma. They reported, however, that patients with aSAH showed the highest serum CRP increases, and they postulated that the SAH and not the surgical trauma itself had most likely contributed to the significant serum CRP elevation. In a previous retrospective clinical study, by analyzing the patients’ admission Hunt and Hess grades, Rothoerl et al.\textsuperscript{56} found that serum CRP levels could provide independent information regarding the severity of brain injury resulting from the initial SAH. They concluded that the higher the serum CRP level, the poorer the clinical outcome was. Furthermore, the time pattern of serum CRP increase in their cohort was similar to the one observed in our current series. They noted a peak serum CRP concentration on the 3rd and 4th postictal days and gradual, slow decrease of the measurements obtained after the 5th postictal day, a pattern identical to the one observed in our series. Likewise, Takizawa et al.\textsuperscript{63} in their study examining CSF samples of patients suffering SAH by using enzyme-linked immunosorbent assays and Western blot analysis, found that CRP levels in CSF were significantly increased, and the CRP in CSF peaked between the 2nd and 3rd postictal days. On the contrary, Berger et al.\textsuperscript{3} reported that serum CRP reached a peak on the 2nd postictal day in their series. Kacira et al.\textsuperscript{28} reported increased high-sensitivity CRP levels in serum and CSF in patients with aSAH compared with healthy controls. Interestingly, they found that elevated serum and CSF high-sensitivity CRP was correlated to increased measurements of caspase-3 and neuron-specific enolase, which are markers of apoptosis and neuronal tissue damage, respectively. In a previous study, Fountas et al.\textsuperscript{23} found that patients with aSAH who had low (< 8) GCS scores and high (> III) Hunt and Hess grades had significantly higher serum CRP levels, and that an increased serum CRP level was positively correlated with poor clinical outcome. Their findings are in agreement with the data in our current study.

Unfortunately, the clinical significance of elevated
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serum CRP measurements in patients sustaining aSAH is confounded by the fact that most of these patients may have other concomitant systemic infections or pathological conditions that could potentially result in increased CRP serum concentrations. Additionally, the surgical manipulation in these patients (arterial catheterization, surgical clipping, endovascular coil occlusion, ventriculostomy insertion) could influence the systemic CRP levels. However, our strict inclusion criteria minimized the influence of other confounding factors such as systemic infection or other concomitant heart conditions. Moreover, obtaining CRP measurements in CSF could further minimize the effect of other confounding factors in our current study.

It has to be emphasized however, that our study carries significant limitations. First, the size of our clinical series was limited, and therefore the statistical strength of our conclusions was also limited. Second, it is well known that clinical outcome in patients with aSAH is multifactorial. The association between CRP levels systemically and in CSF with the clinical outcome may well be influenced by other parameters in a complex and frequently unpredictable way. In addition, CRP represents a sensitive but nonspecific inflammatory marker. Although we attempted to minimize the presence of other systemic or confounding factors in our study by applying strict inclusion criteria, there were certain issues that could not be addressed. The insidious onset of a CNS and/or systemic infection may contribute to serum CRP elevation and significantly confound the CRP predictive value for early vasospasm detection. Furthermore, any neurosurgical intervention per se may well increase the serum CRP levels, although there are no data as far as we know regarding the CRP response in CSF to standard neurosurgical procedures. It has been reported, however, that CRP levels in CSF are elevated in patients with severe head trauma,20,27 a finding that may further support our current results that the postictal elevated CRP levels in CSF are mainly caused by the aSAH insult to the brain parenchyma. A large-scale, multiinstitutional, prospective clinical study is necessary to validate our results and to determine the role of CRP in serum and/or CSF in the identification of patients at high risk for developing cerebral vasospasm.

Conclusions

Our prospective clinical study showed that patients with aSAH who were admitted with low GCS scores and high Hunt and Hess and Fisher grades had elevated CRP levels in serum and CSF. The CRP measurements in the CSF were higher than the serum CRP values in all our patients throughout the entire postictal period. Patients developing angiographic vasospasm demonstrated increased levels of CRP in serum and CSF in our cohort. In addition, the CRP levels in serum and CSF were associated with poor clinical outcome in a statistically significant fashion in our study. Further validation of our results is necessary to define the prognostic role of CRP in identifying patients who are at high risk for developing postictal cerebral vasospasm secondary to aSAH.

Disclaimer

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

References


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