Title: Delayed Cerebral Ischemia after Subarachnoid Hemorrhage: Beyond Vasospasm and Towards a Multifactorial Pathophysiology

Authors: Joseph R. Geraghty, B.S.[1,*] and Fernando D. Testai, M.D., Ph.D., FAHA[1]

Affiliations: [1]Department of Neurology and Rehabilitation, University of Illinois at Chicago College of Medicine, Chicago, IL

***Corresponding Author:**
Joseph R. Geraghty, B.S.
Department of Neurology & Rehabilitation
College of Medicine
University of Illinois at Chicago
912 S. Wood St. Suite 174N
Chicago, IL 60612
Tel: 312-996-1757
Fax: 312-996-4169
Email: Jgerag2@uic.edu

Abstract
Purpose of Review: Delayed cerebral ischemia (DCI) is common after subarachnoid hemorrhage (SAH) and represents a significant cause of poor functional outcome. DCI was mainly thought to be caused by cerebral vasospasm; however, recent clinical trials have been unable to confirm this hypothesis. Studies in humans and animal models have since supported the notion of a multifactorial pathophysiology of DCI. This review summarizes some of the main mechanisms under investigation including cerebral vascular dysregulation, microthrombosis, cortical spreading depolarizations, and neuroinflammation.
Recent Findings: Recent guidelines have differentiated between DCI and angiographic vasospasm and have highlighted roles of the microvasculature, coagulation and fibrinolytic systems, cortical spreading depressions, and the contribution of the immune system to DCI. Many therapeutic interventions are underway in both preclinical and clinical studies to target these novel mechanisms as well as studies connecting these mechanisms to one another.
Summary: Clinical trials to date have been largely unsuccessful at preventing or treating DCI after SAH. The only successful pharmacologic intervention is the calcium channel antagonist, nimodipine. Recent studies have provided evidence that cerebral vasospasm is not the sole contributor to DCI and that additional mechanisms may play equal if not more important roles.

Keywords: Subarachnoid hemorrhage, delayed cerebral ischemia, vasospasm, microthrombosis, cortical spreading depolarization, neuroinflammation

Introduction

Subarachnoid hemorrhage (SAH) is a neurological emergency with considerable morbidity and mortality. It accounts for up to 5% of strokes each year, with about 7.2-10.5 per 100,000 people each year [1,2]. The most common non-traumatic cause of SAH is the spontaneous rupture of cerebral aneurysms. Despite it being less common than ischemic stroke, SAH has a higher mortality rate, with older studies demonstrating case-fatality rates between 32-67% and more recent studies demonstrating stable incidence of SAH but some reductions in case-fatality due to improved diagnosis and management [3,4]. Overall, the mortality is still estimated to be approximately 40% [5]. For those who survive, there are both short- and long-term consequences that can significantly reduce quality of life. With advances in both the detection and management of patients suffering from SAH, there is a growing number of SAH survivors who require lifelong care, cannot return to work, and suffer from both physical and cognitive long-term impairments [6-9]. As SAH typically affects younger patient populations compared to ischemic stroke, these long-term impairments can have larger personal and economic impacts on individual patients, their families, and society.

Brain injury after SAH can be divided into early and delayed stages (Figure 1). One of the earliest consequences of SAH is early brain injury (EBI), which typically occurs within the first 72 hours [10,11]. As blood rushes into the subarachnoid space under high arterial pressure there is a rise in intracranial pressure (ICP). Blood and its breakdown products can also further contribute to this rise in ICP from obstruction of flow of cerebrospinal fluid (CSF), resulting in hydrocephalus. The sharp rise in ICP results in a significant reduction in both cerebral perfusion pressure (CPP) and cerebral blood flow (CBF) that can lead to both loss of consciousness and global cerebral ischemia. Additionally, there are many other pathophysiological responses important to consider at this stage, including neuroinflammation, activation of platelets and clotting factors, endothelial injury, and excitotoxic effects of blood and its breakdown products on neurons [11].

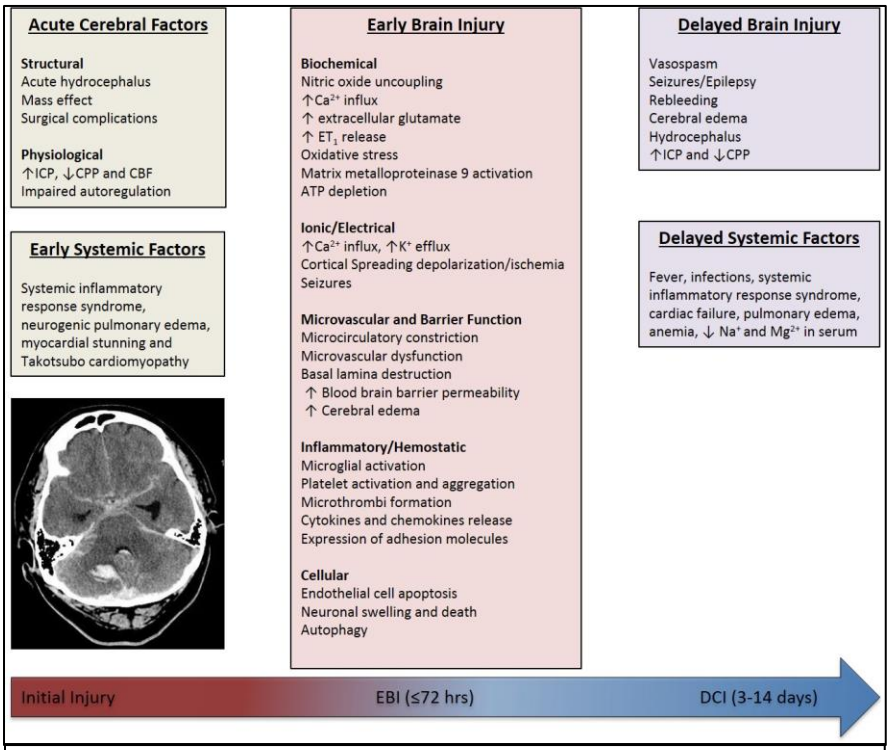

Figure 1. Factors contributing to brain injury in SAH. Following aneurysm rupture, injury can be divided into early and delayed stages, which include both systemic and cerebral factors. Insert: Head CT scan of a 40-year-old woman with subarachnoid hemorrhage showing right cerebellar hemorrhage with local mass effect, intraventricular extension, and early hydrocephalus represented by the distention noted at the temporal horns of the lateral ventricles.

All of the events that take place in the acute setting after SAH likely set the stage for the development of further delayed brain injury. One of the most commonly studied mechanisms of delayed injury involves delayed cerebral ischemia (DCI), a major cause of morbidity and mortality for those who survive EBI. DCI presents with delayed neurological deterioration and focal deficits that may progress to infarction [1]. Until recently, DCI has been associated with the development of cerebral vasospasm after SAH, and it was thought that DCI was a direct result of this arterial narrowing [12]. However, recent studies have begun to differentiate these two events and show that DCI may be a result of several underlying and inter-related mechanisms [13,14]. In this review, we will discuss four principal mechanisms that may contribute to the development of DCI (Figure 2): 1) cerebral vascular dysfunction (both macro- and micro-vascular), 2) microthrombosis, 3) cortical spreading depolarizations, and 4) neuroinflammation. While each of these have been studied in animal models, controlled clinical trials targeting these mechanisms have been limited.

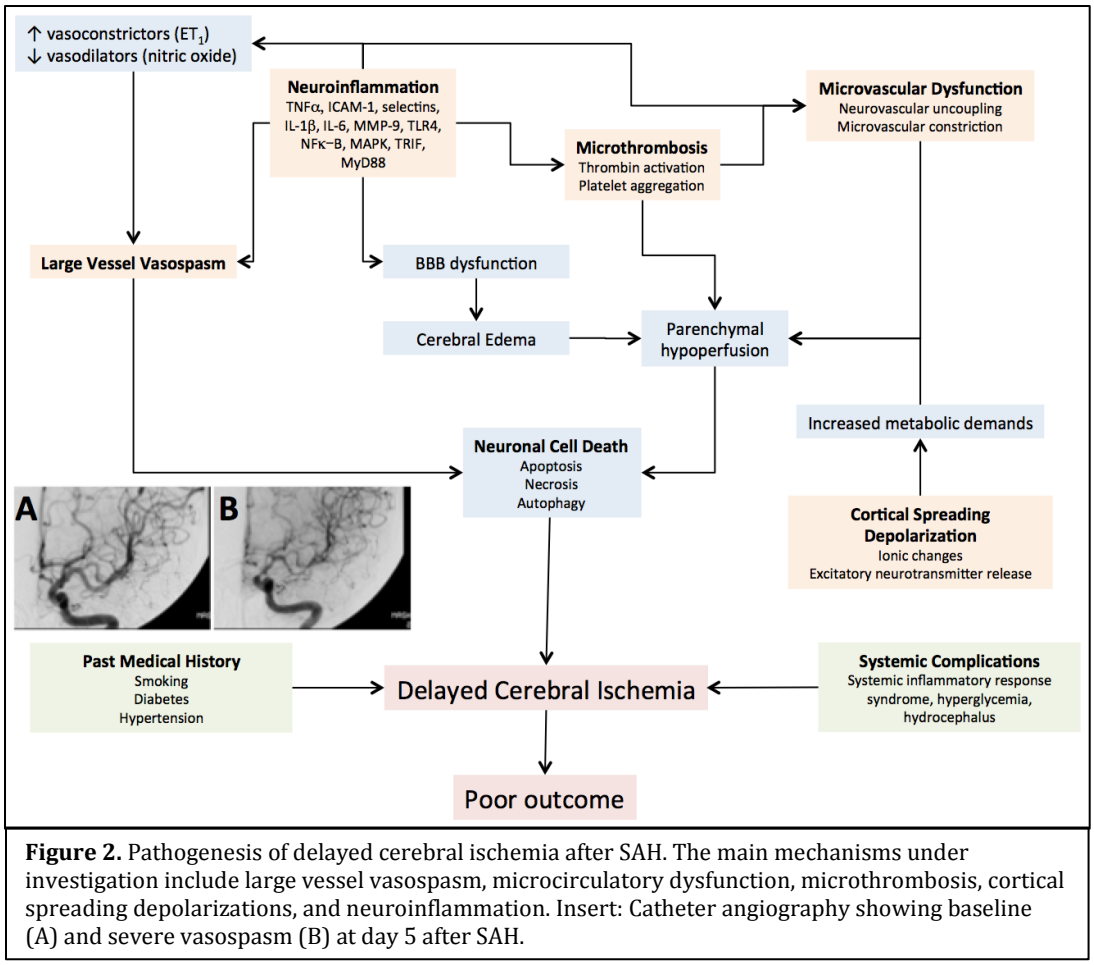

Figure 2. Pathogenesis of delayed cerebral ischemia after SAH. The main mechanisms under investigation include large vessel vasospasm, microcirculatory dysfunction, microthrombosis, cortical spreading depolarizations, and neuroinflammation. Insert: Catheter angiography showing baseline (A) and severe vasospasm (B) at day 5 after SAH.

Current Diagnosis and Management of DCI

There are limited therapeutic options available to address long-term impairment following SAH. DCI plays a highly significant role in long-term outcomes and develops in approximately 30% of SAH patients and usually occurs between 3 and 14 days following aneurysmal rupture [15,16]. Some factors that increase the likelihood of developing DCI include smoking, alcohol use, hyperglycemia, hydrocephalus, early systemic inflammatory response, and poor clinical condition upon initial presentation [17]. Some of these factors are considered markers of severity of the initial insult. However, it has been hypothesized that they correlate with the development of a pro-inflammatory environment that facilitates the occurrence of delayed complications such as DCI.

Following occlusion of the ruptured intracranial aneurysm either by surgical clipping or endovascular coiling, there are several mainstays of treatment designed to detect and prevent the development of DCI. Diagnostic approaches including clinical assessments (i.e., Glasgow Coma Scale), Transcranial Doppler ultrasonography (TCD), and angiography can be used to detect vasospasm which is frequently seen in association with DCI [18]. Additionally, CT and MR scans, together with non-invasive perfusion imaging, can be utilized to identify cerebral ischemia, hypoperfusion, infarction, edema, and herniation. More recently, continuous electroencephalography (EEG) has been shown to provide valuable information in monitoring for DCI [19,20]; however, its use in clinical practice has not been universally accepted.

Prevention of DCI has mainly been directed at cerebral vasospasm and includes both peri-operative and post-operative approaches. During surgical clipping or endovascular coiling, preventive measures can include clot removal, intracisternal agents such as thombolytics or prolonged-release vasodilatory agents [21]. Since outcomes investigated were mainly related to vasospasm, direct effects on DCI remain unclear although some did show improvements in symptomatic vasospasm. The main post-operative prophylactic measures include hypovolemia, lumbar drains, and the calcium channel antagonist nimodipine [22]. Nimodipine is the only drug shown to reduce DCI and improve outcomes after SAH, and several studies have attempted to optimize the

route of administration and dosage while reducing side effects [23-25]. The beneficial effect of nimodipine in SAH is unclear and has been associated to a cytoprotective effect. This Food and Drug Administration (FDA) approved drug is administered orally and its main adverse effect is hypotension, which can precipitate cerebral infarction in patients with vasospasm and hemodynamic compromise. Other pharmacologic interventions have been studied with largely disappointing results [25-35].

Once DCI has developed, treatment options are largely supportive and rely on ensuring cerebral perfusion that may be compromised in patients with vasospasm. “Triple-H” therapy including hypertension, hypervolemia, and hemodilution has been used as the mainstay of treating these patients for decades [21]. The goal of this approach is to preserve CPP in patients with vasospasm. The beneficial effect of each of the individual components of “Triple-H” therapy has not been established; in addition, hypervolemia has been associated with increased costs and complication rates [36-38]. Thus, current SAH treatment guidelines recommend the use of induced hypertension and maintenance of euvolemia, although the efficacy of this approach also has not been conclusively demonstrated [18,39,40]. In refractory cases, endovascular angioplasty as well as intra-arterial treatment with vasodilators including verapamil, nimodipine, nicardipine, and milrinone, may revert vasospasm [41-43]. Thus, while there are some options for managing DCI, there exists conflicting evidence in terms of efficacy.

Mechanisms Underlying DCI

The lack of proven interventions to prevent or treat DCI after SAH has driven investigation into novel mechanisms of DCI. While historically cerebral vasospasm has been viewed as the principal underlying mechanism of DCI, it is likely a combination of several mechanisms that results in DCI and poor outcome [11]. Numerous studies have looked at these mechanisms, but one of the problems in the current literature has been the lack of clear definitions that distinguish DCI, cerebral vasospasm, and cerebral infarction. In 2010, an international panel of experts assembled to propose definitions for these terms (Table 1) [44]. Future studies looking at either vasospasm or DCI following SAH should abide by these definitions, which will allow for easier cross-study analysis and guide development of future therapeutic interventions.

Table 1. Consensus Definitions of Delayed Cerebral Ischemia, Cerebral Vasospasm, and Cerebral Infarction Set by a Multidisciplinary Research Group Consisting of Experts on SAH and DCI [1].

Term	Definition	Similar Terms
Delayed Cerebral Ischemia (DCI)	Occurrence of focal neurological impairments or decrease of at least 2 points on Glasgow Coma Scale that last for at least 1 hour, is not apparent immediately after aneurysm occlusion, and cannot be attributed to other causes by means of clinical assessment, CT, MRI, and other lab studies.	Delayed ischemic neurological deficit (DIND), delayed ischemic deficit
Cerebral Vasospasm	Arterial narrowing of large cerebral vessels observed on a radiological test such as CT angiography, MRA, or digital subtraction angiography.	Angiographic vasospasm, radiographic vasospasm, arterial narrowing
Cerebral Infarction	Death of brain tissue due to ischemia present on CT or MRI within 6 weeks of SAH, or on latest scan made before death within 6 weeks, or proven at autopsy, not present on scans between 24-48 hours after aneurysm occlusion, and not attributable to other causes such as surgical or endovascular treatment.	N/A

1) Cerebral Vascular Dysregulation

For decades, the main focus of research into DCI was dysregulation of the cerebral vasculature, mainly at the level of larger extraparenchymal vessels. Mechanistically, this phenomenon has been associated with the presence of vasoactive blood products in the subarachnoid space, imbalance in the production of endogenous vasodilators (nitric oxide, NO) and vasoconstrictors (endothelin-1), and inflammation. Cerebral vasospasm has been the target of extensive study, yet only recently has its role in DCI come into question. New studies have begun to look at alterations in smaller intraparenchymal vessels that have an important role in regulation of CBF, neurovascular coupling, and maintenance of the blood-brain barrier.

1.1) Cerebral Vasospasm

As shown in Table 1, cerebral vasospasm is defined as "arterial narrowing of large cerebral vessels observed on a radiological test such as CT angiography, MRA, or digital subtraction angiography" [44]. The more precise term we will use here is angiographic vasospasm in order to differentiate from symptomatic vasospasm, which includes angiographic vasospasm associated with signs and symptoms of parenchymal injury. Angiographic vasospasm has been shown to develop in up to 70% of patients after SAH [45]. Meanwhile, DCI is only observed in 30% of patients and does not always fall within the vascular distribution of the angiographic vasospasm [46,47]. Thus, DCI can occur without the presence of angiographic vasospasm and likely is driven by other factors. Further, the only FDA approved drug to prevent DCI after SAH, nimodipine, has had no observed effect on angiographic vasospasm [48,49].

Despite this information, many recent clinical trials have targeted angiographic vasospasm in hopes of preventing DCI. After studies in animal models identified a role of endothelin receptors on vasoconstriction of large extraparenchymal vessels, landmark randomized clinical trials using the endothelin-receptor antagonist clazosentan entitled "Clazosentan to overcome neurological ischaemia and infarct occurring after subarachnoid hemorrhage," or CONSCIOUS [13]. The randomized CONSCIOUS-1 trial demonstrated a significant reduction in angiographic vasospasm. This finding was confirmed in the higher powered, phase III, randomized, double-blind, placebo-controlled CONSCIOUS-2 trial. However, this study failed to show a statistically significant reduction in morbidity, mortality, or functional outcome [13,49,50]. While there were some limitations to these studies, they suggest that angiographic vasospasm is not the sole contributor to DCI and long-term outcomes after SAH.

1.2) Microcirculatory Dysfunction

In addition to angiographic vasospasm that occurs at the level of larger extraparenchymal vessels, the smaller microvasculature of the brain parenchyma can also show alterations. Compared to angiographic vasospasm, microcirculatory dysfunction cannot be as easily detected clinically by angiography or TCD [51]. Most of these studies have been in animal models and few therapeutic interventions have been designed to target the microvasculature. Within the brain parenchyma, the main alterations that take place occur at the levels of the arterioles and capillaries and can include disruption of cerebral autoregulation, neurovascular coupling, and blood brain barrier (BBB) function [52-54]. Further, while nimodipine was shown to have no significant effect on large vessel vasospasm, it does inhibit arteriolar vasoconstriction [55]. This may relate to its therapeutic effect on DCI.

Maintenance of CBF within the brain parenchyma is dependent on both cerebral autoregulation and neurovascular coupling, both of which can be disturbed following SAH. Cerebral autoregulation is responsible for maintaining constant CBF despite changes in arterial blood pressures while neurovascular coupling allows local changes in CBF to occur in response to varying degrees of neuronal activity [56]. After SAH, fluctuations in arterial pressures have been observed to result in more dynamic changes in both CBF and CPP, indicating impairment of cerebral autoregulation [55]. Disturbance of neurovascular coupling is also observed and relates to impairment at the level of the neurovascular unit (NVU), made up of endothelia, pericytes, smooth muscle cells, neurons, and glia. Under normal physiological circumstances, the NVU should cause microvessel dilation in response to higher neuronal activity. Neurons release glutamate, which binds to metabotropic glutamate receptors (mGluRs) on astrocytes, triggering an increase in intracellular calcium that in turn releases vasodilatory substances at astrocytic end feet surrounding arterioles. However, several studies have shown that after SAH this neurovascular coupling is inverted, wherein instead of vasodilation in response to neuronal activity, there is either transient or sustained vasoconstriction [57-59]. This is not observed 3 hours after SAH, but occurs as a delayed response and can be progressive [59]. Following Poiseuille's law, which states that flow through a cylinder is proportional to the fourth power of the radius, this vasoconstriction can therefore have drastic effects on the delivery of essential oxygen and nutrients to brain parenchyma [55]. Thus, vasoconstriction due to inverted neurovascular coupling can result in ischemic damage that may manifest as DCI.

This arteriolar vasoconstriction or "microspasm" has been well documented in animal models although only recently have molecular details emerged. Activation of smooth muscle cells and pericytes can result in microvascular constriction and patchy areas of hypoperfusion, which then may shunt blood away from areas and cause hyperperfusion in other regions [11]. This constriction may be a result of numerous vasoactive substances released following SAH that travel along the vasculature and reach smaller vessels, including alterations in the NO pathway, oxidative stress, cellular-adhesion molecules, and inflammation [54]. Nitric oxide in particular is an

attractive candidate for future studies as decreased bioavailability has been observed within minutes after SAH that persist up to 24 hours [54]. These changes in NO can be the result of decreased synthesis by the endothelial NO synthase (eNOS) or binding of NO by oxidative species and hemoglobin [54].

At the level of the BBB, several alterations have been shown that promote cerebral edema and neuroinflammation. Changes in capillary morphology observed after SAH include luminal endothelial protrusion and swelling of astrocytic end feet that can compress the lumen of the vessel, constriction of pericytes, opening of tight junctions, and damage to basement membranes by matrix metalloproteinases (MMPs) [54,60]. Many of these changes can be long lasting. Taken together, these alterations further support the notion that ischemic injury in the brain after SAH, both during EBI and thereafter, is not solely dependent on larger vessels but may be heavily influenced by impairments in the microvasculature as well.

2) Microthrombosis

Another novel mechanism under investigation for its role in DCI is the development of microscopic thrombi after SAH. Increased microthrombi can have detrimental effects on neurological function by creating corresponding areas of microinfarction throughout the brain. Many studies have shown alterations in both the coagulation and fibrinolytic cascades following SAH [51,61]. Further, this may be closely related to arteriolar microspasm by creating a pro-coagulable environment [52]. Stasis and turbulence of blood flow are factors important in Virchow's triad resulting in thrombosis, and along with endothelial dysfunction can follow arteriolar vasoconstriction. Further, nimodipine has been shown to affect fibrinolytic activity following SAH, potentially promoting the breakdown of microclots within the brain [62]. This may underlie the therapeutic mechanism of the drug along with its ability to reduce arteriolar vasoconstriction.

The first case study to observe microthrombosis in patients after SAH was done in autopsy studies in 1983 [63], and since then additional experiments have been conducted in animal models and humans. Sabri *et. al.* showed that after SAH, mice had a higher burden of microthrombi-filled arterioles that correlated with arteriolar constriction, low NO, increased P-selectin, and neuronal apoptosis [64]. Microthrombi likely form *in situ* within microvessels following arteriolar vasoconstriction related to changes in NO, P-selectin, and shifts in other coagulation and fibrinolytic factors. The microthrombi observed are not merely emboli resulting from endothelial injury at the site of aneurysmal rupture as they are seen globally in both cerebral hemispheres [64,65]. Further, the peak burden of microthrombi in these animals was shown to be consistent with the corresponding timing of DCI in humans [65]. In humans, microthrombi formation has been observed in both EBI and DCI, and higher levels of platelet activation correlate with the development of DCI in SAH patients [66,67]. It is thus possible that the neurological deficits seen in DCI may be a result of microthrombosis, and indeed some studies have shown a relationship between microthrombi formation and long-term cognitive deficits commonly observed in SAH [68]. This relationship between microthrombosis and cognitive deficits warrants further study.

Alterations in both the coagulation and fibrinolysis cascades have been observed after SAH in both human and animal studies. Coagulation begins with the formation of a weak platelet plug. The coagulation cascade is a highly regulated series of reactions involved in hemostasis that results in the formation of thrombin, which cleaves soluble fibrinogen to form insoluble fibrin that seals the weak platelet plug. Alterations in molecules important in this cascade including platelet-activating factor, P-selectin, and overall platelet activity have been observed after SAH [61,67]. Further, alterations in von Willebrand Factor (vWF) and its cleaving factor ADAMTS13, known to be involved in other microthrombotic disorders such as thrombotic thrombocytopenic purpura (TTP), have been demonstrated after SAH [69]. These changes have resulted in efforts to target this system therapeutically; however, one of the limitations has been the lack of well-powered, randomized controlled trials [61]. For example, studies looking at aspirin and enoxaparin had negligible and contradictory results, respectively [61]. Some studies of the antithrombotic effects of statins have shown reductions in vasospasm but no reduction in the incidence of DCI or overall mortality while others have seen no change in the development of vasospasm [70,71]. In animal models, some of these therapies, including the administration of ADAMTS13, have shown benefit which is reduced after SAH [72,73].

At the level of the fibrinolytic cascade, conversion of plasminogen to active fibrin-cleaving plasmin is an important component in the breakdown of fibrin clots. Activators such as tissue plasminogen activator (tPA) and inhibitors such as plasminogen activator inhibitor-1 (PAI-1) are altered after SAH such that the balance is shifted toward reduced fibrinolysis [61]. Intracisternal administration of fibrinolytics such as urokinase or tPA show some efficacy at reducing clot burden, but there is conflicting evidence on its ability to reduce DCI and improve

functional outcome [74,75]. Larger randomized, double-blinded, placebo controlled trials will likely provide additional evidence as to the role of the coagulation and fibrinolytic cascades.

3) Cortical Spreading Depolarizations (CSD)

CSDs were initially discovered by Leão in 1944 and have since been implicated in several pathological conditions, most notably migraine [76,77]. In fact, many of the observations initially done in migraine seem to share common features with that of stroke, suggesting a common underlying pathophysiology related to dysfunction in the cerebral vasculature [78]. CSDs are related to alterations in the microvasculature including arteriolar vasoconstriction and inverse neurovascular coupling [79]. This provides a link between some of the multiple potential mechanisms underlying DCI after SAH.

CSD is defined as a slowly propagating wave of depolarization that spreads in all directions from a region of onset, is accompanied by a spreading depression of electrocorticographic (ECoG) activity, increased metabolic activity, and a significant disruption in ion homeostasis within and outside of cells [79,80]. This disruption can result in an osmotic imbalance that causes neuronal swelling, distortion of dendritic architecture, and release of large amounts of neurotransmitters. Shifts in the intracellular and extracellular ion concentration gradients lead to dysfunction between neighboring neuronal networks and an electrical silence within the brain until the period of spreading depression ceases [79]. Release of glutamate can additionally result in glutamate-induced neurotoxicity by binding to N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and kainate receptors, resulting in excessive stimulation and cell death.

In addition to neurotransmitter-induced excitotoxicity, there are changes in local CBF related to CSDs. Conditions that induce brief periods of CSD can raise local CBF resulting in a spreading hyperemia [79]. After the rise in CBF, there is a period of spreading oligemia with decreased perfusion and poor neurovascular coupling [81]. Under pathological conditions that result in CSDs with a prolonged period of depression, this spreading oligemia is worsened and can lead to sustained hypoperfusion with inverse neurovascular coupling [79]. Arteriolar vasoconstriction following CSDs may further exacerbate the injury via hypoperfusion and development of a spreading ischemia.

The first study to demonstrate the presence of CSDs after SAH occurred in 1980 in a cat model [82]. Over twenty years later, CSDs were demonstrated in human SAH patients by experiments done by Dreier *et. al.* in 2006 [83]. This prospective, multi-center study using ECoG not only observed CSDs in 72% (13 of 18) of patients, but showed that CSDs correlated strongly with the development of DCI and had high predictive values for DCI, especially those with progressively prolonged depression periods [83]. Since then, additional studies such as those of the influential Co-Operative Study on Brain Injury Depolarizations (COSBID) have shown CSDs in a majority of SAH patients, and that these can occur in the absence of angiographic vasospasm [84]. Some limitations to several of these studies include small patient sample sizes and lack of adequate control groups. It seems very likely that CSDs play a role in delayed injury after SAH, but whether they are a key mechanism or a byproduct of other alterations remains uncertain.

Experiments in animal models have given rise to several hypotheses regarding the mechanisms underlying CSDs after SAH. Following aneurysmal rupture, there are decreased levels of glucose, NO, and oxygen along with increased levels of potassium and hemoglobin in the subarachnoid space [80]. These alterations can result in depolarization of neurons, with release of high concentrations of neurotransmitters and further shifts in ion homeostasis. One interesting and largely unexplored area is the relationship between CSDs and epileptogenesis, both of which are seen after SAH and may share certain features. CSDs and ictal epileptic events have been viewed as distinct entities, but have similar toxic effects including increased metabolic demand, inverse neurovascular coupling, and BBB dysfunction [85]. Case studies have additionally shown that a higher frequency of CSDs is associated with higher risk of developing epilepsy after SAH [85]. Further studies in both patients with SAH and animal models of SAH are needed to determine whether these events are truly related to one another.

Given the high incidence of CSDs after SAH and their ability to predict the development of DCI, it is tempting to target CSDs pharmacologically in order to prevent DCI. Potential targets under investigation for reducing the frequency of CSDs include NMDA antagonists, GABA inhibition, anesthetics, the anticonvulsant topiramate, and calcitonin gene-related peptide (CGRP) antagonists [81,86-88]. Most of these therapies are undergoing preclinical testing in animal models and may show promise for application in SAH.

4) Neuroinflammation

Neuroinflammation within the CNS is an emerging concept in many neurological and psychiatric diseases. Historically, the CNS was viewed as "immune-privileged;" however, many studies over the last few decades have challenged that notion and have implicated the role of the immune system in both normal physiologic activity and during pathological conditions [89,90]. Following aneurysmal rupture in SAH, a robust inflammatory response ensues that some studies have correlated with both EBI and DCI [25,51,67,91]. Further, in a mouse model of SAH subjected to high-throughput screening, the most differentially expressed transcripts were genes involved in inflammation [92]. Many promising studies and potential pharmacologic interventions have emerged from animal models, and some have gone on to human clinical trials.

Clinical studies have focused on identifying markers in both the cerebrospinal fluid (CSF) and plasma in order to predict the occurrence of DCI [93-96]. These studies have looked at circulating inflammatory markers such as interleukin-6 (IL-6), C-reactive protein (CRP), interleukin-1 receptor (IL-1R) as well as hematologic factors such as leukocytosis and anemia. Most studies have shown a correlation between some of these factors, especially IL-6 and leukocytosis, and the occurrence of DCI and poor outcome [93,94]. Some limitations to these studies have been variation of markers within individuals and low specificity that limit translation to clinical practice, although larger, multi-center, randomized clinical studies are needed to further solidify the use of these lab values as predictors of DCI.

Inflammation within the CNS after SAH begins with arterial rupture, as blood enters the subarachnoid space and releases signals that promote an inflammatory response. Products normally found within erythrocytes such as hemoglobin are released into the subarachnoid space and can serve as damage-associated molecular patterns (DAMPs) recognized by innate immune cells [97]. Heme can be metabolized by heme oxygenase in immune cells and generate bioactive and pro-inflammatory compounds [98]. To prevent this damaging interaction, haptoglobin can bind hemoglobin and prevent its metabolism, and indeed some studies have shown that haptoglobin genotypes may play a role in brain injury [99]. Heme and other similar red blood cell (RBC) degradation products can then bind to pattern recognition receptors (PRRs) found on innate immune cells, and in particular microglia, the resident immune cells of the brain. One such PRR is Toll-like receptor 4 (TLR4), which has been correlated to the development of DCI and poor outcome [97,100]. Another DAMP currently under investigation for its role in DCI and vasospasm is the high mobility group box-1 (HMGB1) protein that is released by necrotic cells and activated immune cells after SAH [101]. These molecules play an important role in activating inflammatory responses in the brain.

Studies investigating the role of microglia after SAH have found a highly significant role in DCI. Schneider *et. al.* showed that microglia play a prominent role as the major inflammatory cell in the brain after SAH and depletion of these cells reduces neuronal cell death [102]. The presence of microglia and neuroinflammation appears long-lasting as studies have shown their presence within the brain in experimental models as far as day 21 and that it is associated with gray and white matter damage and sensorimotor impairment [103]. Activated microglia have been observed in both cerebral hemispheres following SAH, although are more prominent on the side of initial injury [103]. Experimental depletion of microglia also resulted in a reduction of vasospasm and neuronal apoptosis in mice [104]. This occurs via a TLR4-dependent pathway and results in activation of the MyD88, TRIF, MAPK, and NF-κB signal transduction pathways all involved in the transcription of pro-inflammatory genes [97,104]. An important consideration in studying microglia is the dual role they have in CNS damage and neuroprotection. Similar to macrophages in the periphery, microglia can be polarized to pro-inflammatory (M1) or anti-inflammatory (M2) phenotypes, a continuum between these two phenotypes likely exists [105]. Microglial polarization and its time course will be important to further elucidate their complex role in post-SAH pathophysiology.

While microglia play a prominent role as the resident immune cells of the CNS, many other factors work in conjunction to set up a robust inflammatory response after SAH. Increased expression of cellular adhesion molecules such as ICAM-1 and extracellular matrix remodeling proteins such as MMP-9 can contribute to increased permeability and breakdown of the BBB [97,106]. Peripheral immune cells including neutrophils, monocytes/macrophages, and lymphocytes can then transmigrate across the BBB and gain access to the subarachnoid space and brain parenchyma. Neutrophils and macrophages have been observed in some studies to enter the subarachnoid space hours after SAH and can further promote inflammation [107]. Other studies have shown that neutrophils in particular do not directly invade the subarachnoid space, but through secretion of cytokines can influence the immune response in the CNS [108]. Indeed, depletion of neutrophils resulted in a

reduction in tissue inflammation, vasospasm, and long-term potentiation (LTP) dysfunction in mouse models [108]. It is clear that the complex interplay between various immune cells warrants further study, in particular focusing on the predominant immune cells over time and the cytokines they produce.

Understanding the prominent immune cell populations that contribute to acute and chronic neuroinflammation will be helpful in elucidating novel therapeutic targets that either prevent or treat DCI. Some exciting new targets under study in preclinical rodent models are listed in Table 2 [109-114]. We have focused on the therapeutic potential of fingolimod (FTY720) in the treatment of SAH and other forms of stroke as it is already FDA-approved and is well tolerated [114,115]. Additional studies are needed, especially over a longer period of time after the initial injury to determine the effect of these potential interventions on DCI and other long-term impairments after SAH.

Table 2. Examples of anti-inflammatory targets under development in preclinical models of experimental SAH.

Target	Drug/Molecule	Outcomes	Reference
MAPK	U0126 (MEK1/2 inhibitor)	Prevented upregulation of cytokines and MMP-9 in cerebral vessels, improved neurological outcome	[99]
mTOR	Rapamycin or AZD8055	Reduced microglial activation, promoted M2 phenotype polarization, reduced neuronal apoptosis, necrosis, brain edema and BBB permeability	[100]
Mincle/Syk	Albumin	Reduced microglial activation and M1 polarization, decreased MPO, ICAM-1 and chemokines involved in neutrophil invasion	[101]
PARP	PJ34	Reduced BBB permeability, brain edema, neuronal cell death, improved neurological function, decreased expression of MMP-9 and pro-inflammatory cytokines	[102]
RAGE	FPS-ZM1	Reduced neuroinflammation and brain edema, improved neurological function at day 1 but not at day 3; also sensitized neurons towards cell death	[103]
S1PR	FTY720 (Fingolimod)	Reduced intravascular leukocyte adhesion to pial venules, preserved pial arteriolar reactivity, improved neurological outcome	[104]

Abbreviations: MAPK (mitogen-activated protein kinase); mTOR (mechanistic target of rapamycin); Mincle (microglia macrophage-inducible C-type lectin); Syk (spleen tyrosine kinase); PARP (poly(ADP-ribose) polymerase); RAGE (receptor for advanced glycation endproducts); S1PR (sphingosine 1-phosphate receptor)

Some anti-inflammatory drugs have been studied clinically, often with conflicting results or low power due to small patient sample sizes. Examples of either anti-inflammatory or immunomodulatory agents studied in SAH patients have included NSAIDs, thromboxane inhibitors, corticosteroids, methylprednisolone, cyclosporine A, complement inhibitors, statins, and monoclonal antibodies targeting cytokine receptors or cellular adhesion molecules [25,51,116,117]. While some of these drugs failed to demonstrate efficacy, some have shown promise and warrant continued study. For example, despite a small number of patients, IL-1R antagonist (IL-1Ra) in both intravenous and subcutaneous forms appears safe in SAH patients and there was a reduction in CSF and plasma concentrations of IL-6 [117,118]. While some of these interventions have shown no efficacy, those that have shown promise further support the notion that neuroinflammation plays a role in DCI after SAH.

Future Directions and Conclusions

DCI is common after SAH and considered to be the main contributor to long-term poor functional outcome. Cerebral vasospasm was long viewed as the principal contributor to DCI; however, recent studies have challenged this notion. Microarteriolar dysfunction, microthrombosis, CSDs, and neuroinflammation are promising candidates with preclinical and clinical evidence supporting their role in this multifactorial disease process. It is also possible that these mechanisms are connected to one another and thus not entirely independent. Microvascular vasoconstriction within the CNS parenchyma supports a pro-thrombotic environment, while CSDs can impair neurovascular coupling to induce vasoconstriction instead of vasodilation [52,79]. New roles of microglia in the pathogenesis and regulation of CSDs have also been suggested. Activated

microglia may be capable of both sensing and inducing CSDs, further exacerbating the injury by contributing to imbalances in ion homeostasis and synaptic pruning [119]. Another exciting area that warrants further investigation is disturbances in the flow of CSF and clearance of waste products through both the glymphatic system and recently discovered meningeal lymphatic system [120-124]. It is likely that these distinct systems are altered following SAH and their impairment may further contribute to DCI. While each of these mechanisms requires further study to elucidate their contribution, it is possible that drugs targeting more than one of these mechanisms may be more beneficial in preventing DCI and improving long-term outcomes.

Compliance with Ethical Standards

Conflicts of Interest – Joseph R. Geraghty and Fernando D. Testai declare no conflicts of interest.

Human and Animal Rights and Informed Consent – This article does not contain any studies with human or animal subjects performed by any of the authors.

References Cited

[1] Suarez JI, Tarr RW, Selman WR. Aneurysmal subarachnoid hemorrhage. N Engl J Med. 2006 Jan 26;354(4):387-96.
[2] Rincon F, Rossenwasser RH, Dumont A. The epidemiology of admissions of nontraumatic subarachnoid hemorrhage in the United States. Neurosurgery. 2013 Aug;73(2):217,22; discussion 212-3.
[3] Hop JW, Rinkel GJ, Algra A, van Gijn J. Case-fatality rates and functional outcome after subarachnoid hemorrhage: a systematic review. Stroke. 1997 Mar;28(3):660-4.
[4] Mackey J, Khoury JC, Alwell K, Moomaw CJ, Kissela BM, Flaherty ML, et al. Stable incidence but declining case-fatality rates of subarachnoid hemorrhage in a population. Neurology. 2016 Nov 22;87(21):2192-7.
[5] Lovelock CE, Rinkel GJ, Rothwell PM. Time trends in outcome of subarachnoid hemorrhage: Population-based study and systematic review. Neurology. 2010 May 11;74(19):1494-501.
[6] Taufique Z, May T, Meyers E, Falo C, Mayer SA, Agarwal S, et al. Predictors of Poor Quality of Life 1 Year After Subarachnoid Hemorrhage. Neurosurgery. 2016 Feb;78(2):256-64.
[7] Boerboom W, Heijenbrok-Kal MH, Khajeh L, van Kooten F, Ribbers GM. Differences in cognitive and emotional outcomes between patients with perimesencephalic and aneurysmal subarachnoid haemorrhage. J Rehabil Med. 2014 Jan;46(1):28-32.
[8] Kreiter KT, Rosengart AJ, Claassen J, Fitzsimmons BF, Peery S, Du YE, et al. Depressed mood and quality of life after subarachnoid hemorrhage. J Neurol Sci. 2013 Dec 15;335(1-2):64-71.
[9] Wong GK, Lam SW, Ngai K, Wong A, Siu D, Poon WS, et al. Cognitive domain deficits in patients with aneurysmal subarachnoid haemorrhage at 1 year. J Neurol Neurosurg Psychiatry. 2013 Sep;84(9):1054-8.
[10] Fujii M, Yan J, Rolland WB, Soejima Y, Caner B, Zhang JH. Early brain injury, an evolving frontier in subarachnoid hemorrhage research. Transl Stroke Res. 2013 Aug;4(4):432-46.
[11] Foreman B. The Pathophysiology of Delayed Cerebral Ischemia. J Clin Neurophysiol. 2016 Jun;33(3):174-82.
[12] Macdonald RL. Origins of the Concept of Vasospasm. Stroke. 2016 Jan;47(1):e11-5.
[13] Macdonald RL, Kassell NF, Mayer S, Ruefenacht D, Schmiedek P, Weidauer S, et al. Clazosentan to overcome neurological ischemia and infarction occurring after subarachnoid hemorrhage (CONSCIOUS-1): randomized, double-blind, placebo-controlled phase 2 dose-finding trial. Stroke. 2008 Nov;39(11):3015-21.
[14] Macdonald RL, Higashida RT, Keller E, Mayer SA, Molyneux A, Raabe A, et al. Randomized trial of clazosentan in patients with aneurysmal subarachnoid hemorrhage undergoing endovascular coiling. Stroke. 2012 Jun;43(6):1463-9.
[15] Dorsch NW, King MT. A review of cerebral vasospasm in aneurysmal subarachnoid haemorrhage Part I: Incidence and effects. J Clin Neurosci. 1994 Jan;1(1):19-26.
[16] Pegoli M, Mandrekar J, Rabinstein AA, Lanzino G. Predictors of excellent functional outcome in aneurysmal subarachnoid hemorrhage. J Neurosurg. 2015 Feb;122(2):414-8.
[17] de Rooij NK, Rinkel GJ, Dankbaar JW, Frijns CJ. Delayed cerebral ischemia after subarachnoid hemorrhage: a systematic review of clinical, laboratory, and radiological predictors. Stroke. 2013 Jan;44(1):43-54.

[18] Francoeur CL, Mayer SA. Management of delayed cerebral ischemia after subarachnoid hemorrhage. Crit Care. 2016 Oct 14;20(1):277.
[19] Bouzat P, Payen JF, Crippa IA, Taccone FS. Noninvasive Vascular Methods for Detection of Delayed Cerebral Ischemia After Subarachnoid Hemorrhage. J Clin Neurophysiol. 2016 Jun;33(3):260-7.
[20] Gaspard N. Current Clinical Evidence Supporting the Use of Continuous EEG Monitoring for Delayed Cerebral Ischemia Detection. J Clin Neurophysiol. 2016 Jun;33(3):211-6.
[21] Alaraj A, Charbel FT, Amin-Hanjani S. Peri-operative measures for treatment and prevention of cerebral vasospasm following subarachnoid hemorrhage. Neurol Res. 2009 Jul;31(6):651-9.
[22] Raya AK, Diringer MN. Treatment of subarachnoid hemorrhage. Crit Care Clin. 2014 Oct;30(4):719-33.
[23] Allen GS, Ahn HS, Preziosi TJ, Battye R, Boone SC, Boone SC, et al. Cerebral arterial spasm--a controlled trial of nimodipine in patients with subarachnoid hemorrhage. N Engl J Med. 1983 Mar 17;308(11):619-24.
[24] Hanggi D, Etminan N, Aldrich F, Steiger HJ, Mayer SA, Diringer MN, et al. Randomized, Open-Label, Phase 1/2a Study to Determine the Maximum Tolerated Dose of Intraventricular Sustained Release Nimodipine for Subarachnoid Hemorrhage (NEWTON [Nimodipine Microparticles to Enhance Recovery While Reducing Toxicity After Subarachnoid Hemorrhage]). Stroke. 2017 Jan;48(1):145-51.
**[25] Macdonald RL. Delayed neurological deterioration after subarachnoid haemorrhage. Nat Rev Neurol. 2014 Jan;10(1):44-58. Excellent review of delayed neurological injury after SAH including mechanisms, clinical trials and updated management schemes.
[26] Dankbaar JW, Slooter AJ, Rinkel GJ, Schaaf IC. Effect of different components of triple-H therapy on cerebral perfusion in patients with aneurysmal subarachnoid haemorrhage: a systematic review. Crit Care. 2010;14(1):R23.
[27] Dorhout Mees SM, Rinkel GJ, Feigin VL, Algra A, van den Bergh WM, Vermeulen M, et al. Calcium antagonists for aneurysmal subarachnoid haemorrhage. Cochrane Database Syst Rev. 2007 Jul 18;(3)(3):CD000277.
[28] Dorhout Mees SM, van den Bergh WM, Algra A, Rinkel GJ. Antiplatelet therapy for aneurysmal subarachnoid haemorrhage. Cochrane Database Syst Rev. 2007 Oct 17;(4)(4):CD006184.
[29] Feigin VL, Anderson N, Rinkel GJ, Algra A, van Gijn J, Bennett DA. Corticosteroids for aneurysmal subarachnoid haemorrhage and primary intracerebral haemorrhage. Cochrane Database Syst Rev. 2005 Jul 20;(3)(3):CD004583.
[30] Golan E, Vasquez DN, Ferguson ND, Adhikari NK, Scales DC. Prophylactic magnesium for improving neurologic outcome after aneurysmal subarachnoid hemorrhage: systematic review and meta-analysis. J Crit Care. 2013 Apr;28(2):173-81.
[31] Guo J, Shi Z, Yang K, Tian JH, Jiang L. Endothelin receptor antagonists for subarachnoid hemorrhage. Cochrane Database Syst Rev. 2012 Sep 12;(9):CD008354. doi(9):CD008354.
[32] Kramer AH, Fletcher JJ. Locally-administered intrathecal thrombolytics following aneurysmal subarachnoid hemorrhage: a systematic review and meta-analysis. Neurocrit Care. 2011 Jun;14(3):489-99.
[33] Liu GJ, Wang ZJ, Wang YF, Xu LL, Wang XL, Liu Y, et al. Systematic assessment and meta-analysis of the efficacy and safety of fasudil in the treatment of cerebral vasospasm in patients with subarachnoid hemorrhage. Eur J Clin Pharmacol. 2012 Feb;68(2):131-9.
[34] Vergouwen MD, de Haan RJ, Vermeulen M, Roos YB. Effect of statin treatment on vasospasm, delayed cerebral ischemia, and functional outcome in patients with aneurysmal subarachnoid hemorrhage: a systematic review and meta-analysis update. Stroke. 2010 Jan;41(1):e47-52.
[35] Zhang S, Wang L, Liu M, Wu B. Tirilazad for aneurysmal subarachnoid haemorrhage. Cochrane Database Syst Rev. 2010 Feb 17;(2):CD006778. doi(2):CD006778.
[36] Egge A, Waterloo K, Sjoholm H, Solberg T, Ingebrigtsen T, Romner B. Prophylactic hyperdynamic postoperative fluid therapy after aneurysmal subarachnoid hemorrhage: a clinical, prospective, randomized, controlled study. Neurosurgery. 2001 Sep;49(3):593,605; discussion 605-6.
[37] Muench E, Horn P, Bauhuf C, Roth H, Philipps M, Hermann P, et al. Effects of hypervolemia and hypertension on regional cerebral blood flow, intracranial pressure, and brain tissue oxygenation after subarachnoid hemorrhage. Crit Care Med. 2007 Aug;35(8):1844,51; quiz 1852.
[38] Lennihan L, Mayer SA, Fink ME, Beckford A, Paik MC, Zhang H, et al. Effect of hypervolemic therapy on cerebral blood flow after subarachnoid hemorrhage : a randomized controlled trial. Stroke. 2000 Feb;31(2):383-91.

[39] Gathier CS, Dankbaar JW, van der Jagt M, Verweij BH, Oldenbeuving AW, Rinkel GJ, et al. Effects of Induced Hypertension on Cerebral Perfusion in Delayed Cerebral Ischemia After Aneurysmal Subarachnoid Hemorrhage: A Randomized Clinical Trial. Stroke. 2015 Nov;46(11):3277-81.
[40] Connolly ES,Jr, Rabinstein AA, Carhuapoma JR, Derdeyn CP, Dion J, Higashida RT, et al. Guidelines for the management of aneurysmal subarachnoid hemorrhage: a guideline for healthcare professionals from the American Heart Association/american Stroke Association. Stroke. 2012 Jun;43(6):1711-37.
[41] Shankar JJ, dos Santos MP, Deus-Silva L, Lum C. Angiographic evaluation of the effect of intra-arterial milrinone therapy in patients with vasospasm from aneurysmal subarachnoid hemorrhage. Neuroradiology. 2011 Feb;53(2):123-8.
[42] Venkatraman A, Khawaja AM, Gupta S, Hardas S, Deveikis JP, Harrigan MR, et al. Intra-arterial vasodilators for vasospasm following aneurysmal subarachnoid hemorrhage: a meta-analysis. J Neurointerv Surg. 2017 Jun 29.
[43] Hollingworth M, Chen PR, Goddard AJ, Coulthard A, Soderman M, Bulsara KR. Results of an International Survey on the Investigation and Endovascular Management of Cerebral Vasospasm and Delayed Cerebral Ischemia. World Neurosurg. 2015 Jun;83(6):1120,1126.e1.
[44] Vergouwen MD, Vermeulen M, van Gijn J, Rinkel GJ, Wijdicks EF, Muizelaar JP, et al. Definition of delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage as an outcome event in clinical trials and observational studies: proposal of a multidisciplinary research group. Stroke. 2010 Oct;41(10):2391-5.
[45] Crowley RW, Medel R, Dumont AS, Ilodigwe D, Kassell NF, Mayer SA, et al. Angiographic vasospasm is strongly correlated with cerebral infarction after subarachnoid hemorrhage. Stroke. 2011 Apr;42(4):919-23.
[46] Hijdra A, Van Gijn J, Stefanko S, Van Dongen KJ, Vermeulen M, Van Crevel H. Delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage: clinicoanatomic correlations. Neurology. 1986 Mar;36(3):329-33.
[47] Vergouwen MD, Ilodigwe D, Macdonald RL. Cerebral infarction after subarachnoid hemorrhage contributes to poor outcome by vasospasm-dependent and -independent effects. Stroke. 2011 Apr;42(4):924-9.
[48] Bederson JB, Connolly ES,Jr, Batjer HH, Dacey RG, Dion JE, Diringer MN, et al. Guidelines for the management of aneurysmal subarachnoid hemorrhage: a statement for healthcare professionals from a special writing group of the Stroke Council, American Heart Association. Stroke. 2009 Mar;40(3):994-1025.
[49] Macdonald RL, Higashida RT, Keller E, Mayer SA, Molyneux A, Raabe A, et al. Clazosentan, an endothelin receptor antagonist, in patients with aneurysmal subarachnoid haemorrhage undergoing surgical clipping: a randomised, double-blind, placebo-controlled phase 3 trial (CONSCIOUS-2). Lancet Neurol. 2011 Jul;10(7):618-25.
[50] Meyers PM, Connolly ES,Jr. Stroke: disappointing results for clazosentan in CONSCIOUS-2. Nat Rev Neurol. 2011 Oct 18;7(12):660-1.
[51] Budohoski KP, Guilfoyle M, Helmy A, Huuskonen T, Czosnyka M, Kirollos R, et al. The pathophysiology and treatment of delayed cerebral ischaemia following subarachnoid haemorrhage. J Neurol Neurosurg Psychiatry. 2014 Dec;85(12):1343-53.
[52] Friedrich B, Muller F, Feiler S, Scholler K, Plesnila N. Experimental subarachnoid hemorrhage causes early and long-lasting microarterial constriction and microthrombosis: an in-vivo microscopy study. J Cereb Blood Flow Metab. 2012 Mar;32(3):447-55.
[53] Sabri M, Ai J, Lakovic K, Macdonald RL. Mechanisms of microthrombosis and microcirculatory constriction after experimental subarachnoid hemorrhage. Acta Neurochir Suppl. 2013;115:185-92.
[54] Sehba FA, Friedrich V. Cerebral microvasculature is an early target of subarachnoid hemorrhage. Acta Neurochir Suppl. 2013;115:199-205.
[55] Wellman GC, Koide M. Impact of subarachnoid hemorrhage on parenchymal arteriolar function. Acta Neurochir Suppl. 2013;115:173-7.
[56] Wolf ME. Functional TCD: regulation of cerebral hemodynamics--cerebral autoregulation, vasomotor reactivity, and neurovascular coupling. Front Neurol Neurosci. 2015;36:40-56.
[57] Koide M, Bonev AD, Nelson MT, Wellman GC. Inversion of neurovascular coupling by subarachnoid blood depends on large-conductance Ca2+-activated K+ (BK) channels. Proc Natl Acad Sci U S A. 2012 May 22;109(21):E1387-95.
[58] Koide M, Bonev AD, Nelson MT, Wellman GC. Subarachnoid blood converts neurally evoked vasodilation to vasoconstriction in rat brain cortex. Acta Neurochir Suppl. 2013;115:167-71.

*[59] Balbi M, Koide M, Wellman GC, Plesnila N. Inversion of neurovascular coupling after subarachnoid hemorrhage in vivo. J Cereb Blood Flow Metab. 2017 Jan 01:271678X16686595. Most recent report showing inversion of neurovascular coupling that occurs in a delayed fashion and progresses.
[60] Ostergaard L, Aamand R, Karabegovic S, Tietze A, Blicher JU, Mikkelsen IK, et al. The role of the microcirculation in delayed cerebral ischemia and chronic degenerative changes after subarachnoid hemorrhage. J Cereb Blood Flow Metab. 2013 Dec;33(12):1825-37.
[61] Vergouwen MD, Vermeulen M, Coert BA, Stroes ES, Roos YB. Microthrombosis after aneurysmal subarachnoid hemorrhage: an additional explanation for delayed cerebral ischemia. J Cereb Blood Flow Metab. 2008 Nov;28(11):1761-70.
[62] Roos YB, Levi M, Carroll TA, Beenen LF, Vermeulen M. Nimodipine increases fibrinolytic activity in patients with aneurysmal subarachnoid hemorrhage. Stroke. 2001 Aug;32(8):1860-2.
[63] Suzuki S, Suzuki M, Iwabuchi T, Kamata Y. Role of multiple cerebral microthrombosis in symptomatic cerebral vasospasm: with a case report. Neurosurgery. 1983 Aug;13(2):199-203.
[64] Sabri M, Ai J, Lakovic K, D'abbondanza J, Ilodigwe D, Macdonald RL. Mechanisms of microthrombi formation after experimental subarachnoid hemorrhage. Neuroscience. 2012 Nov 8;224:26-37.
[65] Pisapia JM, Xu X, Kelly J, Yeung J, Carrion G, Tong H, et al. Microthrombosis after experimental subarachnoid hemorrhage: time course and effect of red blood cell-bound thrombin-activated pro-urokinase and clazosentan. Exp Neurol. 2012 Jan;233(1):357-63.
[66] Boluijt J, Meijers JC, Rinkel GJ, Vergouwen MD. Hemostasis and fibrinolysis in delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage: a systematic review. J Cereb Blood Flow Metab. 2015 May;35(5):724-33.
*[67] Frontera JA, Provencio JJ, Sehba FA, McIntyre TM, Nowacki AS, Gordon E, et al. The Role of Platelet Activation and Inflammation in Early Brain Injury Following Subarachnoid Hemorrhage. Neurocrit Care. 2017 Feb;26(1):48-57. Demonstrates association of platelet activation and inflammation in EBI, DCI, and long-term outcomes after SAH.
*[68] El Amki M, Dubois M, Lefevre-Scelles A, Magne N, Roussel M, Clavier T, et al. Long-Lasting Cerebral Vasospasm, Microthrombosis, Apoptosis and Paravascular Alterations Associated with Neurological Deficits in a Mouse Model of Subarachnoid Hemorrhage. Mol Neurobiol. 2017 Apr 28. Demonstrates utility of a model of SAH in studying mechanisms of vasospasm, microthrombosis, and neuronal loss.
[69] Vergouwen MD, Bakhtiari K, van Geloven N, Vermeulen M, Roos YB, Meijers JC. Reduced ADAMTS13 activity in delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage. J Cereb Blood Flow Metab. 2009 Oct;29(10):1734-41.
[70] Shen J, Huang KY, Zhu Y, Pan JW, Jiang H, Weng YX, et al. Effect of statin treatment on vasospasm-related morbidity and functional outcome in patients with aneurysmal subarachnoid hemorrhage: a systematic review and meta-analysis. J Neurosurg. 2016 Oct 07:1-11.
[71] Kramer AH, Gurka MJ, Nathan B, Dumont AS, Kassell NF, Bleck TP. Statin use was not associated with less vasospasm or improved outcome after subarachnoid hemorrhage. Neurosurgery. 2008 Feb;62(2):422,7; discussion 427-30.
[72] Vergouwen MD, Knaup VL, Roelofs JJ, de Boer OJ, Meijers JC. Effect of recombinant ADAMTS-13 on microthrombosis and brain injury after experimental subarachnoid hemorrhage. J Thromb Haemost. 2014 Jun;12(6):943-7.
[73] Muroi C, Fujioka M, Mishima K, Irie K, Fujimura Y, Nakano T, et al. Effect of ADAMTS-13 on cerebrovascular microthrombosis and neuronal injury after experimental subarachnoid hemorrhage. J Thromb Haemost. 2014 Apr;12(4):505-14.
[74] Amin-Hanjani S, Ogilvy CS, Barker FG,2nd. Does intracisternal thrombolysis prevent vasospasm after aneurysmal subarachnoid hemorrhage? A meta-analysis. Neurosurgery. 2004 Feb;54(2):326,34; discussion 334-5.
[75] Etminan N, Beseoglu K, Eicker SO, Turowski B, Steiger HJ, Hanggi D. Prospective, randomized, open-label phase II trial on concomitant intraventricular fibrinolysis and low-frequency rotation after severe subarachnoid hemorrhage. Stroke. 2013 Aug;44(8):2162-8.
[76] LEAO AA. Further observations on the spreading depression of activity in the cerebral cortex. J Neurophysiol. 1947 Nov;10(6):409-14.
[77] Charles AC, Baca SM. Cortical spreading depression and migraine. Nat Rev Neurol. 2013 Nov;9(11):637-44.

**[78] Dreier JP, Reiffurth C. The stroke-migraine depolarization continuum. Neuron. 2015 May 20;86(4):902-22. Excellent review of the pathophysiology of spreading depolarizations, similarities and differences in migraine and stroke, and recent developments in the field.
[79] Dreier JP. The role of spreading depression, spreading depolarization and spreading ischemia in neurological disease. Nat Med. 2011 Apr;17(4):439-47.
[80] Sanchez-Porras R, Zheng Z, Santos E, Scholl M, Unterberg AW, Sakowitz OW. The role of spreading depolarization in subarachnoid hemorrhage. Eur J Neurol. 2013 Aug;20(8):1121-7.
[81] Kramer DR, Fujii T, Ohiorhenuan I, Liu CY. Cortical spreading depolarization: Pathophysiology, implications, and future directions. J Clin Neurosci. 2016 Feb;24:22-7.
[82] Hubschmann OR, Kornhauser D. Cortical cellular response in acute subarachnoid hemorrhage. J Neurosurg. 1980 Apr;52(4):456-62.
[83] Dreier JP, Woitzik J, Fabricius M, Bhatia R, Major S, Drenckhahn C, et al. Delayed ischaemic neurological deficits after subarachnoid haemorrhage are associated with clusters of spreading depolarizations. Brain. 2006 Dec;129(Pt 12):3224-37.
[84] Woitzik J, Dreier JP, Hecht N, Fiss I, Sandow N, Major S, et al. Delayed cerebral ischemia and spreading depolarization in absence of angiographic vasospasm after subarachnoid hemorrhage. J Cereb Blood Flow Metab. 2012 Feb;32(2):203-12.
[85] Winkler MK, Chassidim Y, Lublinsky S, Revankar GS, Major S, Kang EJ, et al. Impaired neurovascular coupling to ictal epileptic activity and spreading depolarization in a patient with subarachnoid hemorrhage: possible link to blood-brain barrier dysfunction. Epilepsia. 2012 Nov;53 Suppl 6:22-30.
[86] Aiba I, Shuttleworth CW. Sustained NMDA receptor activation by spreading depolarizations can initiate excitotoxic injury in metabolically compromised neurons. J Physiol. 2012 Nov 15;590(22):5877-93.
[87] Zarcone D, Corbetta S. Shared mechanisms of epilepsy, migraine and affective disorders. Neurol Sci. 2017 May;38(Suppl 1):73-6.
[88] Tozzi A, de Iure A, Di Filippo M, Costa C, Caproni S, Pisani A, et al. Critical role of calcitonin gene-related peptide receptors in cortical spreading depression. Proc Natl Acad Sci U S A. 2012 Nov 13;109(46):18985-90.
[89] Engelhardt B, Vajkoczy P, Weller RO. The movers and shapers in immune privilege of the CNS. Nat Immunol. 2017 Feb;18(2):123-31.
[90] Louveau A, Harris TH, Kipnis J. Revisiting the Mechanisms of CNS Immune Privilege. Trends Immunol. 2015 Oct;36(10):569-77.
[91] Carr KR, Zuckerman SL, Mocco J. Inflammation, cerebral vasospasm, and evolving theories of delayed cerebral ischemia. Neurol Res Int. 2013;2013:506584.
[92] Peng J, Wu Y, Tian X, Pang J, Kuai L, Cao F, et al. High-Throughput Sequencing and Co-Expression Network Analysis of lncRNAs and mRNAs in Early Brain Injury Following Experimental Subarachnoid Haemorrhage. Sci Rep. 2017 Apr 18;7:46577.
[93] Muroi C, Hugelshofer M, Seule M, Tastan I, Fujioka M, Mishima K, et al. Correlation among systemic inflammatory parameter, occurrence of delayed neurological deficits, and outcome after aneurysmal subarachnoid hemorrhage. Neurosurgery. 2013 Mar;72(3):367,75; discussion 375.
[94] McMahon CJ, Hopkins S, Vail A, King AT, Smith D, Illingworth KJ, et al. Inflammation as a predictor for delayed cerebral ischemia after aneurysmal subarachnoid haemorrhage. J Neurointerv Surg. 2013 Nov;5(6):512-7.
[95] Da Silva IR, Gomes JA, Wachsman A, de Freitas GR, Provencio JJ. Hematologic counts as predictors of delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage. J Crit Care. 2017 Feb;37:126-9.
[96] Chamling B, Gross S, Stoffel-Wagner B, Schubert GA, Clusmann H, Coburn M, et al. Early Diagnosis of Delayed Cerebral Ischemia: Possible Relevance for Inflammatory Biomarkers in Routine Clinical Practice? World Neurosurg. 2017 May 13.
[97] Lucke-Wold BP, Logsdon AF, Manoranjan B, Turner RC, McConnell E, Vates GE, et al. Aneurysmal Subarachnoid Hemorrhage and Neuroinflammation: A Comprehensive Review. Int J Mol Sci. 2016 Apr 2;17(4):497.
*[98] Schallner N, Pandit R, LeBlanc R,3rd, Thomas AJ, Ogilvy CS, Zuckerbraun BS, et al. Microglia regulate blood clearance in subarachnoid hemorrhage by heme oxygenase-1. J Clin Invest. 2015 Jul 1;125(7):2609-25. Evidence of a mechanism by which microglia act in hemoglobin clearance after SAH.

[99] Kantor E, Bayir H, Ren D, Provencio JJ, Watkins L, Crago E, et al. Haptoglobin genotype and functional outcome after aneurysmal subarachnoid hemorrhage. J Neurosurg. 2014 Feb;120(2):386-90.
[100] Ma C, Zhou W, Yan Z, Qu M, Bu X. Toll-like receptor 4 (TLR4) is correlated with delayed cerebral ischemia (DCI) and poor prognosis in aneurysmal subarachnoid hemorrhage. J Neurol Sci. 2015 Dec 15;359(1-2):67-71.
[101] Haruma J, Teshigawara K, Hishikawa T, Wang D, Liu K, Wake H, et al. Anti-high mobility group box-1 (HMGB1) antibody attenuates delayed cerebral vasospasm and brain injury after subarachnoid hemorrhage in rats. Sci Rep. 2016 Nov 24;6:37755.
*[102] Schneider UC, Davids AM, Brandenburg S, Muller A, Elke A, Magrini S, et al. Microglia inflict delayed brain injury after subarachnoid hemorrhage. Acta Neuropathol. 2015 Aug;130(2):215-31. Explores role of microglia in delayed neuronal and axonal injury in an animal model of SAH.
[103] Kooijman E, Nijboer CH, van Velthoven CT, Mol W, Dijkhuizen RM, Kesecioglu J, et al. Long-term functional consequences and ongoing cerebral inflammation after subarachnoid hemorrhage in the rat. PLoS One. 2014 Mar 6;9(6):e90584.
[104] Hanafy KA. The role of microglia and the TLR4 pathway in neuronal apoptosis and vasospasm after subarachnoid hemorrhage. J Neuroinflammation. 2013 Jul 13;10:83,2094-10-83.
[105] Doorn KJ, Breve JJ, Drukarch B, Boddeke HW, Huitinga I, Lucassen PJ, et al. Brain region-specific gene expression profiles in freshly isolated rat microglia. Front Cell Neurosci. 2015 Mar 12;9:84.
[106] Penn DL, Witte SR, Komotar RJ, Sander Connolly E,Jr. Pathological mechanisms underlying aneurysmal subarachnoid haemorrhage and vasospasm. J Clin Neurosci. 2015 Jan;22(1):1-5.
*[107] Kooijman E, Nijboer CH, van Velthoven CT, Kavelaars A, Kesecioglu J, Heijnen CJ. The rodent endovascular puncture model of subarachnoid hemorrhage: mechanisms of brain damage and therapeutic strategies. J Neuroinflammation. 2014 Jan 3;11:2,2094-11-2. Review of rodent SAH models, current therapies and targets being tested.
[108] Provencio JJ, Swank V, Lu H, Brunet S, Baltan S, Khapre RV, et al. Neutrophil depletion after subarachnoid hemorrhage improves memory via NMDA receptors. Brain Behav Immun. 2016 May;54:233-42.
[109] Maddahi A, Povlsen GK, Edvinsson L. Regulation of enhanced cerebrovascular expression of proinflammatory mediators in experimental subarachnoid hemorrhage via the mitogen-activated protein kinase kinase/extracellular signal-regulated kinase pathway. J Neuroinflammation. 2012 Dec 21;9:274,2094-9-274.
[110] You W, Wang Z, Li H, Shen H, Xu X, Jia G, et al. Inhibition of mammalian target of rapamycin attenuates early brain injury through modulating microglial polarization after experimental subarachnoid hemorrhage in rats. J Neurol Sci. 2016 Aug 15;367:224-31.
[111] Xie Y, Guo H, Wang L, Xu L, Zhang X, Yu L, et al. Human albumin attenuates excessive innate immunity via inhibition of microglial Mincle/Syk signaling in subarachnoid hemorrhage. Brain Behav Immun. 2017 Feb;60:346-60.
[112] Chen T, Wang W, Li JR, Xu HZ, Peng YC, Fan LF, et al. PARP inhibition attenuates early brain injury through NF-kappaB/MMP-9 pathway in a rat model of subarachnoid hemorrhage. Brain Res. 2016 Aug 1;1644:32-8.
[113] Li H, Yu JS, Zhang DD, Yang YQ, Huang LT, Yu Z, et al. Inhibition of the Receptor for Advanced Glycation End-Products (RAGE) Attenuates Neuroinflammation While Sensitizing Cortical Neurons Towards Death in Experimental Subarachnoid Hemorrhage. Mol Neurobiol. 2017 Jan;54(1):755-67.
[114] Xu HL, Pelligrino DA, Paisansathan C, Testai FD. Protective role of fingolimod (FTY720) in rats subjected to subarachnoid hemorrhage. J Neuroinflammation. 2015 Jan 27;12:16,015-0234-7.
[115] Li W, Xu H, Testai FD. Mechanism of Action and Clinical Potential of Fingolimod for the Treatment of Stroke. Front Neurol. 2016 Aug 26;7:139.
[116] Nassiri F, Ibrahim GM, Badhiwala JH, Witiw CD, Mansouri A, Alotaibi NM, et al. A Propensity Score-Matched Study of the Use of Non-steroidal Anti-inflammatory Agents Following Aneurysmal Subarachnoid Hemorrhage. Neurocrit Care. 2016 Dec;25(3):351-8.
[117] Singh N, Hopkins SJ, Hulme S, Galea JP, Hoadley M, Vail A, et al. The effect of intravenous interleukin-1 receptor antagonist on inflammatory mediators in cerebrospinal fluid after subarachnoid haemorrhage: a phase II randomised controlled trial. J Neuroinflammation. 2014 Jan 3;11:1,2094-11-1.

*[118] Galea J, Ogungbenro K, Hulme S, Patel H, Scarth S, Hoadley M, et al. Reduction of inflammation after administration of interleukin-1 receptor antagonist following aneurysmal subarachnoid hemorrhage: results of the Subcutaneous Interleukin-1Ra in SAH (SCIL-SAH) study. J Neurosurg. 2017 Feb 24:1-9. Important study showing safety and levels of inflammation after administration of IL-1Ra.

*[119] Shibata M, Suzuki N. Exploring the role of microglia in cortical spreading depression in neurological disease. J Cereb Blood Flow Metab. 2017 Apr;37(4):1182-91. Review connecting microglia and neuroinflammation to cortical spreading depression, referencing how these two phenomena may be related.

[120] Gaberel T, Gakuba C, Goulay R, Martinez De Lizarrondo S, Hanouz JL, Emery E, et al. Impaired glymphatic perfusion after strokes revealed by contrast-enhanced MRI: a new target for fibrinolysis? Stroke. 2014 Oct;45(10):3092-6.

[121] Golanov EV, Bovshik EI, Wong KK, Pautler RG, Foster CH, Federley RG, et al. Subarachnoid hemorrhage - Induced block of cerebrospinal fluid flow: Role of brain coagulation factor III (tissue factor). J Cereb Blood Flow Metab. 2017 Jan 01:271678X17701157.

[122] Goulay R, Flament J, Gauberti M, Naveau M, Pasquet N, Gakuba C, et al. Subarachnoid Hemorrhage Severely Impairs Brain Parenchymal Cerebrospinal Fluid Circulation in Nonhuman Primate. Stroke. 2017 May 19.

*[123] Louveau A, Smirnov I, Keyes TJ, Eccles JD, Rouhani SJ, Peske JD, et al. Structural and functional features of central nervous system lymphatic vessels. Nature. 2015 Jul 16;523(7560):337-41. Landmark study demonstrating the existence of CNS lymphatics lining dural sinuses and draining to deep cervical lymph nodes.

[124] Raper D, Louveau A, Kipnis J. How Do Meningeal Lymphatic Vessels Drain the CNS? Trends Neurosci. 2016 Sep;39(9):581-6