Cytokines and subarachnoid hemorrhage
FNU Ruchika, Siddharth Shah*, Maliya Delawan, Neupane Durga, Brandon Lucke-Wold

Department of Neurosurgery, University of Florida, Gainesville, 32608, USA
* Corresponding author: Siddharth Shah, siddharth.dr99@gmail.com

ABSTRACT: Subarachnoid hemorrhage (SAH) remains a potentially devastating cerebrovascular disease with a high morbidity and mortality rate, irrespective of treatment. The disease still has a 40-50% mortality rate with a 70% rate of cerebral vasospasm in those patients. The release of cytokines has been implicated in the development and progression of SAH. In this paper, we will explore the role of cytokines in aneurysmal subarachnoid hemorrhage (aSAH), including their effects on the inflammatory response, cerebral vasospasm, blood-brain barrier disruption, and neuronal damage. We also identify the role of the glymphatic system in progression of aSAH. The review will also briefly touch upon current research on potential therapeutic targets aimed at modulating cytokine activity in patients with aSAH. This review aims to give an in-depth review of the cytokines involved in aSAH and serve as a catalyst to research directed towards treatment options for aSAH.

KEYWORDS: subarachnoid hemorrhage; cytokines; inflammation; TNF-α; interleukin; secondary brain injury

1. Introduction

Cytokines are peptides involved in endocrine, autocrine, and paracrine signaling as immunomodulating agents. They are mediators of immune and inflammatory response. The rupture of an intracranial aneurysm leads to the deposition of blood resulting in physical disturbances and secondary complex inflammatory pathways. In the population that survives the event, the leading cause of death and disability is subsequent vasospasm and delayed ischemia. Inflammation post-SAH is believed to be the driver of most secondary insults in this often critically ill population[1]. Peripheral immune cells are recruited and activated in the damaged parenchyma. This leads to the elevation of inflammatory transcription pathways and increased expression of cytokines and chemokines.

Numerous studies correlate serum inflammatory cytokine concentration to cerebral vasospasm and delayed ischemia after aSAH[1,2]. Furthermore, an analysis of CSF cytokine concentration has demonstrated an association between Interleukin 6 (IL-6) and TNF-α with the risk of cerebral vasospasm and delayed cerebral neurological deficits[3,4]. These cytokines also lead to dysfunction in the blood-brain barrier function and glymphatic system, causing further damage[5]. Understanding the trajectory and time course of various cytokines involved in inflammation after aSAH is essential to manage unregulated inflammation and improving patient outcomes.

1.1. Subarachnoid hemorrhage and inflammatory cytokines

Until recently, cerebral vasospasm (CV) was believed to be the main contributor to poor outcomes. However, the latest research has shown that treating vasospasm has not led to improved outcomes.
advocating a need for a deeper understanding of the pathophysiology and underlying mechanisms to improve pharmacological interventions⁸.

Brain injury following aSAH is multimodal, with delayed cerebral ischemia (DCI) being an important factor contributing to poor clinical outcomes. The incidence of DCI is around 30% among survivors and can be a consequence of angiographic cerebral vasospasm (CV), which occurs in about 70% of patients during the first two weeks after aSAH, as well as other mechanisms such as altered autoregulation, cortical spreading depression (CSD) and microthrombosis⁷. Here, systemic and local inflammation is critical in mediating all these complications.

Blood in the subarachnoid space leads to an increase in the global intracranial pressure (ICP), leading to the release of certain molecules from damaged brain tissue. Molecules from damaged central and peripheral nerve tissue, such as damage-associated molecular pattern molecules (DAMPs) and extravasated blood, seem to be the early initiators of the inflammatory cascade; they trigger the expression of adhesion molecules, infiltration of leukocytes (particularly macrophages) and activation of resident microglia. The activated microglia and infiltrating macrophages subsequently release pro-inflammatory cytokines. Cytokines that have been implicated in neuroinflammation include IL-1β, TNF, IL-6, IL-12, IL-17, IL-23. Increased levels of IL-2, IL-6, IL-10, and TNF-α are associated with poor prognosis⁴,⁵,⁸. Increased blood–brain barrier (BBB) permeability further facilitates the passage of circulating leukocytes and cytokines to the brain interstitium or parenchyma, contributing to global cerebral edema⁹.

In the acute event following aSAH, macrophages and neutrophils enter the subarachnoid space to phagocytize the extravasated red blood cells in an effort to promote recovery. But changes in the CSF flow and restoration of the endothelial tight junction of the BBB may cause trapping of macrophages and neutrophils in the subarachnoid space in the subacute or chronic phase following aSAH. These leukocytes release pro-inflammatory mediators such as endothelins and oxidative radicals, leading to vasoconstriction, meningitis, and cerebritis¹⁰. Interleukins IL-1β, IL-6, and tumor necrosis factor-alpha (TNFα) are well-known pro-inflammatory mediators contributing to the pathophysiology of aSAH. They are produced in high amounts by the microglia and released into both serum and CSF, causing destabilization of the BBB and further downstream activation of pro-inflammatory proteins. In addition, TNFα and IL-1β are known to promote the activation of NF-κB, which in turn expresses pro-inflammatory mediators such as cyclooxygenase-2 (COX-2), prostaglandin E2 (PGE-2) and molecules that facilitate macrophage recruitment and adhesion¹¹,¹². Although the role that IL-1β, IL-6, and TNFα play in aSAH warrants further investigation, it is well known that they bring about the clinical signs of aSAH, such as neutrophilia, pyrexia, and general cerebral edema¹³.

The high mobility group box 1 (HMGB1) protein is a cytokine that is found to mediate neuroinflammation in early brain injury (EBI) after aSAH. In aSAH, overexpression of HMGB1 has been found in the extracellular milieu¹⁴. Once in the extracellular space, HMGB1 functions as a DAMP protein, initiating the production of several inflammatory mediators, including TNFα, IL-6, and IL-1β via TLRs/NF-κB signaling cascades. In addition, HMGB1 also contributes to the rupture of the BBB. It functions to interact with TLR4 and the receptor for advanced glycation end products (RAGE), facilitating cell migration and further production of pro-inflammatory cytokines¹⁴. **Figure 1** represents the development of DCI secondary to aSAH.
1.2. Role of cytokines in cerebral vasospasm

Cerebral Vasospasm (CV) is a common complication of aSAH. CV refers to a transient narrowing of the intracranial arteries several days after an aSAH, leading to ischemia and causing significant brain damage. Inflammation and oxidative stress play a vital role in the pathophysiology of cerebral vasospasm. In aSAH, there is extravasation of blood which is responsible for a cascade of reactions involving the release of various vasoactive and pro-inflammatory factors from blood and vascular components in the subarachnoid space leading to local vasospasm. In a clinical trial measuring the inflammatory mediator levels in plasma and cerebrospinal fluid (CSF) in the days following an aneurysmal Subarachnoid hemorrhage (aSAH), they found that the levels of IL-1β, IL-4, IL-6, IL-8, IL-10, IL-15, IL-17, IL-18, macrophage chemotactic protein (MCP)-1, vascular endothelial growth factor (VEGF) and tumor necrosis factor (TNF)-α were significantly higher in aSAH compared to controls in the first seven days. Out of these cytokines, IL-1β, IL-6, IL-8, and TNF-α are associated with the development of hemodynamic abnormalities in the basal cerebral arteries and determine the clinical outcome.

IL-1β is responsible for significantly increasing the leukocyte rolling and adhesion with the upregulation of inflammation. In a study focusing on the role of IL-1β in aSAH, they found that in 50% of animals, IL-1β activity was inhibited by intracerebroventricular administration of anti-rat IL-1β antibodies. Furthermore, they discovered that neutralizing IL-1β activity significantly reduced the vasospasm and blood vessel density only 24 h after aSAH. The study proved the role of IL-1β in the development of vascular pathologies after aSAH.

IL-6 levels reach very high concentrations in CSF after aSAH and play a role in inducing vasospasm. In a study on the effects of IL-6 in vasospasm after aSAH, they found it produces long-
lasting vasoconstriction in the canine cerebral artery, which may be partly related to the activation of the prostaglandin cascade\textsuperscript{[22]}. They used IL-6 to induce vasospasm by intracisternal injection of IL-6 and found elevated levels of prostaglandins E\textsubscript{2} and I\textsubscript{2} in CSF for the first 4.5 h\textsuperscript{[22]}. Multiple studies have linked the presence of IL-6 in the CSF to the development of cerebral vasospasm\textsuperscript{[22–24]}.

IL-8 is another cytokine linked to CV in aSAH. IL-8 is a pro-inflammatory cytokine and is mainly responsible for recruiting neutrophils to the site of inflammation. A study analyzing the expression change of the IL-8 gene in the basilar artery in rabbits showed that the expression of the IL-8 gene increased on days 4–7 suggesting its role in cerebral vasospasm as an immunological inflammatory factor\textsuperscript{[25]}. Griessenauer and colleagues studied soluble Fms-like tyrosine kinase 1 (sFlt-1) and soluble transforming growth factor β coreceptor, soluble endoglin (sEng) as important markers of CV pathophysiology. They found that sFlt-1 was increased in patients with aSAH who were at risk of severe CV\textsuperscript{[26]}.

Sung Ho Ahn and colleagues studied cytokines in serum after aSAH and concluded that persistent elevations in Eotaxin is associated with persistent cerebral edema post aSAH\textsuperscript{[27]}. Additionally, Some groups have attempted to profile inflammatory cells as biomarkers to assess prognosis in aSAH\textsuperscript{[28]}. Overall a better understanding of the mechanisms of cerebral vasospasm is critical for developing targeted treatment strategies and improved outcomes for aSAH patients. Figure 2 represents the role of cytokines in CV after SAH.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{role_of_cytokines.png}
\caption{Role of cytokines in CV after SAH.}
\end{figure}

1.3. BBB and cytokines in SAH

The luminal and subluminal aspects of the neurovascular unit (NVU) consist of microvascular endothelial cells that line the cerebrovascular capillary network in conjunction with non-vascular cells (neurons, microglia, astrocytes, pericytes) located on the basolateral side of the capillary endothelium respectively. The NVU carefully regulates the bi-directional traffic of fluids and solutes between circulating blood and the neural microenvironment, which is the primary function of the blood–brain barrier (BBB), thereby facilitating homeostasis of the central nervous system (CNS)\textsuperscript{[29]}. Within the
capillary wall, BBB integrity partly derives from the coordinated assembly of inter-endothelial adherens by promoting cell–cell adhesion and tight junction complexes regulating molecular traffic through the paracellular space\textsuperscript{30}. Both junctional complexes are intracellularly connected to the actin cytoskeleton and mutually cooperate to modify endothelial barrier function in response to circulatory insults such as biomechanical shear stress\textsuperscript{31}. Dysregulation of the BBB leading to elevated permeability has been attributed to various neurological disorders, including aSAH\textsuperscript{32–34}.

SAH leads to a neuroinflammatory response as a result of initial bleeding that leads to BBB failure in these circumstances. Furthermore, this BBB disruption leads to elevated levels of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), which originate either from local sources within the NVU (including the endothelium), from peripheral circulating cells or other systemic injury sites. Unfortunately, a comprehensive understanding of precisely how cytokines directly interact with and damage the microvascular endothelium of the BBB is still relatively unknown. Moreover, current models arguably exhibit a disproportionate focus on the contribution of TNF-α to BBB dysregulation, with very few studies focusing on other equally relevant cytokines such as interleukin (IL)-1, IL-6 and interferon-γ (IFN-γ)\textsuperscript{35}.

TNF-α is a pro-inflammatory adipokine associated with innate immunity and acute phase reactions. It exhibits many pleiotropic homoeostatic roles, including a neuroprotective role within the brain\textsuperscript{36}. However, its excess production, whether acute or chronic, has long been associated with systemic disease pathogenesis and brain dysfunction. In vitro studies have demonstrated the permeabilizing ability of TNF-α in brain microvascular endothelial cells of bovine and mouse origin, non-transformed and immortalized human brain microvascular endothelial cell (HBMvEC) models, with further confirmation in a variety of in vivo mouse models\textsuperscript{37,38}. IL-6 is a cytokine secreted by T-cells and macrophages, and non-immune cell types to elicit pro-inflammatory responses. Its role is well established in vascular inflammation, actively modulating vascular cell adaptive and pathological responses to stress\textsuperscript{23,39}. However, it has received surprisingly little attention regarding BBB regulation compared to TNF-α.

Though not profoundly studied, possible signaling mechanisms underlying the reactive oxygen species (ROS)-dependent/-independent pathways that lead to BBB destabilization by pro-inflammatory cytokines may include activation of mitogen-activated protein (MAP) kinase cascades and transcription factors such as nuclear factor kappa B (NF-κB). This leads to reduced gene expression, enhancement of junctional protein turnover via the ubiquitin-proteasome system, degradation of paracellular junctions by up-regulated matrix metalloproteinases, and even dysregulation of actin cytoskeletal signaling\textsuperscript{40–42}. Figure 3 represents the pathway mechanism of SAH leading to BBB dysfunction.
1.4. The glymphatic system and aSAH

The glymphatic system (GS) comprises perivascular channels formed by astrocytes that mediate the clearance of metabolic waste from the CNS. The GS plays a pivotal role in stroke pathophysiology, cerebral edema, BBB dysfunction, immune cell inflammation, neuronal apoptosis, and neuroinflammation.

Following aSAH, blood components, particularly fibrin and fibrinogen, accumulate in the perivascular space (PVS), blocking the GS flow and ultimately worsening cerebral edema and ischemia\[43\]. A study in 2016 demonstrated that subarachnoid blood invaded the PVS within 5 min of aSAH and gradually penetrated the parenchyma over the following hours\[44\]. The study further showed that GS dysfunction following aSAH resulted in vasculitis, widespread microinfarction, and neuroinflammation\[44\]. When microthrombi form, the microcirculation has no perfusion and no outflow, paralyzing the clearance function of GS and leading to neuronal cell death. Hence, aSAH leads to GS dysfunction.

Along with blood components, peripheral immune cells enter the PVS and drive neuronal inflammation. Elevated pro-inflammatory monocytes and T-cells are associated with high levels of pro-inflammatory cytokines. In the context of aSAH, GS dysfunction aggravates damage by suppressing cytokine clearance from the brain\[44,45\].

Interestingly, CV following aSAH has been linked to GS dysfunction. Using recombinant tissue plasminogen activator (rt-PA) in patients with aSAH has been shown to clear coagulation in PVS and reduce inflammation. rt-PA used intrathecally in aSAH patients has been shown to reduce the incidence of CV and improve neurological outcomes\[46\].

Under physiological conditions, the clearance of metabolites in the GS is regulated by Aquaporin-4 (APQ4), which is highly expressed in the astrocyte processes. In aSAH, APQ4 expression is up-regulated, and its polarization is disrupted\[47\]. APQ4 mislocation away from vascular endfeet during an inflammatory process polarizes the glymphatic flow\[45\]. After aSAH, APQ4 knockout mice showed a significant decrease in CSF flow through cerebral parenchyma and interstitium. Furthermore, those mice had persistent neuroinflammation compared to the wild-type control mice after seven days\[48\]. This shows the GS undertakes a vital function of scavenging harmful metabolites and proteins post aSAH and plays...
a role in rehabilitating neurological function. **Figure 4** represents the mechanism of Glymphatic system dysfunction in aSAH.

**Figure 4.** Glymphatic system dysfunction and aSAH.

### 1.5. Cytokine targeted therapy for aSAH

The immediate treatment of aSAH is aimed at lowering blood pressure using drugs such as nicardipine, labetalol and sodium nitroprusside with the goal of maintaining the blood pressure at <160 mmHg. With advancement in both microvascular and endovascular surgical techniques patients are selected using regularly refined algorithms. Nimodipine, a calcium channel blocker, was confirmed as the only drug associated with improved neurological outcomes but it failed to consistently show its efficacy for vasospasm. Secondary injury as a consequence of inflammation and cytokines plays a major role in death and disability despite the current treatment strategies. Various preclinical and clinical studies have shown the utility of targeting cytokines in the treatment of aSAH-induced inflammation.

A recent study described the utility Eupatilin, a Chinese herbal medicine, in markedly reducing the levels of IL-1β, IL-6, and TNF-α, and suppressing the expression levels of MyD88, TLR4, and p-NF-κB p65 in SAH induced rats. It showed efficacy in ameliorating early brain injury following SAH. The IL receptor antagonist (IL-1Ra) is a naturally occurring selective antagonist of IL-1, a key inflammatory cytokine. Studies have shown the utility of using IL-1Ra as a promising candidate in the treatment of cerebral ischemia.

A recent study described increased extracellular glutamate levels can activate astrocytes and promote pro-inflammatory factor production particularly, TNF-α, IL-1β, complement component 3 (C3) showing, antagonization of glutamate is a feasible treatment option. A study in rats demonstrated significantly reduced brain edema and rescued microcirculation impairment with concomitant anti-inflammatory benefits after SAH with the use of dental pulp stem cell conditioned medium (DPSC-CM). Minocycline showed efficacy in attenuating the upregulation of TNF-α and IL-1 in addition to decreased neuronal cell death, cerebral vasospasm and improved outcomes in rats. Yong Giang and colleagues found TNFα antibody had a neuroprotective effect on apoptosis following aSAH in a rat model. Another study showed that mitogen-activated protein kinase (MAPK) pathway inhibition reduced inflammation in a rodent model of aSAH. Tamoxifen, a drug used in breast cancer, also showed efficacy in a rat aSAH model with decreased inflammation and no evidence of early brain damage such as cortical edema and BBB disruption. There was also a complete reversal of their aSAH-induced spatial working memory dysfunction compared to vehicle-treated controls. A study showed that Fingolimod, an immunomodulator, can down-regulate IL-6 and TNF-α and up-regulate IL-10 and TGF-β1 in serum in a rodent model of SAH. Haoliang Xu and colleagues targeted peripheral immune cell adhesion and inflammation using the drug LJP-1586 which improved cognitive and functional...
performance after aSAH in rodent models\(^{60}\). The team also showed improved outcomes with fingolimod\(^{61}\). Melatonin has also shown efficacy in attenuating early brain injury following SAH by regulating pro-inflammatory cytokines\(^{62}\).

Potential targets for the treatment of cerebral vasospasm include neutrophils, macrophages, and myeloid lineage cells. In addition, preclinical therapeutics that block E-selectin\(^{63}\) or antibodies against CD8/CD11\(^{64}\) cause a reduction in the severity of CV. Antibodies against LY6G/C, a surface marker on CD8 cells, have shown efficacy in reducing CV and improving behavioral tests\(^{65}\). The antioxidant rosiglitazone also effectively reduced inflammation-mediated CV in animal models\(^{66,67}\).

Tosun and colleagues studied glibenclamide (glyburide), an inhibitor of the Sur1-Trpm4 receptor, attenuates neuroinflammation\(^{68}\). It has showed reduced inflammation and improved behavior deficits in rodent models\(^{68,69}\). A clinical trial is evaluating glibenclamide as a treatment for acute aSAH (NCT05137678).

Human studies have shown the efficacy of targeted cytokine therapies, as depicted in Table 1. James Galea and colleagues performed a randomized trial using 100 mg subcutaneous IL-1Ra (Anakinra). They found decreased peripheral inflammation and improved Glasgow come scale in the active group, but this finding was not statistically significant\(^{70}\). Puerarin, an anti-inflammatory agent, showed efficacy in the prophylaxis and treatment of CV in patients after aSAH\(^{40}\).

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Control group (n)</th>
<th>Active group (n)</th>
<th>Intervention</th>
<th>Duration</th>
<th>Outcome</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galea J(^{[52]})</td>
<td>68</td>
<td>68</td>
<td>100 mg sc IL-1Ra</td>
<td>6 months</td>
<td>Reduced IL6 and CRP</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Wang JW(^{[40]})</td>
<td>24</td>
<td>30</td>
<td>Puerarin</td>
<td>N/A</td>
<td>Decreased incidence of CV</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Simon M(^{[70]})</td>
<td>6</td>
<td>7</td>
<td>Anakinra</td>
<td>-</td>
<td>Decreased IL6 in CSF and plasma</td>
<td>&lt;0.08</td>
</tr>
</tbody>
</table>

### 2. Conclusion

To conclude, aneurysmal subarachnoid hemorrhage is a serious medical condition that can lead to neuronal damage and significant morbidity and mortality. The release of cytokines in response to the presence of blood in the subarachnoid space plays a crucial role in initiating and propagating neuroinflammation. Elevated levels of cytokines have been observed in patients with aSAH and are associated with poor outcomes. Further research is needed to better understand the role of cytokines in the pathophysiology of aSAH and to develop targeted therapies to modulate their expression and activity. The study of cytokines in neuroinflammation holds promise for improving treatment strategies and outcomes of patients with aSAH.

### Conflict of interest

The authors declare no conflict of interest.

### References


