

8-3-2021

## Pathophysiology of Delayed Cerebral Ischemia After Subarachnoid Hemorrhage: A Review.

William S. Dodd  
*University of Florida Gainesville*

Dimitri Laurent  
*University of Florida Gainesville*

Aaron S. Dumont  
*Tulane University*

David M. Hasan  
*University of Iowa*

Pascal M. Jabbour  
*Thomas Jefferson University*  
Follow this and additional works at: <https://jdc.jefferson.edu/neurosurgeryfp>

 Part of the [Neurology Commons](#), and the [Surgery Commons](#)

*See next page for additional authors*

[Let us know how access to this document benefits you](#)

---

### Recommended Citation

Dodd, William S.; Laurent, Dimitri; Dumont, Aaron S.; Hasan, David M.; Jabbour, Pascal M.; Starke, Robert M.; Hosaka, Koji; Polifka, Adam J.; Hoh, Brian L.; and Chalouhi, Nohra, "Pathophysiology of Delayed Cerebral Ischemia After Subarachnoid Hemorrhage: A Review." (2021). *Department of Neurosurgery Faculty Papers*. Paper 161.  
<https://jdc.jefferson.edu/neurosurgeryfp/161>

This Article is brought to you for free and open access by the Jefferson Digital Commons. The Jefferson Digital Commons is a service of Thomas Jefferson University's [Center for Teaching and Learning \(CTL\)](#). The Commons is a showcase for Jefferson books and journals, peer-reviewed scholarly publications, unique historical collections from the University archives, and teaching tools. The Jefferson Digital Commons allows researchers and interested readers anywhere in the world to learn about and keep up to date with Jefferson scholarship. This article has been accepted for inclusion in Department of Neurosurgery Faculty Papers by an authorized administrator of the Jefferson Digital Commons. For more information, please contact: [JeffersonDigitalCommons@jefferson.edu](mailto:JeffersonDigitalCommons@jefferson.edu).






---

**Authors**

William S. Dodd, Dimitri Laurent, Aaron S. Dumont, David M. Hasan, Pascal M. Jabbour, Robert M. Starke, Koji Hosaka, Adam J. Polifka, Brian L. Hoh, and Nohra Chalouhi

CONTEMPORARY REVIEW

# Pathophysiology of Delayed Cerebral Ischemia After Subarachnoid Hemorrhage: A Review

William S. Dodd , BS; Dimitri Laurent , MD; Aaron S. Dumont, MD; David M. Hasan , MD; Pascal M. Jabbour , MD; Robert M. Starke, MD; Koji Hosaka, PhD; Adam J. Polifka, MD; Brian L. Hoh, MD; Nohra Chalouhi , MD

**ABSTRACT:** Delayed cerebral ischemia is a major predictor of poor outcomes in patients who suffer subarachnoid hemorrhage. Treatment options are limited and often ineffective despite many years of investigation and clinical trials. Modern advances in basic science have produced a much more complex, multifactorial framework in which delayed cerebral ischemia is better understood and novel treatments can be developed. Leveraging this knowledge to improve outcomes, however, depends on a holistic understanding of the disease process. We conducted a review of the literature to analyze the current state of investigation into delayed cerebral ischemia with emphasis on the major themes that have emerged over the past decades. Specifically, we discuss microcirculatory dysfunction, glymphatic impairment, inflammation, and neuroelectric disruption as pathological factors in addition to the canonical focus on cerebral vasospasm. This review intends to give clinicians and researchers a summary of the foundations of delayed cerebral ischemia pathophysiology while also underscoring the interactions and interdependencies between pathological factors. Through this overview, we also highlight the advances in translational studies and potential future therapeutic opportunities.

**Key Words:** delayed cerebral ischemia ■ intracranial aneurysm ■ stroke ■ subarachnoid hemorrhage

**A**neurysmal subarachnoid hemorrhage (aSAH) is a particularly devastating event; the case-fatality rate is ~40% to 50% and many survivors remain dependent on others for activities of daily living.<sup>1–4</sup> The disproportionate impact on people younger than 65 years old relative to ischemic stroke also imposes a burden on the healthcare system and society through increased costs and loss of productive life-years.<sup>5</sup> The prognosis for patients with SAH is heavily influenced by the development of delayed cerebral ischemia (DCI),<sup>1,6</sup> but adequate treatments to prevent DCI remain elusive.<sup>7</sup> Advances in critical care management and refinement of surgical techniques have helped the overall morbidity and mortality from aSAH decline slightly over the past few decades<sup>8</sup>; however, translationally focused scientific inquiry in this field remains vital to paradigm-shifting discoveries.

The conceptual framework of DCI after SAH has undergone vast transformations over the last century. Ischemic cerebral lesions were documented after aneurysmal SAH as far back as the 1940s,<sup>9</sup> around the same time that researchers noted relationships between hemorrhage, delayed infarctions, and cerebral vasospasm.<sup>10,11</sup> The associations between these phenomena, especially the time course of onset, led to the belief that vasospasm was the singular cause of DCI (also referred to as delayed ischemic neurological deficits).<sup>3,12–14</sup> Widespread use of the terms “clinical vasospasm” and “symptomatic vasospasm” reflect the conceptualization of cerebral ischemia after SAH as consequent function of “angiographic vasospasm” rather than a distinct, multifactorial entity. This paradigm began to shift in the early 21st century as it became increasingly apparent that the

Correspondence to: Nohra Chalouhi, MD, Department of Neurosurgery, University of Florida, PO Box 100265, Gainesville, FL 32610. E-mail: nohra.elchalouhi@neurosurgery.ufl.edu

This manuscript was sent to Sean Savitz, MD, Guest Editor, for review by expert referees, editorial decision, and final disposition.

For Sources of Funding and Disclosures, see page 11.

© 2021 The Authors. Published on behalf of the American Heart Association, Inc., by Wiley. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

JAHA is available at: [www.ahajournals.org/journal/jaha](http://www.ahajournals.org/journal/jaha)

ischemic areas did not necessarily correlate with the distribution of spastic arteries and DCI/hypoperfusion could occur without the presence of vasospasm.<sup>15–18</sup> The CONSCIOUS (Clazosentan to Overcome Neurological Ischemia and Infarction Occurring After Subarachnoid Hemorrhage) trials<sup>19–21</sup> were foundational in transforming the understanding of DCI pathology by demonstrating that prevention of vasospasm does not necessarily reduce all-cause mortality or DCI. The renewed interest in clinical investigation also prompted a unified definition of DCI<sup>22</sup>:

*The occurrence of focal neurological impairment ... or a decrease of at least 2 points on the Glasgow Coma Scale ... This should 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 or MRI scanning of the brain, and appropriate laboratory studies.*

This definition is now widely used in clinical studies, facilitating efficient investigation and reliable meta-analysis. The SAHIT (Subarachnoid Hemorrhage International Trialists) Repository has also aided the development of well-designed, harmonized clinical trials by identifying critical data points.<sup>23</sup> Since then, further study into the underlying pathophysiology has revealed previously elusive effects on the microvasculature and inflammatory milieu associated with DCI that can inform future clinical trials and drug development.<sup>24,25</sup>

DCI is currently understood as a multifactorial process that evolves over time. The first 24 to 48 hours after ictus are referred to as the early brain injury phase, largely characterized by the sequelae of increased intracranial pressure and transient global ischemia during ictus. Cerebral edema, blood-brain barrier (BBB) disruption, sympathetic nervous system activation, autoregulatory failure, microthrombosis, spreading depolarizations (SDs), and inflammation have all been observed during this period.<sup>26</sup> Over time, the extravasated blood begins to aggravate and modulate the same core factors, culminating in the clinical manifestation of delayed cerebral ischemia around 4 to 10 days post-SAH.<sup>12,27</sup>

The purpose of this review is to both examine the current state of investigation into DCI as well as analyze the underlying mechanisms of the disease. In addition, we review novel therapeutic strategies as they relate to the novel insights into DCI pathophysiology. The pathological components of DCI are intimately interconnected, but for the purposes of this review we discuss 3 overarching areas: vascular dysfunction, inflammation, and cortical spreading depolarizations.

For each topic we review the foundational studies demonstrating a role in DCI, the most recent advances within the field, and therapeutic strategies gleaned from those developments.

## VASCULAR DYSFUNCTION

Inability of cerebral perfusion to match metabolic demand is the ultimate cause of DCI; thus any pathological event that decreases perfusion or increases metabolic demand can contribute to DCI. In this section, we focus on the former, specifically the mechanisms of inadequate vascular response that increase susceptibility to DCI.

Because of the lasting influence of the vasospasm-centered approach to DCI research, many vasodilatory or otherwise vasoactive agents have been tested in patients with SAH (Table). Triple H therapy (hypertension, hypervolemia, and hemodilution) or permissive hypertension alone are intended to mechanically vasodilate by intravascular volume expansion but are prone to cardiopulmonary and renal complications.<sup>28,29</sup> A meta-analysis of Triple H therapy found that, in addition to methodological issues in standardizing treatments, there was no effect on DCI.<sup>30</sup> As mentioned previously, the CONSCIOUS trials demonstrated that inhibition of the vasoconstrictive endothelin-1 pathway decreases vasospasm but has no effect on functional outcomes.<sup>19–21</sup> Another phase 3 clinical trial with clazosentan, a selective endothelin-1 receptor antagonist, has been announced since the end of CONSCIOUS-3, the REACT trial.<sup>31</sup> Unlike the CONSCIOUS trials that used a composite primary endpoint (all-cause mortality, DCI, or need for vasospasm rescue therapy), REACT will focus on the development of DCI. Additionally, the REACT trial will use the higher of the 2 clazosentan doses administered in CONSCIOUS-3 because of more support for possible efficacy. The MASH-2 (Magnesium for Aneurysmal Subarachnoid Haemorrhage-2) trial showed intravenous magnesium sulfate, putatively acting through inhibition of voltage-gated calcium channels,<sup>32</sup> is also not effective for improving outcomes.<sup>33</sup> Oral administration of the dihydropyridine-type calcium channel blocker nimodipine is the only treatment with consistent, high-quality evidence for decreasing DCI<sup>34</sup> and is now standard of care in patients with aSAH, although these results are principally driven by 1 large trial.<sup>35</sup> Importantly, those early studies showed oral nimodipine reduces DCI and improves outcomes without affecting vasospasm,<sup>36,37</sup> suggesting nimodipine may have important vessel-independent effects. A recent trial (NEWTON [Nimodipine Microparticles to Enhance Recovery While Reducing Toxicity After Subarachnoid Hemorrhage]) of intraventricular nimodipine administration found no improvements over standard oral administration.<sup>38</sup> These clinical trials clearly demonstrate that

**Table 1. Summary of Major Clinical Trials and Meta-Analyses**

Candidate	Mechanism	No. Patients Included in Trial or Meta-Analysis	Summary of Findings
Clazosentan <sup>19-21</sup> (IV)	Endothelin-1 receptor antagonist.	CONSCIOUS-2: 1147 total (764 treatment/383 placebo) CONSCIOUS-3: 571 total (188 high-dose/194 standard dose/189 placebo)	Clazosentan treatment (5 or 15 mg/h) does not improve outcomes after aSAH. Possible increase in pulmonary complications, anemia, and hypotension
Magnesium sulfate <sup>33</sup> (IV)	Inhibition of voltage-gated calcium channels, N-methyl-D-aspartate receptors, & glutamate release	Magnesium for Aneurysmal Subarachnoid Haemorrhage-2: 1201 total (604 treatment/597 placebo)	Intravenous magnesium sulfate therapy does not improve outcomes after aSAH
Simvastatin <sup>39,40</sup> (oral)	$\beta$ -Hydroxy $\beta$ -methylglutaryl-CoA reductase inhibition, but has important pleiotropic effects including improved endothelial function and decreased platelet activation	Simvastatin in Aneurysmal Subarachnoid Haemorrhage: 809 total (391 treatment/412 placebo) High-Dose Simvastatin for Aneurysmal Subarachnoid Hemorrhage: 255 total (124 high dose/131 standard dose)	Simvastatin (40 or 80 mg/d) does not improve short- or long-term outcomes after aSAH
Tirilazad <sup>41</sup> (IV)	Free radical scavenging and cell membrane stabilization	Meta-Analysis: 3821 total across 5 double-blind, placebo-controlled trials	Tirilazad does not reduce mortality or improve outcomes after aSAH
Cilostazol <sup>42</sup> (oral)	Phosphodiesterase enzyme <sub>3</sub> inhibition, leading to increased PKA activity. PKA relaxes vascular smooth muscle and inhibits platelet activation through multiple pathways	Meta-Analysis: 543 total across 5 studies	Cilostazol reduced "symptomatic vasospasm" and improved outcomes after aSAH; however, the component studies included in the meta-analysis were small and mostly not placebo controlled. A larger randomized controlled trial is needed
Calcium-channel blockers <sup>34,35</sup> (including oral nimodipine)	Inhibition of L-type calcium channels	Meta-Analysis: 3361 total across 16 trials; 6 trials specifically for oral nimodipine (969 patients total)	Oral nimodipine reduces incidence of poor outcomes and delayed cerebral ischemia. Importantly, the results are driven primarily by a large single-center study

aSAH indicates aneurysmal subarachnoid hemorrhage; CONSCIOUS, Clazosentan to Overcome Neurological Ischemia and Infarction Occurring After Subarachnoid Hemorrhage; and PKA, protein kinase A.

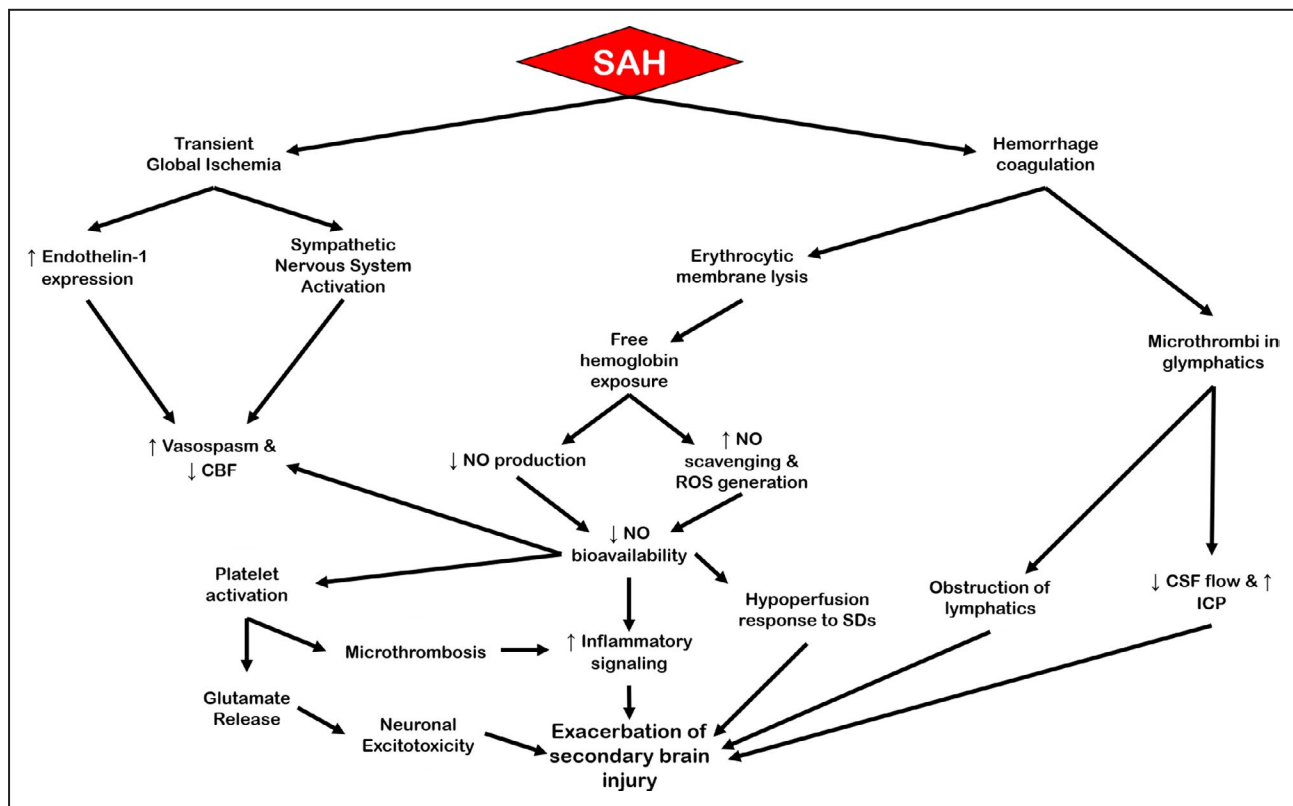
targeting vascular dysfunction through vasodilation alone is not sufficient to reduce DCI.

In this article we highlight the clinical and animal studies that elucidated the fundamental processes of vascular dysfunction after SAH and those that expanded the understanding to include microvessels, arterioles, paravascular spaces, and lymphatic vessels. We also discuss the recently discovered mechanisms of vascular dysfunction that can be leveraged in the development of novel therapies.

### Inciting Factors of Vascular Dysfunction

The first physiologic insult after SAH is a transient global ischemia as intracranial pressure approaches mean arterial pressure.<sup>43,44</sup> This ischemic episode can trigger vascular dysfunction even before the toxic effects of hemoglobin are realized (Figure 1). First, the process is initiated through an induction of the sympathetic nervous system,<sup>45</sup> often referred to as the "sympathetic surge" or "catecholamine surge." Initial activation involves both ischemic injury to the hypothalamus<sup>46</sup> as well as compression of the brain stem.<sup>47</sup> Plasma catecholamines remain elevated for several days post-SA<sup>45</sup> and high concentrations predict poor

outcomes,<sup>48,49</sup> perhaps involving injury to extracerebral organs.<sup>50</sup> A recent study by Takemoto et al demonstrated that attenuation of the sympathetic response via bilateral renal denervation decreases vasospasm and cerebral edema in rats.<sup>51</sup> Behavioral and neurological responses were not affected by this intervention, indicating that reducing sympathetic nervous system activation alone is not sufficient to prevent neurological deficits. Separate from sympathetic nervous system activation, transient global ischemia causes endothelial injury and BBB disruption as well. Endothelial injury and even apoptotic cell death have been reported to occur within the first 24 hours post-SA<sup>52,53</sup> which disrupts the BBB and promotes coagulation by exposing subendothelial collagen.<sup>54</sup> BBB disruption can be measured indirectly in humans, via abnormal tissue enhancement on contrast computed tomography or isotope scintigraphy studies.<sup>55</sup> Endothelial injury, BBB disruption, and the resulting vasogenic edema are all important avenues for future investigation, as all are predictive of patient outcome.<sup>55,56</sup> Yet another consequence of acute global ischemia is stimulation of the endothelin-1 pathway. Using a primate model, Pluta et al found that hypoxia, not oxyhemoglobin, was



**Figure 1. Vascular dysfunction after subarachnoid hemorrhage.**

Transient global ischemia and free hemoglobin toxicity are the ultimate sources of vascular dysfunction leading to microthrombosis and vasospasm. Perturbation of the NO pathway is a pivotal mechanism connecting vascular dysfunction to inflammation and cortical spreading ischemia. The glymphatic system and meningeal lymphatic vessels are also emerging as a possible mediator of delayed cerebral ischemia. CBF indicates cerebral blood flow; CSF, cerebrospinal fluid; ICP, intracranial pressure; ROS, reactive oxygen species; SAH, subarachnoid hemorrhage; and SDs, spreading depolarizations.

responsible for the SAH-induced increase in endothelin-1 expression.<sup>57</sup> Some early clinical studies into this pathway found that plasma endothelin-1 concentrations correlated with DCI<sup>58</sup> and endothelin-1 inhibition reversed SAH-associated reductions in cerebral blood flow.<sup>59</sup> So, although endothelin-1 antagonism alone does not prevent DCI, it is still important to consider the acute phase sequelae of increased intracranial pressure that occur independently of hemoglobin-mediated vascular dysfunction.

Management of elevated intracranial pressure and hydrocephalus is also significant to outcomes after SAH. Aggressive clearance of cerebrospinal fluid (CSF) through continuous external ventricular drainage is a conceptually tempting approach to lower intracranial pressure and accelerate clearance of spasmogenic blood products; however, this strategy does not lead to decreased DCI.<sup>60</sup> Further, intermittent CSF drainage and rapid external ventricular drainage weans are associated with fewer complications and shorter intensive care unit length of stay.<sup>60–62</sup> Lumbar drainage has emerged as an alternative to external ventricular drainage that has the potential to provide the benefits

of CSF drainage, including reduced vasospasm, with a lower rate of complications.<sup>63,64</sup> There is an ongoing trial assessing neurapheresis using a lumbar drainage filtration system as an intervention to reduce DCI.<sup>65</sup> These approaches demonstrate the ability of refined techniques to not only prevent complications but actively suppress DCI pathology as well.

Hemoglobin and other blood products remain sequestered until the erythrocytic membranes become unstable and lyse, releasing oxyhemoglobin and other vasoactive blood products. Oxyhemoglobin and deoxyhemoglobin concentrations in the CSF peak around day 7 post-SAH in primates,<sup>66</sup> roughly corresponding to the onset of secondary brain injury. Even before the precise mechanisms were clarified, the hypothesis that hemoglobin must be the primary spasmogen was supported by reports that hematoma evacuation prevents vasospasm in primates.<sup>67,68</sup> A few years later, purified oxyhemoglobin alone was shown to induce a contractile response in canine cerebral arteries<sup>69</sup> and later studies demonstrated this occurred through the Rho/ROCK (Rho/Rho-associated protein kinase) and PKC (protein kinase C) pathways.<sup>70</sup>

Fasudil, an inhibitor of the Rho/ROCK pathway, has been shown to reduce smooth muscle cell contraction, reduce vasospasm, and improve clinical outcomes after aSAH,<sup>71–73</sup> but it is not approved for use by the Food and Drug Administration or European Medicines Agency. Cilostazol, a phosphodiesterase enzyme<sub>3</sub> inhibitor that relaxes vascular smooth muscle and inhibits platelet activation,<sup>74</sup> has also been shown to reduce vasospasm and improve clinical outcomes in several trials.<sup>42,75</sup> Oxyhemoglobin is also a potent scavenger of NO and reduces the availability of NO in the surrounding cerebral vasculature.<sup>76,77</sup> Production of NO is unable to compensate for this loss owing to a rise in asymmetric dimethylarginine,<sup>78,79</sup> an endogenous nitric oxide synthase (NOS) inhibitor, and decreased expression of endothelial- and neuronal-specific NOS isoforms.<sup>80,81</sup> The remaining NOS enzymes are also damaged through oxidation of essential enzymatic cofactors by reactive oxygen species from hemoglobin metabolism and local inflammation. This results in the “NOS uncoupling” phenomenon whereby consumption of substrates L-arginine and O<sub>2</sub> is “uncoupled” from NO production and instead results in superoxide (O<sub>2</sub><sup>•-</sup>) generation.<sup>82</sup> The presence of superoxide further reduces NO bioavailability by reacting with the remaining NO to form peroxynitrite, a potent oxidizing agent.<sup>82</sup> This perfect storm of vasoconstrictive, NO-depleting, and reactive oxygen species-generating events after SAH is central to the resulting vascular dysfunction (Figure 1). This has been demonstrated experimentally by administration of NO-donors, which improved cerebral hemodynamics in humans and non-humans primates after SAH<sup>83,84</sup>; however, NO donors have little translational potential owing to their adverse effects.<sup>83</sup> Nonspecific antioxidant therapies are also ineffective after SAH, demonstrated most clearly by the failure of tirilazad to improve outcomes in a meta-analysis of 3821 patients.<sup>41</sup> Interestingly, the clinical importance and relative pathological contribution of the “NOS uncoupling” phenomenon is still not completely resolved, as some studies report genetic knockout of endothelial NOS in mice reduces vascular dysfunction after SAH whereas others show phenotypes similar to wild-type mice.<sup>85,86</sup> In any case, future therapeutic application must account for the complexities of the NOS pathway and the context-dependent relationship between NOS activation and NO bioavailability.

### Microthrombosis and Thromboinflammation

The NO-cyclic guanosine monophosphate pathway is central to preserving vascular homeostasis through inhibition of platelet aggregation, leukocyte adhesion, and smooth muscle cell proliferation in addition to maintaining vasodilatory tone.<sup>87–90</sup> The procoagulant

effects of platelet aggregation and spasm within intraparenchymal arterioles, known as microthrombosis, has been an emerging area of intense research because the incidence of microclots was shown to correlate with DCI and clinical outcome.<sup>15,91–94</sup> The endothelial protease ADAMTS13 normally represses platelet adhesion and thrombosis-induced inflammatory change through downregulation of von Willebrand factor and P-selectin,<sup>95</sup> making microvascular endothelial injury and ADAMTS13 dysregulation a potential link between microthrombosis and pathological inflammation. Interestingly, decreased ADAMTS13 activity and increased von Willebrand factor and P-selectin levels all predict the development of DCI in patients with SAH,<sup>96–99</sup> indicating thromboinflammation could be a clinically relevant therapeutic target. Preclinical animal models have demonstrated increased P-selectin expression in the microvascular endothelium after SAH corresponding to areas of microthrombosis and neuronal cell death.<sup>100</sup> Moreover, treatment with a monoclonal anti-P-selectin antibody can reduce platelet-endothelial and leukocyte-endothelial interactions,<sup>101</sup> suggesting P-selectin may be a particularly suitable translational candidate to target thromboinflammatory pathways. In addition to inciting inflammation, activated platelets release glutamate,<sup>102</sup> high concentrations of which can be neurotoxic.<sup>103</sup> In rats, glutamate levels in the CSF increase after SAH<sup>104</sup> and there is an association between the location of microthrombi and regional markers of excitotoxicity,<sup>105</sup> indicating that the platelets within microthrombi could be the source of toxic glutamate. Moreover, glutamate and glutamate receptor activity are important regulators of the incidence and propagation of spreading depolarizations (see “Spreading Depolarizations”). A small, exploratory study in humans found extracellular glutamate concentrations rise after SAH, vary from region to region, and may predict clinical outcome.<sup>106</sup> Although more study is needed to confirm and expand upon these findings, regional variation in glutamate levels could be explained by the presence of microthrombi in areas that progress to delayed ischemia. Taken together, the available evidence strongly suggests microcirculatory dysfunction is central to DCI pathology and an important area for more intense investigation.

There have been several trials aiming to reduce DCI through inhibiting coagulation or platelet aggregation. An important point to consider in the interpretation of these studies is that the effects of anticoagulative therapies on primary hematoma dissolution and prevention of secondary microthrombi cannot be readily discerned from one another. The design of these protocols has to be done carefully as rebleeding of a previously secured aneurysm and other hemorrhagic complications are of primary concern with these treatment modalities. Systemic anticoagulation with

enoxaparin was shown to reduce DCI when given to Hunt-Hess grade I–III patients,<sup>107</sup> but this effect was lost when the patient population was expanded to include more severe hemorrhage.<sup>108</sup> Three retrospective studies have also found that treatment with low-dose intravenous heparin reduces DCI and improves functional outcomes.<sup>109–111</sup> A rat model suggests that heparin may work through reducing the neuroinflammatory response to SAH.<sup>112</sup> Antiplatelet therapy has also been tested through trials of aspirin, ozagrel hydrochloride, dipyridamole, and ticlopidine. A meta-analysis (1385 patients total) of trials with these agents to date showed a modest trend for better outcomes but also a possible increase in hemorrhagic complications.<sup>113</sup> A recent retrospective study found dual therapy of aspirin and clopidogrel was associated with reduced incidence of DCI and no increased risk of hemorrhagic complications,<sup>114</sup> suggesting antiplatelet therapy could be useful with refined protocols. We are currently evaluating the glycoprotein IIb/IIIa inhibitor tirofiban as one such therapeutic. Compared with aspirin and clopidogrel, tirofiban has a narrow therapeutic window, making it an ideal antiplatelet agent for aSAH that may require further neurosurgical interventions.<sup>115</sup> After carefully establishing safety and efficacy profiles in patients with aSAH,<sup>116,117</sup> a small trial showed promising reductions in DCI without increase hemorrhagic complications.<sup>118</sup> Larger clinical trials will be instrumental in definitive evaluation of tirofiban and other antiplatelet therapies as a treatment to prevent DCI.

### Glymphatic and Meningeal Lymphatic System

Lack of a lymphatic system was long believed to be a unique characteristic of the central nervous system until Louveau et al discovered lymphatic vessels within the lining of the dural sinuses that interface with the deep cervical lymph nodes.<sup>119</sup> This discovery led to the hypothesis that alterations in this novel meningeal lymphatic system and the central nervous system (CNS) paravascular glymphatics contribute to the development of DCI. Even before the discovery of meningeal lymphatics, it was demonstrated that cervical lymph node blockage intensified oxidative stress after SAH.<sup>120</sup> More recently, the meningeal lymphatic system was found to be important for clearing the extravasated erythrocytes from SAH and disruption of the lymphatic vessels exacerbated the neuroinflammatory response, especially microglial activation.<sup>121</sup>

Upstream from the meningeal lymphatics, paravascular glymphatic pathways lie next to penetrating arteries and constitute the direct interface between CSF and parenchymal interstitial fluid.<sup>122</sup> This pathway provides a route for blood products from SAH to quickly penetrate brain parenchyma and stimulate neuroinflammation.<sup>123</sup>

Liu et al demonstrated that knockout of AQP4 (aquaporin 4), which is expressed in astrocytes at the interface of the paravascular pathways,<sup>124</sup> worsens outcome after SAH in rats.<sup>125</sup> Their study indicates that AQP4 may be involved in the impaired glymphatic flow observed after SAH. Further, microthrombi formed in the paravascular spaces after SAH can obstruct CSF flow through the glymphatic system and contribute to increased intracranial pressure.<sup>126</sup> Administration of tissue plasminogen activator can clear the thrombi from the paravascular space, increase cerebral blood flow in the early brain injury phase, and improve neurological function in the delayed phase.<sup>123,126</sup>

Investigation into the paravascular space and meningeal lymphatics as a therapeutic target for DCI is still in its infancy; however, the foundational studies demonstrate an exciting avenue for future research. The exposure of brain parenchyma to the toxic effects of hemoglobin, hemoglobin metabolites, and other blood products is clearly regulated by the paravascular pathways. Strategies to limit the dissemination of toxic metabolites into healthy tissue while permitting their clearance through the lymphatic system may prove valuable. Earlier research on fibrinolytic therapies presumed the therapeutic mechanism was clearing the primary hematoma or microthrombi within penetrating arterioles and capillaries. Reinterpretation of these studies with respect to the influence of paravascular microthrombi could prove worthwhile.

### Autoregulatory Failure

Cerebrovascular autoregulation is the process by which cerebral blood flow is held constant over a spectrum of perfusion pressures and blood gas partial pressures.<sup>127,128</sup> There are myogenic, neurogenic, metabolic, and endothelial factors contributing to cerebral autoregulation<sup>129,130</sup>; thus, autoregulatory failure after SAH is best conceptualized as a summative process rather than an independent pathological mechanism. Yundt et al found that patients with SAH, regardless of the presence of vasospasm, have decreased cerebral blood volume compared to age-matched healthy volunteers, whereas healthy volunteers subjected to carotid compression display an increase in cerebral blood volume.<sup>131</sup> This study was important in that it showed not just a diminished autoregulatory capacity but a complete inversion of normal function. Another similar study found diminished autoregulatory capacity, as measured by the transient hyperemic response, predicted the development of DCI.<sup>132</sup> This phenomenon has been replicated in recent years in a variety of settings and using different imaging<sup>133,134</sup> and vascular reactivity stimuli,<sup>135</sup> reaffirming the association between diminished autoregulatory capacity and development of DCI after SAH.



The nature of autoregulation as an integrative process makes its relationship with DCI difficult to delineate through interventional studies. A phase II trial found that pravastatin treatment reduced the duration of impaired autoregulation and improved outcomes compared to placebo.<sup>136</sup> Overall, the current literature supports autoregulation as a useful biomarker in clinical studies, but more research is required to determine if autoregulatory disturbance is required for the development of DCI.

## INFLAMMATION

### Systemic Inflammation After SAH

Inflammation is an extremely broad category of physiological and pathophysiological host responses to infection and tissue injury.<sup>137</sup> The severity of the inflammatory response after SAH predicts DCI and poor outcomes. Retrospective studies find that lactate concentration, CRP (C-reactive protein) levels, erythrocyte sedimentation rate, leukocyte count, negative nitrogen balance, neutrophil-lymphocyte ratio, and systemic inflammatory response syndrome burden, all nonspecific markers of inflammation, predict outcome following SAH.<sup>138–145</sup> Accordingly, systemic immunosuppression with corticosteroids was one of the first experimental treatments to prevent DCI.<sup>146,147</sup> These and more recent studies<sup>148</sup> analyzing steroid treatment have shown no effect on DCI and only a modest benefit toward functional outcome. The mixed effects of these small trials are not sufficient to prove benefit or overcome the multitude of adverse side effects; thus, glucocorticoids are not currently indicated in patients with SAH. Additional clinical trials are also unlikely after a large randomized controlled trial showed corticosteroid treatment increased mortality in the pathophysiologically related setting of traumatic brain injury.<sup>149</sup> Cyclosporine (discussed further in the next section) and nonsteroidal anti-inflammatory drugs have also been used as general anti-inflammatories after SAH. A prospective observational study of 138 patients found that cumulative nonsteroidal anti-inflammatory drug usage correlated with better outcome (Glasgow Outcome Scale score >3) and fewer cerebral infarctions.<sup>150</sup> Another study of 178 patients found nonsteroidal anti-inflammatory drug use led to lower mortality and shorter intensive care unit stay, but the effects on DCI and functional outcome were nonsignificant.<sup>151</sup> Aspirin alone has shown no benefit to DCI or outcome.<sup>152,153</sup> Overall, nonspecific anti-inflammatory therapies have proven disappointing for prevention of DCI and poor outcomes.

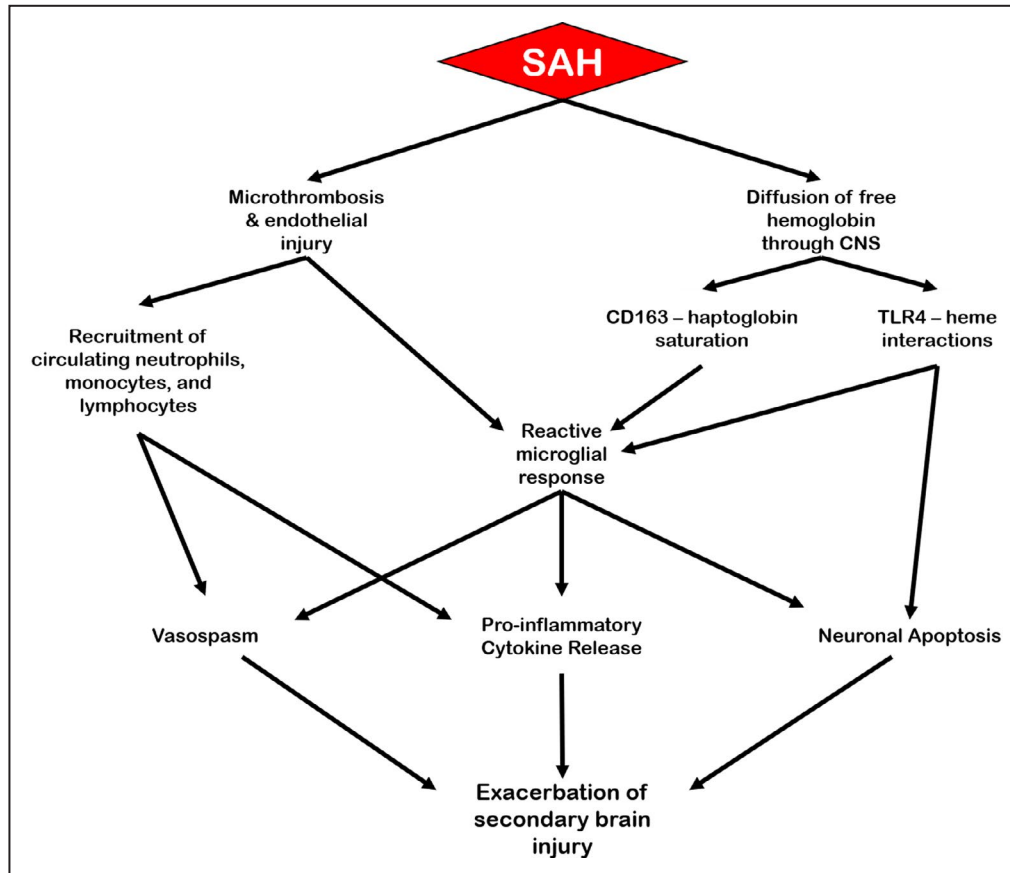
Clear delineation of the complex inflammatory cascades induced after SAH is necessary in order to develop targeted anti-inflammatory therapies. Clinical studies have demonstrated that CSF concentrations

of the classical pro-inflammatory cytokines interleukin-6, interleukin-8, interleukin-1 $\beta$ , tumor necrosis factor-alpha, and MCP-1 (monocyte chemoattractant protein-1) correlate with DCI and poor outcomes.<sup>154–157</sup> Unfortunately, many early mechanistic studies in animal models have focused primarily on vasospasm instead of neuronal cell death or behavioral outcome, obscuring their relevance to translational application. Within the last 15 years, this paradigm has begun to shift, and the cellular and molecular mechanisms of SAH-induced inflammation are emerging from animal studies. We present these advances in the context of the inflammatory cells, resident microglia, and peripheral leukocytes, which mediate host response to SAH and react to inflammatory change within the CNS.

### Cellular Mediators—Glia

Glial involvement in SAH pathology had been suspected since the establishment of free heme as a toll-like receptor 4 (TLR4) activator.<sup>158</sup> TLR4, a pattern recognition receptor central to innate immune function, is expressed in all myeloid-origin cells<sup>159</sup>; however, the function of microglia as resident TLR4-expressing cells makes them well positioned to respond first to TLR4-heme interactions in the CNS. TLR4 expression was shown to increase after SAH,<sup>160</sup> but the evidence for microglial participation in SAH pathology remained speculative until a landmark paper from Hanafy demonstrated microglia-depleted mice have reduced vasospasm and neuronal apoptosis.<sup>161</sup> The same study showed neuronal apoptosis and vasospasm are diminished in TLR4 knockout mice early after SAH but evolve to be driven by TLR4-independent mechanisms in the delayed phase. The contribution of microglia to neuronal cell death after SAH was reaffirmed by Schneider et al through selective depletion of microglia using a ganciclovir-sensitive “suicide gene.”<sup>162</sup> This study left peripheral macrophages intact to differentiate between resident and peripheral myeloid cell involvement. They found that microglia-depleted mice had decreased neuronal cell death as far out as 9 days post-SAH. Together, these studies clearly establish microglia as critical mediators of neuroinflammation and neuronal injury after SAH (Figure 2).

The precise molecular mechanisms of microglia action in the DCI phase remain elusive; the Hanafy study demonstrated neuronal cell death is TLR4-dependent only in the early brain injury phase. There are several other proposed pathways that contribute to microglia-mediated neuroinflammation after hemorrhagic stroke. In cultured microglia cells, the inflammatory reaction to thrombin exposure (ie, interleukin-6, tumor necrosis factor-alpha, CCL2/MCP1 [chemokine ligand 2/monocyte chemoattractant protein 1] production) is muted by TGF $\beta$ 1 (transforming growth factor beta 1)



**Figure 2. Mechanisms of inflammatory response after subarachnoid hemorrhage.**

Subarachnoid hemorrhage elicits an inflammatory response from resident CNS glia directly through TLR4 and CD163 receptor signaling. Reactive microglia then contribute to inflammatory cytokine production, vasospasm, and neuronal apoptosis. The endothelium of the cerebrovasculature also contributes to inflammation by recruiting circulating leukocytes. Neutrophils, monocytes, and lymphocytes all enter the CNS after SAH and promote vasospasm and inflammatory cytokine release. CD163 indicates cluster of differentiation 163; CNS, central nervous system; SAH, subarachnoid hemorrhage; and TLR4, toll-like receptor 4.

treatment.<sup>163</sup> Further, the same study showed reactive microglia express lower levels of TGF $\beta$ 1, and human patients with increased TGF $\beta$ 1 after intracerebral hemorrhage had better outcomes at 90 days; These findings have yet to be replicated in humans after SAH, but simvastatin therapy was found to induce lymphocytic TGF $\beta$ 1 expression in a rat model,<sup>164</sup> suggesting there could be similarities in TGF $\beta$ 1's actions. HMGB1 (high mobility group box 1 protein is a nuclear protein that regulates chromatin remodeling and gene transcription; however, it is also secreted as an inflammatory cytokine by myeloid lineage cells, including microglia.<sup>165</sup> Neutralization of HMGB1 with a monoclonal antibody attenuates microglial reactivity and improves neurological function after SAH.<sup>166</sup> In humans, higher HMGB1 CSF concentrations are correlated with unfavorable outcomes.<sup>156</sup> Unfortunately, these studies are unable to differentiate between microglia-derived and macrophage/monocyte-derived HMGB1, complicating their

interpretation. Future investigation should evaluate the mechanisms of microglial involvement in the delayed phase after SAH.

### Cellular Mediators—Peripheral Leukocytes

An association between peripheral immune response and outcome after SAH has been observed for many years,<sup>167</sup> including recently when outcome measures were updated to match the current consensus definition of DCI.<sup>143</sup> Markers of both myeloid and lymphoid lineage cells have also been directly observed in the CSF and tissue near the subarachnoid space, implying these cells could be directly involved with SAH pathology.<sup>154,168,169</sup> Other correlational studies support this notion by showing the degree of peripheral immune reaction predicts outcome.<sup>140,141,170</sup> One hypothesis to explain this phenomenon is that peripheral immune cells respond when the resident CNS macrophages

(microglia) are overwhelmed by massive hemolysis following SAH. The baseline capacity of the CNS CD163 (cluster of differentiation 163)-haptoglobin scavenger system is much lower than in the periphery and is easily saturated after SAH.<sup>171</sup> Administration of haptoglobin into the subarachnoid space has been shown to reduce vasospasm.<sup>172</sup> Haptoglobin genotype also affects hemoglobin affinity and outcome after SAH indicating that the response after CD163-haptoglobin saturation could be responsible for some of the detrimental effects.<sup>173,174</sup> Cyclosporine A, an immunosuppressant that acts primarily through T cells but also inhibits myeloid cell function,<sup>175</sup> was used to target this immune reaction but has resulted in mixed effects in underpowered trials and animal studies.<sup>176–179</sup> Animal studies have shown generally positive results from peripheral myeloid cell depletion. Monoclonal antibody-mediated neutralization of CD11b/CD18-positive cells resulted in decreased vasospasm in rabbits and nonhuman primates.<sup>180,181</sup> The limitation of these studies is the emphasis on vasospasm as an outcome measure rather than DCI. The expression of CD11b/CD18 on multiple cell types also limits their interpretation. A similar study from Provencio et al improved on previous studies by demonstrating improved functional outcome in addition to decreased vasospasm in mice after treatment with anti-lymphocyte antigen 6 complex antibody.<sup>182</sup> Lymphocyte antigen 6 complex is primarily expressed on neutrophils but certain monocyte, macrophages, and lymphocyte subpopulations also express this marker.<sup>183</sup> Taken together, these data show a strong scientific premise for peripheral myeloid and lymphoid cell involvement in the SAH pathology (Figure 2). More studies with emphasis on DCI and functional outcome are needed before translational therapies can be trialed in human patients.

By responding to inflammatory change on endothelial surfaces, circulating immune cells serve as the link between vascular dysfunction, vascular inflammation, and systemic immune response. Cell adhesion molecules (CAMs) are a family of proteins that facilitate immune cell–endothelium interaction after vascular injury.<sup>184</sup> Increased CAM expression after SAH is well-established, having been demonstrated in the serum and CSF of humans as well as directly in the vascular and cerebral tissues of animal studies.<sup>185–187</sup> A study by Polin et al showed several CAMs (intercellular adhesion molecule-1 [ICAM-1], VCAM-1, and E-selectin) are upregulated in CSF after SAH and E-selectin levels correlate with poor outcomes.<sup>188</sup> Another study similarly showed P-selectin but not ICAM-1, vascular CAM-1, or platelet endothelial CAM was increased in patients with low-grade SAH and DCI.<sup>98</sup> These studies suggest that the selectin subtype of CAMs may be more important in the development of DCI than the immunoglobulin

superfamily subtype (ICAM-1, vascular CAM-1, etc); however, no interventional studies have proven a direct role. One study has shown E-selectin inhibition decreases vasospasm in rodents.<sup>189</sup> Treatments neutralizing ICAM-1 via antibody treatment have shown decreased leukocyte infiltration, demonstrating the putative mechanism of CAMs in DCI pathology.<sup>190–192</sup> One of these studies coadministered an anti-CD18 (ICAM-1 ligand) antibody and found that vasospasm was decreased more than with anti-ICAM-1 treatment alone, suggesting that inflammatory cells contribute to vasospasm in a partially CAM-independent fashion.<sup>192</sup> The use of vasospasm as outcome rather than neurobehavioral function is a major limitation of these studies; nonetheless, the entirety of the current literature supports the hypothesis that peripheral immune cells respond to inflammatory changes in the endothelium after SAH. More investigation into these pathways with a focus on functional outcome is necessary to determine their utility as therapeutic targets.

Statins have been tested extensively owing to their anti-inflammatory effects independent of hepatic  $\beta$ -Hydroxy  $\beta$ -methylglutaryl-CoA reductase inhibition.<sup>193</sup> Some early single-center randomized controlled trials with simvastatin<sup>194</sup> and pravastatin<sup>136</sup> showed reductions in vasospasm and DCI, encouraging more investigation into statin therapy. The largest trials to date (STASH [Simvastatin in Aneurysmal Subarachnoid Haemorrhage] and HDS-SAH [High-Dose Simvastatin for Aneurysmal Subarachnoid Hemorrhage]), however, demonstrated simvastatin does not improve outcomes or reduce DCI.<sup>39,40</sup>

## SPREADING DEPOLARIZATIONS

### Physiology of Spreading Depolarizations

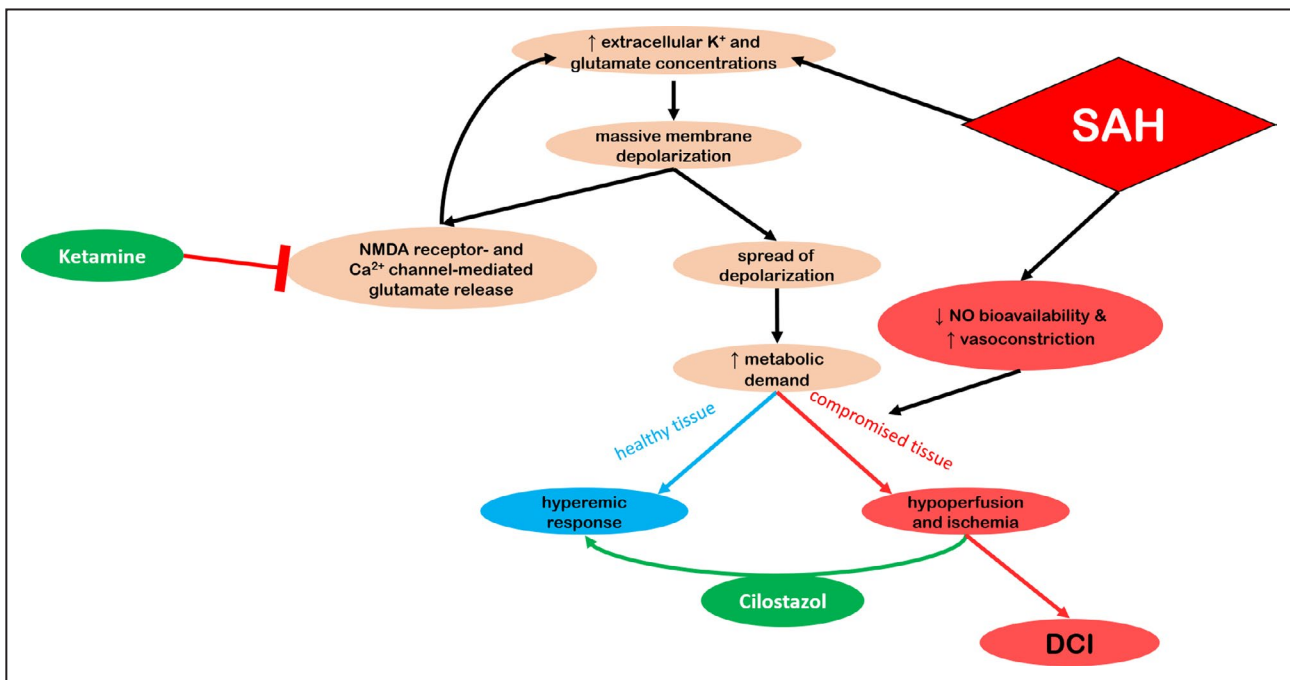
Spreading depolarizations, as the term implies, are slowly propagating waves of almost complete membrane depolarizations in both neuronal and glial cells. Usage of the terms “spreading depolarization” and “spreading depression” often varies between authors and disciplines; for the purposes of this review, we consider “spreading depolarization” to best describe the underlying biophysical phenomenon, “spreading depression” to be its manifestation as decreased neuroelectric activity, and use the initialism “SD” to refer in general to both spreading depolarizations and depressions. Spreading depressions were first discovered by Leão in 1944 while studying epilepsy and he noted its hyperemic effect on pial vasculature soon after.<sup>195,196</sup> In healthy brain tissue, SDs can be elicited by increasing extracellular  $K^+$  concentrations to a point where passive ion channels open and overload the capacity for ATP-dependent  $Na^+$ ,  $K^+$ , and  $Ca^{2+}$  pumps to maintain ion homeostasis.<sup>197</sup> SDs are initiated by similar mechanisms in metabolically compromised brain tissue but

can be more severe and longer lasting.<sup>198</sup> Mechanisms of SD propagation are still under intense investigation, but it is generally accepted that passive diffusion of extracellular K<sup>+</sup> and glutamate provoke depolarization in surrounding grey matter.<sup>198,199</sup> A positive feedback loop mediated by N-methyl-D-aspartate receptor- and Ca<sup>2+</sup> channel-dependent glutamate release and other voltage-gated channels also seems crucial to the self-sustaining nature of SDs.<sup>198</sup> Because of their uniqueness and powerful suppression of normal brain activity, SDs have been studied extensively in the context of many neurological disorders and diseases including stroke.<sup>199</sup>

### Spreading Depolarizations After SAH

SDs evoke a hyperemic response in healthy tissues as a result of the increased metabolic demand during disrupted ion homeostasis.<sup>200</sup> Injured or otherwise compromised brain tissues are more prone to neurovascular uncoupling and often show hypoperfusion after SDs (Figure 3).<sup>200</sup> These depolarizations are also sometimes referred to as “peri-ischemic depolarizations” or “cortical spreading ischemia,” although they are functionally indistinguishable from SDs when they spread into healthy tissue.<sup>201,202</sup> Early investigation into neuroelectric sequelae of cerebrovascular disease revealed that ischemic stroke induces multiple

occurrences of SDs through the cortex.<sup>203</sup> Just a few years later, Dreier et al demonstrated that topical application of K<sup>+</sup> and hemoglobin could induce SDs/cortical spreading ischemia in rats.<sup>204</sup> K<sup>+</sup> cations and free hemoglobin in the subarachnoid space are characteristic of hemolyzed red blood cells after SAH, leading to the hypothesis that SDs induced in this manner might play a pathologic role in the poor outcomes after SAH. Consistent with this notion, Dreier et al again showed that “products of hemolysis” (K<sup>+</sup> and hemoglobin added to artificial CSF) cause SDs and cortical spreading ischemia in the cortex as well as massive neuronal cell death and reactive gliosis (Figure 3).<sup>205</sup> A landmark paper from the same group found that SDs occur after SAH in humans and predict the development of DCI.<sup>206</sup> Later studies also revealed that clusters of SDs magnify the duration of tissue hypoxia and that clustered SDs may be more important to DCI pathology than isolated depolarizations.<sup>207,208</sup> Further, the correlation between SDs and DCI remains even after the successful treatment of angiographic vasospasm.<sup>209</sup> The totality of these studies, driven in large part by the COSBID (Co-Operative Studies on Brain Injury Depolarizations) Study Group, have revolutionized the conceptualization of DCI pathology. SDs and related factors are now rightly at the forefront of investigation into improving SAH outcomes.



**Figure 3. Spreading depolarizations after subarachnoid hemorrhage and potential therapeutic targets.** Spreading depolarizations cause cerebral ischemia by increasing metabolic demand in injured tissue unable to compensate with increased perfusion. SAH itself also promotes the development of spreading depolarizations by the release of K<sup>+</sup> and glutamate from extravasated erythrocytes and platelets. A couple of promising therapeutic agents to prevent spreading depolarizations/cortical spreading ischemia are ketamine and cilostazol. Ketamine works through inhibiting NMDA receptors and the propagation of spreading depolarizations. Cilostazol reduces ischemia by improving neurovascular response to depolarization. DCI indicates delayed cerebral ischemia; NMDA, N-methyl-D-aspartate; and SAH, subarachnoid hemorrhage.

Downloaded from <http://ahajournals.org> by on August 18, 2021

## Spreading Depolarizations as a Therapeutic Target

Inquiry into treatments that target SDs/peri-ischemic depolarizations to prevent DCI is still in its early stages; nonetheless, several promising avenues of investigation have been identified. Vasoactive drugs can modulate the neurovascular response to SD and prevent pathological hypoperfusion. The earliest work was based on the finding from Dreier et al that inhibition of NOS produced similar SD effects as topical hemoglobin application, implying that NO scavenging and vasoconstriction by hemoglobin could be critical to transforming the hyperemic SD response into a cortical spreading ischemia response.<sup>204</sup> Treatment with NO-donors and NO-independent vasodilators reduced the ischemia/hypoperfusion after K<sup>+</sup>-induced SDs.<sup>210</sup> The same group of researchers also showed nimodipine treatment reduces SD-induced ischemia in rats.<sup>211</sup> Years later, the phosphodiesterase enzyme<sub>3</sub> inhibitor cilostazol was shown to reduce spreading ischemia after mimicked SAH-induced SDs (Figure 3).<sup>212</sup> The same study tested cilostazol in a relatively small number of human patients with aSAH and observed a nonsignificant trend for decreased DCI, indicating a larger clinical trial may be worthwhile. These studies exemplify the interdependency between pathological factors after SAH. Given the discrepancy in perfusion response between healthy and injured tissues, SDs/peri-ischemic depolarizations reveal vulnerabilities in the cerebrovasculature that might not have otherwise progressed far enough to cause DCI. The second area of investigation into therapeutics is direct inhibition of SD propagation. N-methyl-D-aspartate receptors have long been known to play a pivotal role in SD propagation in otherwise healthy tissue<sup>213–215</sup>; thus, N-methyl-D-aspartate receptor antagonism was a logical place to explore post-SAH therapies. A 2012 study analyzing various classes of sedatives and analgesics found that the N-methyl-D-aspartate receptor antagonist ketamine decreased the incidence of SDs in patients with traumatic brain injury and SAH, whereas midazolam increased SDs and propofol, fentanyl, and morphine had no effect.<sup>216</sup> More recent studies have found that ketamine reduces SDs in a dose-dependent fashion and can inhibit SD incidence when started in patients with SAH who have already had multiple SDs (Figure 3).<sup>217,218</sup> Larger clinical trials are needed to fully evaluate the efficacy of ketamine in this context. The anticonvulsant valproate has also been investigated based on its SD-inhibiting properties in healthy tissue.<sup>219,220</sup> Valproate treatment was found to reduce cerebral lesion growth after SAH with and without added SD induction (topical potassium chloride application).<sup>221</sup> To our knowledge, these findings have yet to be replicated in human patients

with SAH but still contribute to the mounting evidence that blocking SD propagation could yield favorable outcomes. Additionally, older treatments need to be reassessed with respect to their effects of SDs. As previously discussed, nimodipine reduces SD-induced ischemia and it is tempting to assume this occurs through inhibition of L-type calcium channels in the smooth muscle of the cerebrovasculature; however, nimodipine can work in a vessel-independent fashion to directly alter the ion flux/electrical response to stress in neurons.<sup>222</sup> This finding could help explain why nimodipine reduces secondary ischemia after SAH without reducing vasospasm.<sup>34,36,37</sup> The scientific rationale for targeting SDs to reduce DCI is strong; now the objective of the field is to conduct powerful clinical trials in order to demonstrate a clear benefit in patients.

## CONCLUSIONS

Rigorous investigation into the pathophysiology of delayed cerebral ischemia is imperative to improve outcomes following SAH. The efficacy of current standard of care is suboptimal and large trials of new therapeutics have failed to demonstrate benefit. We believe a deeper understanding of DCI will lead to novel therapeutic strategies and improve the lives of those who suffer from this devastating disease. The goal of this review was to assist in this endeavor by providing an up-to-date examination of the literature in regard to 3 main areas of DCI pathology: vascular dysfunction, inflammation, and spreading depolarizations. Moreover, we pay special attention to the relationships between these areas in order to gain an integrative perspective of DCI and properly interpret study results. This analysis also serves to accentuate the recent discoveries that are the most promising candidates for clinical investigation.

## ARTICLE INFORMATION

Received March 29, 2021; accepted June 9, 2021.

### Affiliations

Department of Neurosurgery, College of Medicine, University of Florida, Gainesville, FL (W.S.D., D.L., K.H., A.J.P., B.L.H., N.C.); Department of Neurological Surgery, School of Medicine, Tulane University, New Orleans, LA (A.S.D.); Department of Neurosurgery, Carver College of Medicine, University of Iowa, Iowa City, IA (D.M.H.); Department of Neurological Surgery, Sidney Kimmel Medical College, Thomas Jefferson University, Philadelphia, PA (P.M.J.); and Department of Neurological Surgery, Miller School of Medicine, University of Miami, FL (R.M.S.).

### Sources of Funding

This work was supported by the National Institutes of Health (R01NS110710), the Brain Aneurysm Foundation, the James and Brigette Marino Family Professorship Endowment, the Eblen Research Endowment, and the Christine Desmond Fund.

### Disclosures

None.

## REFERENCES

- Hop JW, Rinkel GJE, Algra A, van Gijn J. Case-fatality rates and functional outcome after subarachnoid hemorrhage. *Stroke*. 1997;28:660–664. DOI: 10.1161/01.STR.28.3.660.
- Lovelock CE, Rinkel GJE, Rothwell PM. Time trends in outcome of subarachnoid hemorrhage: population-based study and systematic review. *Neurology*. 2010;74:1494–1501. DOI: 10.1212/WNL.0b013e3181dd42b3.
- 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;36:329–333. DOI: 10.1212/WNL.36.3.329.
- Zacharia BE, Hickman ZL, Grobelny BT, DeRosa P, Kotchetkov I, Ducruet AF, Connolly ES. Epidemiology of aneurysmal subarachnoid hemorrhage. *Neurosurg Clin N Am*. 2010;21:221–233. DOI: 10.1016/j.nec.2009.10.002.
- Rivero-Arias O, Gray A, Wolstenholme J. Burden of disease and costs of aneurysmal subarachnoid haemorrhage (aSAH) in the United Kingdom. *Cost Eff Resour Alloc*. 2010;8:6. DOI: 10.1186/1478-7547-8-6.
- Stienen MN, Germans M, Burkhardt J-K, Neidert MC, Fung C, Bervini D, Zumofen D, Roethlisberger M, Marbacher S, Maduri R, et al. Predictors of in-hospital death after aneurysmal subarachnoid hemorrhage: analysis of a nationwide database (Swiss SOS [Swiss Study on Aneurysmal Subarachnoid Hemorrhage]). *Stroke*. 2018;49:333–340. DOI: 10.1161/STROKEAHA.117.019328.
- Velat GJ, Kimball MM, Mocco JD, Hoh BL. Vasospasm after aneurysmal subarachnoid hemorrhage: review of randomized controlled trials and meta-analyses in the literature. *World Neurosurg*. 2011;76:446–454. DOI: 10.1016/j.wneu.2011.02.030.
- Mackey J, Khoury JC, Alwell K, Moomaw CJ, Kissela BM, Flaherty ML, Adeoye O, Woo D, Ferioli S, De Los Rios La Rosa F, et al. Stable incidence but declining case-fatality rates of subarachnoid hemorrhage in a population. *Neurology*. 2016;87:2192–2197. DOI: 10.1212/WNL.0000000000003353.
- Robertson EG. Cerebral lesions due to intracranial aneurysms. *Brain*. 1949;72:150–185. DOI: 10.1093/brain/72.2.150.
- Ecker A, Riemenschneider PA. Arteriographic demonstration of spasm of the intracranial arteries with special reference to saccular arterial aneurysms. *J Neurosurg*. 1951;8:660–667. Available at: <https://thejns.org/view/journals/j-neurosurg/8/6/article-p660.xml>. Accessed August 11, 2019.
- Zucker MB. A study of the substances in blood, serum, and platelets which stimulate smooth muscle. *Am J Physiol Legacy Content*. 1944;142:12–26. DOI: 10.1152/ajplegacy.1944.142.1.12.
- Roos YBWEM, De Haan RJ, Beenen LFM, Groen RJM, Albrecht KW, Vermeulen M. Complications and outcome in patients with aneurysmal subarachnoid haemorrhage: a prospective hospital based cohort study in the Netherlands. *J Neurol Neurosurg Psychiatry*. 2000;68:337–341. DOI: 10.1136/jnnp.68.3.337.
- Dorsch NW. Cerebral arterial spasm—a clinical review. *Br J Neurosurg*. 1995;9:403–412. DOI: 10.1080/02688699550041403.
- Weir B, Grace M, Hansen J, Rothberg C. Time course of vasospasm in man. *J Neurosurg*. 1978;48:173–178. DOI: 10.3171/jns.1978.48.2.0173.
- Romano JG, Rabinstein AA, Arheart KL, Nathan S, Campo-Bustillo I, Koch S, Forteza AM. Microemboli in aneurysmal subarachnoid hemorrhage. *J Neuroimaging*. 2008;18:396–401. DOI: 10.1111/j.1552-6569.2007.00215.x.
- Rabinstein AA, Weigand S, Atkinson JLD, Wijidicks EFM. Patterns of cerebral infarction in aneurysmal subarachnoid hemorrhage. *Stroke*. 2005;36:992–997. DOI: 10.1161/01.STR.0000163090.59350.5a.
- Vergouwen MDI, Ilodigwe D, MacDonald RL. Cerebral infarction after subarachnoid hemorrhage contributes to poor outcome by vasospasm-dependent and -independent effects. *Stroke*. 2011;42:924–929. DOI: 10.1161/STROKEAHA.110.597914.
- Dhar R, Scalfani MT, Blackburn S, Zazulia AR, Videen T, Diringer M. Relationship between angiographic vasospasm and regional hypoperfusion in aneurysmal subarachnoid hemorrhage. *Stroke*. 2012;43:1788–1794. DOI: 10.1161/STROKEAHA.111.646836.
- Macdonald RL, Higashida RT, Keller E, Mayer SA, Molyneux A, Raabe A, Vajkoczy P, Wanke I, Bach D, Frey 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;10:618–625. DOI: 10.1016/S1474-4422(11)70108-9.
- MacDonald RL, Higashida RT, Keller E, Mayer SA, Molyneux A, Raabe A, Vajkoczy P, Wanke I, Bach D, Frey A, et al. Randomized trial of clazosentan in patients with aneurysmal subarachnoid hemorrhage undergoing endovascular coiling. *Stroke*. 2012;43:1463–1469. DOI: 10.1161/STROKEAHA.111.648980.
- MacDonald RL, Kassell NF, Mayer S, Ruefenacht D, Schmiedek P, Weidauer S, Frey A, Roux S, Pasqualin A. 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;39:3015–3021. DOI: 10.1161/STROKEAHA.108.519942.
- Vergouwen MDI, Vermeulen M, van Gijn J, Rinkel GJE, Wijidicks EF, Muizelaar JP, Mendelow AD, Juvela S, Yonas H, Terbrugge KG, 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;41:2391–2395. DOI: 10.1161/STROKEAHA.110.589275.
- Jaja BNR, Attalla D, Macdonald RL, Schweizer TA, Cusimano MD, Etmann N, Hanggi D, Hasan D, Johnston SC, Le Roux P, et al. The Subarachnoid Hemorrhage International Trialists (SAHIT) Repository: advancing clinical research in subarachnoid hemorrhage. *Neurocrit Care*. 2014;21:551–559. DOI: 10.1007/s12028-014-9990-y.
- Clarke JV, Suggs JM, Diwan D, Lee JV, Lipsey K, Vellimana AK, Zipfel GJ. Microvascular platelet aggregation and thrombosis after subarachnoid hemorrhage: a review and synthesis. *J Cereb Blood Flow Metab*. 2020;40:1565–1575. DOI: 10.1177/0271678X20921974.
- de Oliveira Manoel AL, Macdonald RL. Neuroinflammation as a target for intervention in subarachnoid hemorrhage. *Front Neurol*. 2018;9:292. DOI: 10.3389/fneur.2018.00292.
- Flynn L, Andrews P. Advances in the understanding of delayed cerebral ischaemia after aneurysmal subarachnoid haemorrhage. *F1000Res*. 2015;4:1200. DOI: 10.12688/f1000research.6635.1.
- Topkuru B, Egemen E, Solaroglu I, Zhang JH. Early brain injury or vasospasm? An overview of common mechanisms. *Curr Drug Targets*. 2017;18:1424–1429. DOI: 10.2174/1389450117666160905112923.
- Gathier CS, Van Den Bergh WM, Van Der Jagt M, Verweij BH, Dankbaar JW, Müller MC, Oldenbeuving AW, Rinkel GJE, Slooter AJC, Algra A, et al. Induced hypertension for delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage: a randomized clinical trial. *Stroke*. 2018;49:76–83. DOI: 10.1161/STROKEAHA.117.017956.
- Egge A, Waterloo K, Sjøholm H, Solberg T, Ingebrigtsen T, Romner B. Prophylactic hyperdynamic postoperative fluid therapy after aneurysmal subarachnoid hemorrhage: a clinical, prospective, randomized, controlled study. *Neurosurgery*. 2001;49:593–606.
- Treggiari MM, Walder B, Suter PM, Romand JA. Systematic review of the prevention of delayed ischemic neurological deficits with hypertension, hypervolemia, and hemodilution therapy following subarachnoid hemorrhage. *J Neurosurg*. 2003;98:978–984. DOI: 10.3171/jns.2003.98.5.0978.
- ClinicalTrials.gov. Clinical research study with clazosentan to evaluate its effects on preventing complications due to the narrowing of the blood vessels (Vasospasm) in the brain, caused by bleeding onto the surface of the brain—NCT03585270. Available at: <https://clinicaltrials.gov/ct2/show/NCT03585270>. Accessed December 13, 2020.
- McLean RM. Magnesium and its therapeutic uses: a review. *Am J Med*. 1994;96:63–76. DOI: 10.1016/0002-9343(94)90117-1.
- Mees SMD, Algra A, Vandertop WP, Van Kooten F, Kuijsten HAJM, Boiten J, Van Oostenbrugge RJ, Salman R-S, Lavados PM, Rinkel GJE, et al. Magnesium for aneurysmal subarachnoid haemorrhage (MASH-2): a randomised placebo-controlled trial. *Lancet*. 2012;380:44–49. DOI: 10.1016/S0140-6736(12)60724-7.
- Dorhout Mees SM, Rinkel GJE, Feigin VL, Algra A, Van Den Bergh WM, Vermeulen M, Van Gijn J. Calcium antagonists for aneurysmal subarachnoid haemorrhage. *Cochrane Database Syst Rev*. 2007;2007:CD000277. DOI: 10.1002/14651858.CD000277.pub3.
- Pickard JD, Murray GD, Illingworth R, Shaw MDM, Teasdale GM, Foy PM, Humphreys PRD, Lang DA, Nelson R, Richards P, et al. Effect of oral nimodipine on cerebral infarction and outcome after subarachnoid haemorrhage: British aneurysm nimodipine trial. *BMJ*. 1989;298:636–642. DOI: 10.1136/bmj.298.6674.636.
- Philippon J, Grob R, Dageou F, Guggiari M, Rivierez M, Viars P. Prevention of vasospasm in subarachnoid haemorrhage. A controlled

- study with nimodipine. *Acta Neurochir (Wien)*. 1986;82:110–114. DOI: 10.1007/BF01456369.
37. Petruk KC, West M, Mohr G, Weir BKA, Benoit BG, Gentili F, Disney LB, Khan MI, Grace M, Holness RO, et al. Nimodipine treatment in poor-grade aneurysm patients. Results of a multicenter double-blind placebo-controlled trial. *J Neurosurg*. 1988;68:505–517. DOI: 10.3171/jns.1988.68.4.0505.
  38. Carlson AP, Hänggi D, Wong GK, Etminan N, Mayer SA, Aldrich F, Diringer MN, Schmutzhard E, Faleck HJ, Ng D, et al. Single-dose intraventricular nimodipine microparticles versus oral nimodipine for aneurysmal subarachnoid hemorrhage. *Stroke*. 2020;51:1142–1149. DOI: 10.1161/STROKEAHA.119.027396.
  39. Kirkpatrick PJ, Turner CL, Smith C, Hutchinson PJ, Murray GD. Simvastatin in aneurysmal subarachnoid haemorrhage (STASH): a multicentre randomised phase 3 trial. *Lancet Neurol*. 2014;13:666–675. DOI: 10.1016/S1474-4422(14)70084-5.
  40. Wong GKC, Chan DY, Siu DY, Zee BCY, Poon WS, Chan MTV, Gin T, Leung M, Zhu XL, Liang M, et al. High-dose simvastatin for aneurysmal subarachnoid hemorrhage: multicenter randomized controlled double-blinded clinical trial. *Stroke*. 2015;46:382–388. DOI: 10.1161/STROKEAHA.114.007006.
  41. Zhang S, Wang L, Liu M, Wu B. Tirilazad for aneurysmal subarachnoid haemorrhage. *Cochrane Database Syst Rev*. 2010;2:CD006778. DOI: 10.1002/14651858.CD006778.pub2.
  42. Saber H, Desai A, Palla M, Mohamed W, Seraji-Bozorgzad N, Ibrahim M. Efficacy of clobazam in prevention of delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage: a meta-analysis. *J Stroke Cerebrovasc Dis*. 2018;27:2979–2985. DOI: 10.1016/j.jstrokecerebrovasdis.2018.06.027.
  43. Grote E, Hassler W. The critical first minutes after subarachnoid hemorrhage. *Neurosurgery*. 1988;22:654–661. DOI: 10.1227/00006123-198804000-00006.
  44. Hayashi T, Suzuki A, Hatazawa J, Kanno I, Shirane R, Yoshimoto T, Yasui N. Cerebral circulation and metabolism in the acute stage of subarachnoid hemorrhage. *J Neurosurg*. 2000;93:1014–1018. DOI: 10.3171/jns.2000.93.6.1014.
  45. Naredi S, Lambert G, Edén E, Zäll S, Runnerstam M, Rydenhag B, Friberg P. Increased sympathetic nervous activity in patients with non-traumatic subarachnoid hemorrhage. *Stroke*. 2000;31:901–906. DOI: 10.1161/01.STR.31.4.901.
  46. Svendgaard NA, Delgado TJ, Brun A. Effect of selective lesions in the hypothalamic-pituitary region on the development of cerebral vasospasm following an experimental subarachnoid hemorrhage in the rat. *J Cereb Blood Flow Metab*. 1986;6:650–657. DOI: 10.1038/jcbfm.1986.120.
  47. Páztor E, Fedina L, Kocsis B, Berta Z. Activity of peripheral sympathetic efferent nerves in experimental subarachnoid haemorrhage—part I: observations at the time of intracranial hypertension. *Acta Neurochir (Wien)*. 1986;79:125–131. DOI: 10.1007/BF01407456.
  48. Di Battista AP, Rizoli SB, Lejniaks B, Min A, Shiu MY, Peng HT, Baker AJ, Hutchison MG, Churchill N, Inaba K, et al. Sympathoadrenal activation is associated with acute traumatic coagulopathy and endotheliopathy in isolated brain injury. *Shock*. 2016;46:96–103. DOI: 10.1097/SHK.0000000000000642.
  49. Dilraj A, Botha JH, Rambirith V, Miller R, Van Dellen JR. Levels of catecholamine in plasma and cerebrospinal fluid in aneurysmal subarachnoid hemorrhage. *Neurosurgery*. 1992;31:42–51.
  50. Macmillan C, Grant I, Andrews P. Pulmonary and cardiac sequelae of subarachnoid haemorrhage: time for active management? *Intensive Care Med*. 2002;28:1012–1023. DOI: 10.1007/s00134-002-1382-7.
  51. Takemoto Y, Hasegawa Y, Hayashi K, Cao C, Hamasaki T, Kawano T, Mukasa A, Kim-Mitsuyama S. The stabilization of central sympathetic nerve activation by renal denervation prevents cerebral vasospasm after subarachnoid hemorrhage in rats. *Transl Stroke Res*. 2020;11:528–540. DOI: 10.1007/s12975-019-00740-9.
  52. Zubkov AY, Ogihara K, Bernanke DH, Parent AD, Zhang J. Apoptosis of endothelial cells in vessels affected by cerebral vasospasm. *Surg Neurol*. 2000;53:260–266. DOI: 10.1016/S0090-3019(99)00187-1.
  53. Friedrich V, Flores R, Sehba FA. Cell death starts early after subarachnoid hemorrhage. *Neurosci Lett*. 2012;512:6–11. DOI: 10.1016/j.neulet.2012.01.036.
  54. Peeyush Kumar T, McBride DW, Dash PK, Matsumura K, Rubi A, Blackburn SL. Endothelial cell dysfunction and injury in subarachnoid hemorrhage. *Mol Neurobiol*. 2019;56:1992–2006. DOI: 10.1007/s12035-018-1213-7.
  55. Dóczi T. The pathogenetic and prognostic significance of blood-brain barrier damage at the acute stage of aneurysmal subarachnoid haemorrhage. Clinical and experimental studies. *Acta Neurochir (Wien)*. 1985;77:110–132.
  56. Claassen J, Carhuapoma JR, Kreiter KT, Du EY, Connolly ES, Mayer SA. Global cerebral edema after subarachnoid hemorrhage: frequency, predictors, and impact on outcome. *Stroke*. 2002;33:1225–1232. DOI: 10.1161/01.STR.0000015624.29071.1F.
  57. Pluta RM, Boock RJ, Afshar JK, Clouse K, Bacic M, Ehrenreich H, Oldfield EH. Source and cause of endothelin-1 release into cerebrospinal fluid after subarachnoid hemorrhage. *J Neurosurg*. 1997;87:287–293. DOI: 10.3171/jns.1997.87.2.0287.
  58. Juvela S. Plasma endothelin concentrations after aneurysmal subarachnoid hemorrhage. *J Neurosurg*. 2000;92:390–400. DOI: 10.3171/jns.2000.92.3.0390.
  59. Barth M, Capelle HH, Münch E, Thomé C, Fiedler F, Schmiedek P, Vajkocz P. Effects of the selective endothelin A (ETA) receptor antagonist Clazosentan on cerebral perfusion and cerebral oxygenation following severe subarachnoid hemorrhage—preliminary results from a randomized clinical series. *Acta Neurochir (Wien)*. 2007;149:911–918. DOI: 10.1007/s00701-007-1249-3.
  60. Rao SS, Chung DY, Wolcott Z, Sherif F, Khawaja AM, Lee H, Guanci MM, Leslie-Mazwi TM, Kimberly WT, Patel AB, et al. Intermittent CSF drainage and rapid EVD weaning approach after subarachnoid hemorrhage: association with fewer VP shunts and shorter length of stay. *J Neurosurg*. 2020;132:1583–1588. DOI: 10.3171/2019.1.JNS182702.
  61. Olson DM, Zomorodi M, Britz GW, Zomorodi AR, Amato A, Graffagnino C. Continuous cerebral spinal fluid drainage associated with complications in patients admitted with subarachnoid hemorrhage. *J Neurosurg*. 2013;119:974–980. DOI: 10.3171/2013.6.JNS122403.
  62. Chung DY, Mayer SA, Rordorf GA. External ventricular drains after subarachnoid hemorrhage: is less more? *Neurocrit Care*. 2018;28:157–161. DOI: 10.1007/s12028-017-0443-2.
  63. Klimo P, Kestle JRW, Macdonald JD, Schmidt RH. Marked reduction of cerebral vasospasm with lumbar drainage of cerebrospinal fluid after subarachnoid hemorrhage. *J Neurosurg*. 2004;100:215–224. DOI: 10.3171/jns.2004.100.2.0215.
  64. Panni P, Donofrio CA, Barzaghi LR, Giudice L, Albano L, Righi C, Simonato F, Scomazzoni F, Cozzi S, Calvi MR, et al. Safety and feasibility of lumbar drainage in the management of poor grade aneurysmal subarachnoid hemorrhage. *J Clin Neurosci*. 2019;64:64–70. DOI: 10.1016/j.jocn.2019.04.010.
  65. Blackburn SL, Grande AW, Swisher CB, Hauck EF, Jagadeesan B, Provencio JJ. Prospective trial of cerebrospinal fluid filtration after aneurysmal subarachnoid hemorrhage via lumbar catheter (PILLAR). *Stroke*. 2019;50:2558–2561. DOI: 10.1161/STROKEAHA.119.025399.
  66. Pluta RM, Afshar JKB, Boock RJ, Oldfield EH. Temporal changes in perivascular concentrations of oxyhemoglobin, deoxyhemoglobin, and methemoglobin after subarachnoid hemorrhage. *J Neurosurg*. 1998;88:557–561. DOI: 10.3171/jns.1998.88.3.0557.
  67. Nosko M, Weir BKA, Lunt A, Grace M, Allen P, Mielke B. Effect of clot removal at 24 hours on chronic vasospasm after SAH in the primate model. *J Neurosurg*. 1987;66:416–422. DOI: 10.3171/jns.1987.66.3.0416.
  68. Handa Y, Weir BKA, Nosko M, Mosewich R, Tsuji T, Grace M. The effect of timing of clot removal on chronic vasospasm in a primate model. *J Neurosurg*. 1987;67:558–564. DOI: 10.3171/jns.1987.67.4.0558.
  69. Aoki T, Takenaka K, Suzuki S, Kassell NF, Sagher O, Lee KS. The role of hemolysate in the facilitation of oxyhemoglobin-induced contraction in rabbit basilar arteries. *J Neurosurg*. 1994;81:261–266. DOI: 10.3171/jns.1994.81.2.0261.
  70. Wickman G, Lan C, Vollrath B. Functional roles of the Rho/Rho kinase pathway and protein kinase C in the regulation of cerebrovascular constriction mediated by hemoglobin: relevance to subarachnoid hemorrhage and vasospasm. *Circ Res*. 2003;92:809–816. DOI: 10.1161/01.RES.0000066663.12256.B2.
  71. Shibuya M, Suzuki Y, Sugita K, Saito I, Sasaki T, Takakura K, Nagata I, Kikuchi H, Takemae T, Hidaka H, et al. Effect of AT877 on cerebral vasospasm after aneurysmal subarachnoid hemorrhage: results of a prospective placebo-controlled double-blind trial. *J Neurosurg*. 1992;76:571–577. DOI: 10.3171/jns.1992.76.4.0571.

72. Zhao J, Zhou D, Guo J, Ren Z, Zhou L, Wang S, Xu B, Wang R. Effect of fasudil hydrochloride, a protein kinase inhibitor, on cerebral vasospasm and delayed cerebral ischemic symptoms after aneurysmal subarachnoid hemorrhage: results of a randomized trial of fasudil hydrochloride versus nimodipine. *Neurol Med Chir (Tokyo)*. 2006;46:421–427. DOI: 10.2176/nmc.46.421.
73. Zhao J, Zhou D, Guo J, Ren Z, Zhou L, Wang S, Zhang Y, Xu B, Zhao K, Wang R, et al. Efficacy and safety of fasudil in patients with subarachnoid hemorrhage: final results of a randomized trial of fasudil versus nimodipine. *Neurol Med Chir (Tokyo)*. 2011;51:679–683. DOI: 10.2176/nmc.51.679.
74. Gresele P, Momi S, Falcinelli E. Anti-platelet therapy: phosphodiesterase inhibitors. *Br J Clin Pharmacol*. 2011;72:634–646. DOI: 10.1111/j.1365-2125.2011.04034.x.
75. Matsuda N, Naraoka M, Ohkuma H, Shimamura N, Ito K, Asano K, Hasegawa S, Takemura A. Effect of cilostazol on cerebral vasospasm and outcome in patients with aneurysmal subarachnoid hemorrhage: a randomized, double-blind, placebo-controlled trial. *Cerebrovasc Dis*. 2016;42:97–105. DOI: 10.1159/000445509.
76. Kim-Shapiro DB, Schechter AN, Gladwin MT. Unraveling the reactions of nitric oxide, nitrite, and hemoglobin in physiology and therapeutics. *Arterioscler Thromb Vasc Biol*. 2006;26:697–705.
77. Azarov I, He X, Jeffers A, Basu S, Ucer B, Hantgan RR, Levy A, Kim-Shapiro DB. Rate of nitric oxide scavenging by hemoglobin bound to haptoglobin. *Nitric Oxide*. 2008;18:296–302. DOI: 10.1016/j.niox.2008.02.006.
78. Jung CS, Oldfield EH, Harvey-White J, Espey MG, Zimmermann M, Seifert V, Pluta RM. Association of an endogenous inhibitor of nitric oxide synthase with cerebral vasospasm in patients with aneurysmal subarachnoid hemorrhage. *J Neurosurg*. 2007;107:945–950. DOI: 10.3171/JNS-07/11/0945.
79. Appel D, Seeberger M, Schwedhelm E, Czorlich P, Goetz AE, Böger RH, Hannemann J. Asymmetric and symmetric dimethylarginines are markers of delayed cerebral ischemia and neurological outcome in patients with subarachnoid hemorrhage. *Neurocrit Care*. 2018;29:84–93. DOI: 10.1007/s12028-018-0520-1.
80. Pluta RM, Thompson BG, Dawson TM, Snyder SH, Boock RJ, Oldfield EH. Loss of nitric oxide synthase immunoreactivity in cerebral vasospasm. *J Neurosurg*. 1996;84:648–654. DOI: 10.3171/jns.1996.84.4.0648.
81. Hino A, Tokuyama Y, Weir B, Takeda J, Yano H, Bell GI, Macdonald RL. Changes in endothelial nitric oxide synthase mRNA during vasospasm after subarachnoid hemorrhage in monkeys. *Neurosurgery*. 1996;39:562–568.
82. Förstermann U, Münzel T. Endothelial nitric oxide synthase in vascular disease: from marvel to menace. *Circulation*. 2006;113:1708–1714. DOI: 10.1161/CIRCULATIONAHA.105.602532.
83. Raabe A, Zimmermann M, Setzer M, Vatter H, Berkefeld J, Seifert V, Dumont AS, Kassell NF, Salem A, Thomas JE, et al. Effect of intraventricular sodium nitroprusside on cerebral hemodynamics and oxygenation in poor-grade aneurysm patients with severe, medically refractory vasospasm. *Neurosurgery*. 2002;50:1006–1014.
84. Pluta RM, Oldfield EH, Boock RJ. Reversal and prevention of cerebral vasospasm by intracarotid infusions of nitric oxide donors in a primate model of subarachnoid hemorrhage. *J Neurosurg*. 1997;87:746–751. DOI: 10.3171/jns.1997.87.5.0746.
85. Sabri M, Ai J, Lass E, D'abbondanza J, Macdonald RL. Genetic elimination of eNOS reduces secondary complications of experimental subarachnoid hemorrhage. *J Cereb Blood Flow Metab*. 2013;33:1008–1014. DOI: 10.1038/jcbfm.2013.49.
86. Vellimana AK, Milner E, Azad TD, Harries MD, Zhou M-L, Gidday JM, Han BH, Zipfel GJ. Endothelial nitric oxide synthase mediates endogenous protection against subarachnoid hemorrhage-induced cerebral vasospasm. *Stroke*. 2011;42:776–782. DOI: 10.1161/STROKEAHA.110.607200.
87. Alheid U, Frölich J, Förstermann U. Endothelium-derived relaxing factor from cultured human endothelial cells inhibits aggregation of human platelets. *Thromb Res*. 1987;47:561–571. DOI: 10.1016/0049-3848(87)90361-6.
88. Radomski MW, Palmer RMJ, Moncada S. The anti-aggregating properties of vascular endothelium: interactions between prostacyclin and nitric oxide. *Br J Pharmacol*. 1987;92:639–646. DOI: 10.1111/j.1476-5381.1987.tb11367.x.
89. Kubes P, Suzuki M, Granger DN. Nitric oxide: an endogenous modulator of leukocyte adhesion. *Proc Natl Acad Sci USA*. 1991;88:4651–4655. DOI: 10.1073/pnas.88.11.4651.
90. Garg UC, Hassid A. Nitric oxide-generating vasodilators and 8-bromo-cyclic guanosine monophosphate inhibit mitogenesis and proliferation of cultured rat vascular smooth muscle cells. *J Clin Invest*. 1989;83:1774–1777. DOI: 10.1172/JCI114081.
91. McBride DW, Blackburn SL, Peeyush KT, Matsumura K, Zhang JH. The role of thromboinflammation in delayed cerebral ischemia after subarachnoid hemorrhage. *Front Neurol*. 2017;8:555. DOI: 10.3389/fneur.2017.00555.
92. Suzuki S, Kimura M, Souma M, Ohkima H, Iwabuchi T, Shimizu T. Cerebral microthrombosis in symptomatic cerebral vasospasm—a quantitative histological study in autopsy cases. *Neurol Med Chir (Tokyo)*. 1990;30:309–316.
93. Stein SC, Browne KD, Chen XH, Smith DH, Graham DI. Thromboembolism and delayed cerebral ischemia after subarachnoid hemorrhage: an autopsy study. *Neurosurgery*. 2006;59:781–787. DOI: 10.1227/01.NEU.0000227519.27569.45.
94. Ohkuma H, Itoh K, Shibata S, Suzuki S. Morphological changes of intraparenchymal arterioles after experimental subarachnoid hemorrhage in dogs. *Neurosurgery*. 1997;41:230–236. DOI: 10.1097/00006-123-199707000-00036.
95. Chauhan AK, Kisucka J, Brill A, Walsh MT, Scheiflinger F, Wagner DD. ADAMTS13: a new link between thrombosis and inflammation. *J Exp Med*. 2008;205:2065–2074. DOI: 10.1084/jem.20080130.
96. Vergouwen M, Bakhtiari K, Van Geloven N, Vermeulen M, Roos YB, Meijers J. Reduced ADAMTS13 activity in delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage. *J Cereb Blood Flow Metab*. 2009;29:1734–1741. DOI: 10.1038/jcbfm.2009.88.
97. Li W, Hui C, Ju H. Expression and significance of vWF, GMP-140 and ADAMTS13 in patients with aneurysmal subarachnoid hemorrhage. *Eur Rev Med Pharmacol Sci*. 2017;21:4350–4356.
98. Nissen JJ, Mantle D, Gregson B, Mendelow AD. Serum concentration of adhesion molecules in patients with delayed ischaemic neurological deficit after aneurysmal subarachnoid haemorrhage: the immunoglobulin and selectin superfamily. *J Neurol Neurosurg Psychiatry*. 2001;71:329–333. DOI: 10.1136/jnnp.71.3.329.
99. Frijs CJM, Kasius KM, Algra A, Fijnheer R, Rinkel GJE. Endothelial cell activation markers and delayed cerebral ischaemia in patients with subarachnoid haemorrhage. *J Neurol Neurosurg Psychiatry*. 2006;77:863–867. DOI: 10.1136/jnnp.2005.081539.
100. Sabri M, Ai J, Lakovic K, D'abbondanza J, Ildigwe D, Macdonald RL. Mechanisms of microthrombi formation after experimental subarachnoid hemorrhage. *Neuroscience*. 2012;224:26–37. DOI: 10.1016/j.neuroscience.2012.08.002.
101. Ishikawa M, Kusaka G, Yamaguchi N, Sekizuka E, Nakadate H, Minamitani H, Shinoda S, Watanabe E. Platelet and leukocyte adhesion in the microvasculature at the cerebral surface immediately after subarachnoid hemorrhage. *Neurosurgery*. 2009;64:546–553. DOI: 10.1227/01.NEU.0000337579.05110.F4.
102. Tremolizzo L, DiFrancesco JC, Rodriguez-Menendez V, Sirtori E, Longoni M, Cassetti A, Bossi M, El Mestikawy S, Cavaletti G, Ferrarese C. Human platelets express the synaptic markers VGLUT1 and 2 and release glutamate following aggregation. *Neurosci Lett*. 2006;404:262–265. DOI: 10.1016/j.neulet.2006.06.015.
103. Lai TW, Zhang S, Wang YT. Excitotoxicity and stroke: identifying novel targets for neuroprotection. *Prog Neurobiol*. 2014;115:157–188. DOI: 10.1016/j.pneurobio.2013.11.006.
104. Wu CT, Wen LL, Wong CS, Tsai SY, Chan SM, Yeh CC, Borel CO, Cherng CH. Temporal changes in glutamate, glutamate transporters, basilar arteries wall thickness, and neuronal variability in an experimental rat model of subarachnoid hemorrhage. *Anesth Analg*. 2011;112:666–673. DOI: 10.1213/ANE.0b013e318207c51f.
105. Bell JD, Thomas TC, Lass E, Ai J, Wan H, Lifshitz J, Baker AJ, MacDonald RL. Platelet-mediated changes to neuronal glutamate receptor expression at sites of microthrombosis following experimental subarachnoid hemorrhage: laboratory investigation. *J Neurosurg*. 2014;124:1424–1431. DOI: 10.3171/2014.3.JNS132130.
106. Nilsson OG, Säveland H, Boris-Möller F, Brandt L, Wieloch T. Increased levels of glutamate in patients with subarachnoid haemorrhage as measured by intracerebral microdialysis. *Acta Neurochir Suppl*. 1996;1996:45–47.
107. Wurm G, Tomancok B, Nussbaumer K, Adewöhrer C, Holl K. Reduction of ischemic sequelae following spontaneous subarachnoid hemorrhage: a double-blind, randomized comparison of enoxaparin



- versus placebo. *Clin Neurol Neurosurg.* 2004;106:97–103. DOI: 10.1016/j.clineuro.2004.01.006.
108. Siironen J, Juvela S, Varis J, Porras M, Poussa K, Ilveskero S, Hernesniemi J, Lassila R. No effect of enoxaparin on outcome of aneurysmal subarachnoid hemorrhage: a randomized, double-blind, placebo-controlled clinical trial. *J Neurosurg.* 2003;99:953–959. DOI: 10.3171/jns.2003.99.6.0953.
  109. Simard JM, Aldrich EF, Schreibman D, James RF, Polifka A, Beaty N. Low-dose intravenous heparin infusion in patients with aneurysmal subarachnoid hemorrhage: a preliminary assessment—clinical article. *J Neurosurg.* 2013;119:1611–1619. DOI: 10.3171/2013.8.JNS1337.
  110. James RF, Khattar NK, Aljuboori ZS, Page PS, Shao EY, Carter LM, Meyer KS, Daniels MW, Craycroft J, Gaughen JR, et al. Continuous infusion of low-dose unfractionated heparin after aneurysmal subarachnoid hemorrhage: a preliminary study of cognitive outcomes. *J Neurosurg.* 2019;130:1460–1467. DOI: 10.3171/2017.11.JNS17894.
  111. Kole MJ, Wessell AP, Ugiliweneza B, Cannarsa GJ, Fortuny E, Stokum JA, Shea P, Chryssikos T, Khattar NK, Crabill GA, et al. Low-dose intravenous heparin infusion after aneurysmal subarachnoid hemorrhage is associated with decreased risk of delayed neurological deficit and cerebral infarction. *Neurosurgery.* 2021;88:523–530. DOI: 10.1093/neuros/nyaa473.
  112. Simard JM, Tosun C, Ivanova S, Kurland DB, Hong C, Radecki L, Gisriel C, Mehta R, Schreiberman D, Gerzanich V. Heparin reduces neuroinflammation and transsynaptic neuronal apoptosis in a model of subarachnoid hemorrhage. *Transl Stroke Res.* 2012;3:155–165. DOI: 10.1007/s12975-012-0166-9.
  113. Dorhout Mees S, Van Den Bergh WM, Algra A, Rinkel GJE. Antiplatelet therapy for aneurysmal subarachnoid haemorrhage. *Cochrane Database Syst Rev.* 2007;4:CD006184. DOI: 10.1002/14651858.CD006184.pub2.
  114. Nagahama Y, Allan L, Nakagawa D, Zanaty M, Starke RM, Chalouhi N, Jabbour P, Brown RD, Derdeyn CP, Leira EC, et al. Dual antiplatelet therapy in aneurysmal subarachnoid hemorrhage: association with reduced risk of clinical vasospasm and delayed cerebral ischemia. *J Neurosurg.* 2018;129:702–710. DOI: 10.3171/2017.5.JNS17831.
  115. Altenburg A, Haage P. Antiplatelet and anticoagulant drugs in interventional radiology. *Cardiovasc Intervent Radiol.* 2012;35:30–42.
  116. Samaniego EA, Gibson E, Nakagawa D, Ortega-Gutierrez S, Zanaty M, Roa JA, Jabbour P, Hasan DM. Safety of tirofiban and dual antiplatelet therapy in treating intracranial aneurysms. *Stroke Vasc Neurol.* 2019;4:36–42. DOI: 10.1136/svn-2018-000192.
  117. Limaye K, Zanaty M, Hudson J, Nakagawa D, Al Kasab S, Alvarez C, Dandapat S, Kung DK, Ortega-Gutierrez S, Jabbour P, et al. The safety and efficacy of continuous tirofiban as a monoantiplatelet therapy in the management of ruptured aneurysms treated using stent-assisted coiling or flow diversion and requiring ventricular drainage. *Neurosurgery.* 2019;85:E1037–E1042. DOI: 10.1093/neuros/nyz226.
  118. Zanaty M, Osorno-Cruz C, Byer S, Roa JA, Limaye K, Ishii D, Nakagawa D, Torner J, Yongjun LU, Ortega-Gutiérrez S, et al. Tirofiban protocol protects against delayed cerebral ischemia: a case-series study. *Neurosurgery.* 2020;87:E552–E556. DOI: 10.1093/neuros/nyaa170.
  119. Louveau A, Smirnov I, Keyes TJ, Eccles JD, Rouhani SJ, Peske JD, Derecki NC, Castle D, Mandell JW, Lee KS, et al. Structural and functional features of central nervous system lymphatic vessels. *Nature.* 2015;523:337–341. DOI: 10.1038/nature14432.
  120. Sun BL, Xie FM, Yang MF, Cao MZ, Yuan H, Wang HT, Wang JR, Jia L. Blocking cerebral lymphatic drainage deteriorates cerebral oxidative injury in rats with subarachnoid hemorrhage. *Acta Neurochir Suppl.* 2011;110:49–53.
  121. Chen J, Wang L, Xu H, Xing L, Zhuang Z, Zheng Y, Li X, Wang C, Chen S, Guo Z, et al. Meningeal lymphatics clear erythrocytes that arise from subarachnoid hemorrhage. *Nat Commun.* 2020;11:3159. DOI: 10.1038/s41467-020-16851-z.
  122. Louveau A, Plog BA, Antila S, Alitalo K, Nedergaard M, Kipnis J. Understanding the functions and relationships of the glymphatic system and meningeal lymphatics. *J Clin Invest.* 2017;127:3210–3219. DOI: 10.1172/JCI90603.
  123. Luo C, Yao X, Li J, He B, Liu Q, Ren H, Liang F, Li M, Lin H, Peng J, et al. Paravascular pathways contribute to vasculitis and neuroinflammation after subarachnoid hemorrhage independently of glymphatic control. *Cell Death Dis.* 2016;7:e2160. DOI: 10.1038/cddis.2016.63.
  124. Nagelhus EA, Ottersen OP. Physiological roles of aquaporin-4 in brain. *Physiol Rev.* 2013;93:1543–1562. DOI: 10.1152/physrev.00011.2013.
  125. Liu E, Sun L, Zhang Y, Wang A, Yan J. Aquaporin4 knockout aggravates early brain injury following subarachnoid hemorrhage through impairment of the glymphatic system in rat brain. *Acta Neurochir Suppl.* 2020;127:59–64.
  126. Siler DA, Gonzalez JA, Wang RK, Cetas JS, Alkayed NJ. Intracisternal administration of tissue plasminogen activator improves cerebrospinal fluid flow and cortical perfusion after subarachnoid hemorrhage in mice. *Transl Stroke Res.* 2014;5:227–237. DOI: 10.1007/s12975-014-0329-y.
  127. Lassen NA. Cerebral blood flow and oxygen consumption in man. *Physiol Rev.* 1959;39:183–238. DOI: 10.1152/physrev.1959.39.2.183.
  128. Kety SS, Schmidt CF. The effects of altered arterial tensions of carbon dioxide and oxygen on cerebral blood flow and cerebral oxygen consumption of normal young men. *J Clin Invest.* 1948;27:484–492.
  129. Armstead WM. Cerebral blood flow autoregulation and dysautoregulation. *Anesthesiol Clin.* 2016;34:465–477. DOI: 10.1016/j.anclin.2016.04.002.
  130. Andresen J, Shafi NI, Bryan RM. Endothelial influences on cerebrovascular tone. *J Appl Physiol.* 2006;100:318–327. DOI: 10.1152/jappphysiol.0.00937.2005.
  131. Yundt KD, Grubb RL, Diringer MN, Powers WJ. Autoregulatory vasodilation of parenchymal vessels is impaired during cerebral vasospasm. *J Cereb Blood Flow Metab.* 1998;18:419–424. DOI: 10.1097/00004647-199804000-00010.
  132. Lam JMK, Smielewski P, Czosnyka M, Pickard JD, Kirkpatrick PJ. Predicting delayed ischemic deficits after aneurysmal subarachnoid hemorrhage using a transient hyperemic response test of cerebral autoregulation. *Neurosurgery.* 2000;47:819–826. DOI: 10.1097/00006123-200010000-00004.
  133. Jaeger M, Soehle M, Schuhmann MU, Meixensberger J. Clinical significance of impaired cerebrovascular autoregulation after severe aneurysmal subarachnoid hemorrhage. *Stroke.* 2012;43:2097–2101. DOI: 10.1161/STROKEAHA.112.659888.
  134. Budohoski KP, Czosnyka M, Smielewski P, Kasprzewicz M, Helmy A, Bulders D, Pickard JD, Kirkpatrick PJ. Impairment of cerebral autoregulation predicts delayed cerebral ischemia after subarachnoid hemorrhage: a prospective observational study. *Stroke.* 2012;43:3230–3237. DOI: 10.1161/STROKEAHA.112.669788.
  135. Bothun ML, Haaland ØA, Moen G, Logallo N, Svendsen F, Thomassen L, Helland CA. Impaired cerebrovascular reactivity may predict delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage. *J Neural Sci.* 2019;407:116539. DOI: 10.1016/j.jns.2019.116539.
  136. Tseng MY, Czosnyka M, Richards H, Pickard JD, Kirkpatrick PJ. Effects of acute treatment with pravastatin on cerebral vasospasm, autoregulation, and delayed ischemic deficits after aneurysmal subarachnoid hemorrhage: a phase II randomized placebo-controlled trial. *Stroke.* 2005;36:1627–1632. DOI: 10.1161/01.STR.0000176743.67564.5d.
  137. Medzhitov R. Origin and physiological roles of inflammation. *Nature.* 2008;454:428–435. DOI: 10.1038/nature07201.
  138. Anan M, Nagai Y, Fudaba H, Fujiki M. Lactate and lactate dehydrogenase in cistern as biomarkers of early brain injury and delayed cerebral ischemia of subarachnoid hemorrhage. *J Stroke Cerebrovasc Dis.* 2020;29:104765. DOI: 10.1016/j.jstrokecerebrovasdis.2020.104765.
  139. Rasmussen R, Bache S, Stavngaard T, Møller K. Plasma levels of IL-6, IL-8, IL-10, ICAM-1, VCAM-1, IFN $\gamma$ , and TNF $\alpha$  are not associated with delayed cerebral ischemia, cerebral vasospasm, or clinical outcome in patients with subarachnoid hemorrhage. *World Neurosurg.* 2019;128:e1131–e1136. DOI: 10.1016/j.wneu.2019.05.102.
  140. McGirt MJ, Mavropoulos JC, McGirt LY, Alexander MJ, Friedman AH, Laskowitz DT, Lynch JR. Leukocytosis as an independent risk factor for cerebral vasospasm following aneurysmal subarachnoid hemorrhage. *J Neurosurg.* 2003;98:1222–1226. DOI: 10.3171/jns.2003.98.6.1222.
  141. McMahon CJ, Hopkins S, Vail A, King AT, Smith D, Illingworth KJ, Clark S, Rothwell NJ, Tyrrell PJ. Inflammation as a predictor for delayed cerebral ischemia after aneurysmal subarachnoid haemorrhage. *J Neurointerv Surg.* 2013;5:512–517. DOI: 10.1136/neurintsurg-2012-010386.
  142. Badjatia N, Monahan A, Carpenter A, Zimmerman J, Schmidt JM, Claassen J, Connolly ES, Mayer SA, Karmally W, Seres D. Inflammation, negative nitrogen balance, and outcome after aneurysmal subarachnoid hemorrhage. *Neurology.* 2015;84:680–687. DOI: 10.1212/WNL.0000000000001259.
  143. Al-Mufti F, Amuluru K, Damodara N, Dodson V, Roh D, Agarwal S, Meyers PM, Connolly ES, Schmidt MJ, Claassen J, et al. Admission

- neutrophil-lymphocyte ratio predicts delayed cerebral ischemia following aneurysmal subarachnoid hemorrhage. *J Neurointerv Surg*. 2019;11:1135–1140. DOI: 10.1136/neurintsurg-2019-014759.
144. Tam AKH, Ildigwe D, Mocco J, Mayer S, Kassell N, Ruefenacht D, Schmiedek P, Weidauer S, Pasqualin A, MacDonald RL. Impact of systemic inflammatory response syndrome on vasospasm, cerebral infarction, and outcome after subarachnoid hemorrhage: exploratory analysis of CONSCIOUS-1 database. *Neurocrit Care*. 2010;13:182–189. DOI: 10.1007/s12028-010-9402-x.
  145. Dhar R, Diringner MN. The burden of the systemic inflammatory response predicts vasospasm and outcome after subarachnoid hemorrhage. *Neurocrit Care*. 2008;8:404–412. DOI: 10.1007/s12028-008-9054-2.
  146. Chyatte D, Fode NC, Nichols DA, Sundt TM. Preliminary report: effects of high dose methylprednisolone on delayed cerebral ischemia in patients at high risk for vasospasm after aneurysmal subarachnoid hemorrhage. *Neurosurgery*. 1987;21:157–160. DOI: 10.1227/00006123-198708000-00004.
  147. Hashi K, Takakura K, Sano K, Ohta T, Saito I, Okada K. Intravenous hydrocortisone in large doses in the treatment of delayed ischemic neurological deficits following subarachnoid hemorrhage—results of a multi-center controlled double-blind clinical study. *No To Shinkei*. 1988;40:373–382.
  148. Mohny N, Williamson CA, Rothman E, Ball R, Sheehan KM, Pandey AS, Fletcher JJ, Jacobs TL, Thompson BG, Rajajee V. A propensity score analysis of the impact of dexamethasone use on delayed cerebral ischemia and poor functional outcomes after subarachnoid hemorrhage. *World Neurosurg*. 2018;109:e655–e661. DOI: 10.1016/j.wneu.2017.10.051.
  149. Oлдashi F, Muzha I, Filipi N, Lede R, Copertari P, Traverso C, Copertari A, Vergara EA, Montenegro C, De Huidobro RR, et al. Effect of intravenous corticosteroids on death within 14 days in 10008 adults with clinically significant head injury (MRC CRASH trial): randomised placebo-controlled trial. *Lancet*. 2004;364:1321–1328.
  150. Muroi C, Hugelshofer M, Seule M, Keller E. The impact of nonsteroidal anti-inflammatory drugs on inflammatory response after aneurysmal subarachnoid hemorrhage. *Neurocrit Care*. 2014;20:240–246. DOI: 10.1007/s12028-013-9930-2.
  151. Nassiri F, Ibrahim GM, Badhiwala JH, Witw CD, Mansouri A, Alotaibi NM, Macdonald RL. A propensity score-matched study of the use of non-steroidal anti-inflammatory agents following aneurysmal subarachnoid hemorrhage. *Neurocrit Care*. 2016;25:351–358. DOI: 10.1007/s12028-016-0266-6.
  152. Hop JW, Rinkel GJE, Algra A, Berkelbach Van Der Sprenkel JW, Van Gijn J. Randomized pilot trial of postoperative aspirin in subarachnoid hemorrhage. *Neurology*. 2000;54:872–878. DOI: 10.1212/WNL.54.4.872.
  153. Van Den Bergh WM. Randomized controlled trial of acetylsalicylic acid in aneurysmal subarachnoid hemorrhage: the MASH study. *Stroke*. 2006;37:2326–2330. DOI: 10.1161/01.STR.0000236841.16055.0f.
  154. Mathiesen T, Andersson B, Loftenius A, Von Holst H. Increased interleukin-6 levels in cerebrospinal fluid following subarachnoid hemorrhage. *J Neurosurg*. 1993;78:562–567. DOI: 10.3171/jns.1993.78.4.0562.
  155. Hendryk S, Jarzab B, Josko J. Increase of the IL-1 $\beta$  and IL-6 levels in CSF in patients with vasospasm following aneurysmal SAH. *Neuro Endocrinol Lett*. 2004;25:141–147.
  156. Nakahara T, Tsuruta R, Kaneko T, Yamashita S, Fujita M, Kasaoka S, Hashiguchi T, Suzuki M, Maruyama I, Maekawa T. High-mobility group box 1 protein in CSF of patients with subarachnoid hemorrhage. *Neurocrit Care*. 2009;11:362–368. DOI: 10.1007/s12028-009-9276-y.
  157. Ahn SH, Savarraj J, Parsha K, Hergenroeder GW, Chang TR, Kim DH, Kitagawa RS, Blackburn SL, Choi HA. Inflammation in delayed ischemia and functional outcomes after subarachnoid hemorrhage. *J Neuroinflammation*. 2019;16:213. DOI: 10.1186/s12974-019-1578-1.
  158. Figueiredo RT, Fernandez PL, Mourao-Sa DS, Porto BN, Dutra FF, Alves LS, Oliveira MF, Oliveira PL, Graça-Souza AV, Bozza MT. Characterization of heme as activator of Toll-like receptor 4. *J Biol Chem*. 2007;282:20221–20229. DOI: 10.1074/jbc.M610737200.
  159. Vaure C, Liu Y. A comparative review of Toll-like receptor 4 expression and functionality in different animal species. *Front Immunol*. 2014;5:316. DOI: 10.3389/fimmu.2014.00316.
  160. Zhou M-L, Wu W, Ding Y-S, Zhang F-F, Hang C-H, Wang H-D, Cheng H-L, Yin H-X, Shi J-X. Expression of Toll-like receptor 4 in the basilar artery after experimental subarachnoid hemorrhage in rabbits: a preliminary study. *Brain Res*. 2007;1173:110–116. DOI: 10.1016/j.brainres.2007.07.059.
  161. Hanafy KA. The role of microglia and the TLR4 pathway in neuronal apoptosis and vasospasm after subarachnoid hemorrhage. *J Neuroinflammation*. 2013;10:868. DOI: 10.1186/1742-2094-10-83.
  162. Schneider UC, Davids A-M, Brandenburg S, Müller A, Elke A, Magrini S, Atangana E, Turkowski K, Finger T, Gutenberg A, et al. Microglia inflict delayed brain injury after subarachnoid hemorrhage. *Acta Neuropathol*. 2015;130:215–231. DOI: 10.1007/s00401-015-1440-1.
  163. Taylor RA, Chang C-F, Goods BA, Hammond MD, Grory BM, Ai Y, Steinschneider AF, Renfro SC, Askenase MH, McCullough LD, et al. TGF- $\beta$ 1 modulates microglial phenotype and promotes recovery after intracerebral hemorrhage. *J Clin Invest*. 2017;127:280–292. DOI: 10.1172/JCI88647.
  164. Ayer RE, Ostrowski RP, Sugawara T, Ma Q, Jafarian N, Tang J, Zhang JH. Statin-induced T-lymphocyte modulation and neuroprotection following experimental subarachnoid hemorrhage. *Acta Neurochir Suppl*. 2013;115:259–266.
  165. Yang H, Wang H, Andersson U. Targeting inflammation driven by HMGB1. *Front Immunol*. 2020;11:484. DOI: 10.3389/fimmu.2020.00484.
  166. Wang L, Zhang Z, Liang L, Wu Y, Zhong J, Sun X. Anti-high mobility group box-1 antibody attenuated vascular smooth muscle cell phenotypic switching and vascular remodeling after subarachnoid hemorrhage in rats. *Neurosci Lett*. 2019;708:134338. DOI: 10.1016/j.neulet.2019.134338.
  167. Neil-dwyer G, Cruickshank J. The blood leucocyte count and its prognostic significance in subarachnoid haemorrhage. *Brain*. 1974;97:79–86. DOI: 10.1093/brain/97.1.79.
  168. Kubota T, Handa Y, Tsuchida A, Kaneko M, Kobayashi H, Kubota T. The kinetics of lymphocyte subsets and macrophages in subarachnoid space after subarachnoid hemorrhage in rats. *Stroke*. 1993;24:1993–2000. DOI: 10.1161/01.STR.24.12.1993.
  169. Mathiesen T, Lefvert AK. Cerebrospinal fluid and blood lymphocyte subpopulations following subarachnoid hemorrhage. *Br J Neurosurg*. 1996;10:89–92. DOI: 10.1080/02688699650040584.
  170. Lim M, Bower RS, Wang Y, Sims L, Bower MR, Camara-Quintana J, Li G, Cheshier S, Harsh GR, Steinberg GK, et al. The predictive value of serum myeloperoxidase for vasospasm in patients with aneurysmal subarachnoid hemorrhage. *Neurosurg Rev*. 2012;35:413–419. DOI: 10.1007/s10143-012-0375-4.
  171. Galea J, Cruickshank G, Teeling JL, Boche D, Garland P, Perry VH, Galea I. The intrathecal CD163-haptoglobin-hemoglobin scavenging system in subarachnoid hemorrhage. *J Neurochem*. 2012;121:785–792. DOI: 10.1111/j.1471-4159.2012.07716.x.
  172. Hugelshofer M, Buzzi RM, Schaer CA, Richter H, Akeret K, Anagnostakou V, Mahmoudi L, Vaccani R, Vallengel F, Deuel JW, et al. Haptoglobin administration into the subarachnoid space prevents hemoglobin-induced cerebral vasospasm. *J Clin Invest*. 2019;129:5219–5235. DOI: 10.1172/JCI130630.
  173. Chaichana KL, Levy AP, Miller-Lotan R, Shakur S, Tamargo RJ. Haptoglobin 2–2 genotype determines chronic vasospasm after experimental subarachnoid hemorrhage. *Stroke*. 2007;38:3266–3271.
  174. Blackburn SL, Kumar PT, McBride D, Zeineddine HA, Leclerc J, Choi HA, Dash PK, Grotta J, Aronowski J, Cardenas JC, et al. Unique contribution of haptoglobin and haptoglobin genotype in aneurysmal subarachnoid hemorrhage. *Front Physiol*. 2018;9:592.
  175. Liddicoat AM, Lavelle EC. Modulation of innate immunity by cyclosporine A. *Biochem Pharmacol*. 2019;163:472–480. DOI: 10.1016/j.bcp.2019.03.022.
  176. Ryba M, Pastuszko M, Iwanska K, Bidzinski J, Dziewiecki C. Cyclosporine A prevents neurological deterioration of patients with SAH—a preliminary report. *Acta Neurochir (Wien)*. 1991;112:25–27. DOI: 10.1007/BF01402450.
  177. Manno EM, Gress DR, Ogilvy CS, Stone CM, Zervas NT. The safety and efficacy of cyclosporine A in the prevention of vasospasm in patients with Fisher grade 3 subarachnoid hemorrhages: a pilot study. *Neurosurgery*. 1997;40:289–293. DOI: 10.1097/00006123-199702000-00010.
  178. Handa Y, Hayashi M, Takeuchi H, Kobayashi H, Kawano H, Kabuto M. Effect of cyclosporine on the development of cerebral vasospasm in a primate model—PubMed. *Neurosurgery*. 1991;28:380–385.
  179. Peterson JW, Nishizawa S, Hackett JD, Bun T, Teramura A, Zervas NT. Cyclosporine A reduces cerebral vasospasm after subarachnoid hemorrhage in dogs. *Stroke*. 1990;21:133–137.

180. Pradilla G, Wang PP, Legnani FG, Ogata L, Dietsch GN, Tamargo RJ. Prevention of vasospasm by anti-CD11/CD18 monoclonal antibody therapy following subarachnoid hemorrhage in rabbits. *J Neurosurg.* 2004;101:88–92.
181. Clatterbuck RE, Gailloud P, Ogata L, Gebremariam A, Dietsch GN, Murphy KJ, Tamargo RJ. Prevention of cerebral vasospasm by a humanized anti-CD11/CD18 monoclonal antibody administered after experimental subarachnoid hemorrhage in nonhuman primates. *J Neurosurg.* 2003;99:376–382.
182. Provencio JJ, Altay T, Smithson S, Moore SK, Ransohoff RM. Depletion of Ly6G/C+ cells ameliorates delayed cerebral vasospasm in subarachnoid hemorrhage. *J Neuroimmunol.* 2011;232:94–100. DOI: 10.1016/j.jneuroim.2010.10.016.
183. Daley JM, Thomay AA, Connolly MD, Reichner JS, Albina JE. Use of Ly6G-specific monoclonal antibody to deplete neutrophils in mice. *J Leukoc Biol.* 2008;83:64–70. DOI: 10.1189/jlb.0407247.
184. Lawson C, Wolf S. ICAM-1 signaling in endothelial cells. *Pharmacol Rep.* 2009;61:22–32. DOI: 10.1016/S1734-1140(09)70004-0.
185. Kaynar MY, Tanriverdi T, Kafadar AM, Kacira T, Uzun H, Aydin S, Gumustas K, Dirican A, Kuday C. Detection of soluble intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 in both cerebrospinal fluid and serum of patients after aneurysmal subarachnoid hemorrhage. *J Neurosurg.* 2004;101:1030–1036. DOI: 10.3171/jns.2004.101.6.1030.
186. Handa Y, Kubota T, Kaneko M, Tsuchida A, Kobayashi H, Kawano H, Kubota T. Expression of intercellular adhesion molecule 1 (ICAM-1) on the cerebral artery following subarachnoid haemorrhage in rats. *Acta Neurochir (Wien).* 1995;132:92–97. DOI: 10.1007/BF01404854.
187. Sills AK, Clatterbuck RE, Thompson RC, Cohen PL, Tamargo RJ. Endothelial cell expression of intercellular adhesion molecule 1 in experimental posthemorrhagic vasospasm. *Neurosurgery.* 1997;41:453–461. DOI: 10.1097/00006123-199708000-00025.
188. Polin RS, Bavbek M, Shaffrey ME, Billups K, Bogaev CA, Kassell NF, Lee KS. Detection of soluble E-selectin, ICAM-1, VCAM-1, and L-selectin in the cerebrospinal fluid of patients after subarachnoid hemorrhage. *J Neurosurg.* 1998;89:559–567.
189. Lin CL, Dumont AS, Calisaneller T, Kwan AL, Hwong SL, Lee KS. Monoclonal antibody against E selectin attenuates subarachnoid hemorrhage-induced cerebral vasospasm. *Surg Neurol.* 2005;64:201–205. DOI: 10.1016/j.surneu.2005.04.038.
190. Clatterbuck RE, Hoffman PA, Dietsch GN, Pardoll DM, Tamargo RJ. Inhibition of vasospasm with lymphocyte function-associated antigen-1 monoclonal antibody in a femoral artery model in rats. *J Neurosurg.* 2002;97:676–682. DOI: 10.3171/jns.2002.97.3.0676.
191. Oshiro EM, Hoffman PA, Dietsch GN, Watts MC, Pardoll DM, Tamargo RJ. Inhibition of experimental vasospasm with anti-intercellular adhesion molecule-1 monoclonal antibody in rats. *Stroke.* 1997;28:2031–2038. DOI: 10.1161/01.STR.28.10.2031.
192. Bavbek M, Polin R, Kwan AL, Arthur AS, Kassell NF, Lee KS. Monoclonal antibodies against ICAM-1 and CD18 attenuate cerebral vasospasm after experimental subarachnoid hemorrhage in rabbits. *Stroke.* 1998;29:1930–1935. DOI: 10.1161/01.STR.29.9.1930.
193. Blanco-Colio LM, Tuñón J, Martín-Ventura JL, Egido J. Anti-inflammatory and immunomodulatory effects of statins. *Kidney Int.* 2003;63:12–23. DOI: 10.1046/j.1523-1755.2003.00744.x.
194. Lynch JR, Wang H, McGirt MJ, Floyd J, Friedman AH, Coon AL, Blessing R, Alexander MJ, Graffagnino C, Warner DS, et al. Simvastatin reduces vasospasm after aneurysmal subarachnoid hemorrhage: results of a pilot randomized clinical trial. *Stroke.* 2005;36:2024–2026. DOI: 10.1161/01.STR.0000177879.11607.10.
195. Leao AAP. Spreading depression of activity in the cerebral cortex. *J Neurophysiol.* 1944;7:359–390. DOI: 10.1152/jn.1944.7.6.359.
196. Leao AAP, Morison RS. Propagation of spreading cortical depression. *J Neurophysiol.* 1945;8:33–45. DOI: 10.1152/jn.1945.8.1.33.
197. Dreier JP. The role of spreading depression, spreading depolarization and spreading ischemia in neurological disease. *Nat Med.* 2011;17:439–447. DOI: 10.1038/nm.2333.
198. Pietrobon D, Moskowitz MA. Chaos and commotion in the wake of cortical spreading depression and spreading depolarizations. *Nat Rev Neurosci.* 2014;15:379–393. DOI: 10.1038/nrn3770.
199. Cozzolino O, Marchese M, Trovato F, Pracucci E, Ratto GM, Buzzi MG, Sicca F, Santorelli FM. Understanding spreading depression from headache to sudden unexpected death. *Front Neurol.* 2018;9:19. DOI: 10.3389/fneur.2018.00019.
200. Ayata C, Lauritzen M. Spreading depression, spreading depolarizations, and the cerebral vasculature. *Physiol Rev.* 2015;95:953–993. DOI: 10.1152/physrev.00027.2014.
201. Fabricius M, Fuhr S, Bhatia R, Boutelle M, Hashemi P, Strong AJ, Lauritzen M. Cortical spreading depression and peri-infarct depolarization in acutely injured human cerebral cortex. *Brain.* 2006;129:778–790.
202. Hossmann KA. Periinfarct depolarizations. *Cerebrovasc Brain Metab Rev.* 1996;8:195–208.
203. Dietrich WD, Feng ZC, Leistra H, Watson BD, Rosenthal M. Photothrombotic infarction triggers multiple episodes of cortical spreading depression in distant brain regions. *J Cereb Blood Flow Metab.* 1994;14:20–28. DOI: 10.1038/jcbfm.1994.4.
204. Dreier JP, Körner K, Ebert N, Görner A, Rubin I, Back T, Lindauer U, Wolf T, Villringer A, Einhüpl KM, et al. Nitric oxide scavenging by hemoglobin or nitric oxide synthase inhibition by N-nitro-L-arginine induces cortical spreading ischemia when K<sup>+</sup> is increased in the subarachnoid space. *J Cereb Blood Flow Metab.* 1998;18:978–990.
205. Dreier JP, Ebert N, Priller J, Megow D, Lindauer U, Klee R, Reuter U, Imai Y, Einhüpl KM, Victorov I, et al. Products of hemolysis in the subarachnoid space inducing spreading ischemia in the cortex and focal necrosis in rats: a model for delayed ischemic neurological deficits after subarachnoid hemorrhage? *J Neurosurg.* 2000;93:658–666. DOI: 10.3171/jns.2000.93.4.0658.
206. Dreier JP, Woitzik J, Fabricius M, Bhatia R, Major S, Drenckhahn C, Lehmann TN, Sarrafzadeh A, Willumsen L, Hartings JA, et al. Delayed ischaemic neurological deficits after subarachnoid haemorrhage are associated with clusters of spreading depolarizations. *Brain.* 2006;129:3224–3237. DOI: 10.1093/brain/awl297.
207. Dreier JP, Major S, Manning A, Woitzik J, Drenckhahn C, Steinbrink J, Toliás C, Oliveira-Ferreira AI, Fabricius M, Hartings JA, et al. Cortical spreading ischaemia is a novel process involved in ischaemic damage in patients with aneurysmal subarachnoid haemorrhage. *Brain.* 2009;132:1866–1881. DOI: 10.1093/brain/awp102.
208. Bosche B, Graf R, Ernestus RI, Dohmen C, Reithmeier T, Brinker G, Strong AJ, Dreier JP, Woitzik J. Recurrent spreading depolarizations after subarachnoid hemorrhage decreases oxygen availability in human cerebral cortex. *Ann Neurol.* 2010;67:607–617. DOI: 10.1002/ana.21943.
209. Woitzik J, Dreier JP, Hecht N, Fiss I, Sandow N, Major S, Winkler M, Dahlem YA, Manville J, Diepers M, et al. Delayed cerebral ischemia and spreading depolarization in absence of angiographic vasospasm after subarachnoid hemorrhage. *J Cereb Blood Flow Metab.* 2012;32:203–212. DOI: 10.1038/jcbfm.2011.169.
210. Dreier JP, Petzold G, Tille K, Lindauer U, Arnold G, Heinemann U, Einhüpl KM, Dirnagl U. Ischaemia triggered by spreading neuronal activation is inhibited by vasodilators in rats. *J Physiol.* 2001;531:515–526. DOI: 10.1111/j.1469-7793.2001.05151.x.
211. Dreier J, Windmuller O, Petzold G, Lindauer U, Einhäupl K, Dirnagl U. Ischemia triggered by red blood cell products in the subarachnoid space is inhibited by nimodipine administration or moderate volume expansion/hemodilution in rats—PubMed. *Neurosurgery.* 2002;51:1465–1467.
212. Sugimoto K, Nomura S, Shirao S, Inoue T, Ishihara H, Kawano R, Kawano A, Oka F, Suehiro E, Sadahiro H, et al. Cilostazol decreases duration of spreading depolarization and spreading ischemia after aneurysmal subarachnoid hemorrhage. *Ann Neurol.* 2018;84:873–885. DOI: 10.1002/ana.25361.
213. Hernández-Cáceres J, Macías-González R, Brožek G, Bureš J. Systemic ketamine blocks cortical spreading depression but does not delay the onset of terminal anoxic depolarization in rats. *Brain Res.* 1987;437:360–364.
214. Lauritzen M, Hansen AJ. The effect of glutamate receptor blockade on anoxic depolarization and cortical spreading depression. *J Cereb Blood Flow Metab.* 1992;12:223–229. DOI: 10.1038/jcbfm.1992.32.
215. Marrannes R, Willems R, De Prins E, Wauquier A. Evidence for a role of the N-methyl-D-aspartate (NMDA) receptor in cortical

- spreading depression in the rat. *Brain Res.* 1988;457:226–240. DOI: 10.1016/0006-8993(88)90690-7.
216. Hertle DN, Dreier JP, Woitzik J, Hartings JA, Bullock R, Okonkwo DO, Shutter LA, Vidgeon S, Strong AJ, Kowoll C, et al. Effect of analgesics and sedatives on the occurrence of spreading depolarizations accompanying acute brain injury. *Brain.* 2012;135:2390–2398. DOI: 10.1093/brain/aws152.
217. Carlson AP, Abbas M, Alunday RL, Qeadan F, William SC. Spreading depolarization in acute brain injury inhibited by ketamine: a prospective, randomized, multiple crossover trial. *J Neurosurg.* 2019;130:1513–1519. DOI: 10.3171/2017.12.JNS171665.
218. Santos E, Olivares-Rivera A, Major S, Sánchez-Porras R, Uhlmann L, Kunzmann K, Zerelles R, Kentar M, Kola V, Aguilera AH, et al. Lasting s-ketamine block of spreading depolarizations in subarachnoid hemorrhage: a retrospective cohort study. *Crit Care.* 2019;23:427. DOI: 10.1186/s13054-019-2711-3.
219. Ayata C, Jin H, Kudo C, Dalkara T, Moskowitz MA. Suppression of cortical spreading depression in migraine prophylaxis. *Ann Neurol.* 2006;59:652–661. DOI: 10.1002/ana.20778.
220. Bogdanov VB, Multon S, Chauvel V, Bogdanova OV, Prodanov D, Makarchuk MY, Schoenen J. Migraine preventive drugs differentially affect cortical spreading depression in rat. *Neurobiol Dis.* 2011;41:430–435. DOI: 10.1016/j.nbd.2010.10.014.
221. Hamming AM, Van Der Toorn A, Rudrapatna US, Ma L, Van Os HJA, Ferrari MD, Van Den Maagdenberg AMJM, Van Zwet E, Poinatte K, Stowe AM, et al. Valproate reduces delayed brain injury in a rat model of subarachnoid hemorrhage. *Stroke.* 2017;48:452–458. DOI: 10.1161/STROKEAHA.116.014738.
222. Pisani A, Calabresi P, Tozzi A, D'Angelo V, Bernardi G. L-type  $Ca^{2+}$  channel blockers attenuate electrical changes and  $Ca^{2+}$  rise induced by oxygen/glucose deprivation in cortical neurons. *Stroke.* 1998;29:196–202.