



# Rethinking the initial changes in subarachnoid haemorrhage: Focusing on real-time metabolism during early brain injury

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## Summary

Over the last two decades, neurological researchers have uncovered many pathophysiological mechanisms associated with subarachnoid haemorrhage (SAH), with early brain injury and delayed cerebral ischaemia both contributing to morbidity and mortality. The current dilemma in SAH management inspired us to rethink the nature of the insult in SAH: sudden bleeding into the subarachnoid space and hypoxia due to disturbed cerebral circulation and increased intracranial pressure, generating exogenous stimuli and subsequent pathophysiological processes. Exogenous stimuli are defined as factors which the brain tissue is not normally exposed to when in the healthy state. Intersections of these initial pathogenic factors lead to secondary brain injury with related metabolic changes after SAH. Herein, we summarized the current understanding of efforts to monitor and analyse SAH-related metabolic changes to identify those precise pathophysiological processes and potential therapeutic strategies; in particular, we highlight the restoration of normal cerebrospinal fluid circulation and the normalization of brain-blood interface physiology to alleviate early brain injury and delayed neurological deterioration after SAH.

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## Introduction

Subarachnoid haemorrhage (SAH) is an acute cerebrovascular disorder and is mainly caused by rupture of intracranial aneurysms. Although ruptured aneurysms can be effectively treated by surgical clamping or interventional embolization, the inpatient mortality for

patients with ruptured aneurysms has remained unchanged in the United States, ranging from 13.7% in 2006 to 13.1% in 2018.<sup>1</sup> In recent years, the milestone CONSCIOUS-2 (NCT00558311) and CONSCIOUS-3 (NCT00940095) clinical trials have inspired us to focus on early brain injury, in addition to delayed cerebral ischaemia caused by vasospasm after SAH.<sup>2</sup> However, there is still a lack of in-depth understanding and effective treatment of secondary brain injury, which leads to a poor prognosis in patients with SAH, often including cognitive dysfunction.<sup>2,3</sup>

Current theory in SAH research and management includes consideration of the two distinct physiological insults that occur immediately after SAH: 1) sudden bleeding into the subarachnoid space and 2) hypoxia due to disturbed cerebral circulation and increased intracranial pressure. Early brain injury may follow

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exogenous materials in subarachnoid space and cerebrospinal fluid (CSF). While the goal of neurocritical care management is restoring effective intracranial perfusion, CSF stimuli can induce injuries to the vascular neural network, resulting in various reported pathophysiological changes. The goal of restoring effective intracranial perfusion has led to recent advances in treatments in the acute and subacute periods after SAH. In addition, these two factors intersect in several ways and create a vicious cycle for SAH patients. Therefore, monitoring and intervention directed towards the initial changes and subsequent consequences might be promising for early brain injury treatment after SAH.

It should be noted that we do not discuss the role of intracerebral haematoma formation in high-grade SAH patients, which might be more similar to the pathophysiological process after intracerebral haemorrhage. This review provides an in-depth update on the pathological processes that follow SAH by focusing on real-time metabolic changes related to early brain injury to uncover novel avenues for research and improve the clinical management of SAH patients.

### Intersections in the pathophysiological changes induced by two initial factors

#### Exogenous stimuli in CSF induce microcirculatory dysfunction (Figure 1)

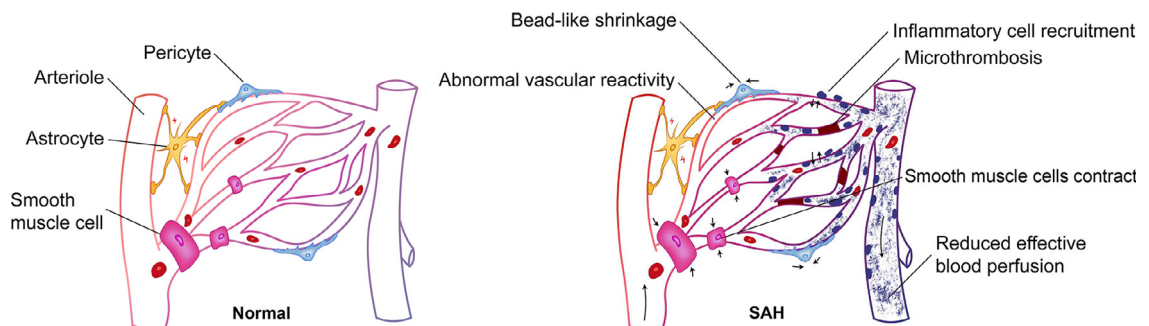
It is well known that the CSF circulation helps maintain brain haemostasis and normal metabolism. Sudden bleeding into the subarachnoid space could cause disruption of flow in the cerebral ventricles, along perivascular spaces and the glymphatic system by the exogenous stimuli mentioned above. Several decades of experimental and clinical data indicate that CSF haemoglobin induces secondary brain injury after SAH,<sup>4</sup> as we speculated before. Targeting CSF haemoglobin reduces

delayed cerebral ischaemia and improves neurological outcomes after SAH in animal models and has potential for clinical application in patients.<sup>5</sup>

However, there are not just blood and haemolytic products in CSF; at least two types of materials should be specifically considered and monitored. One is haemolytic product-induced secondary neuroinflammatory factors, such as inflammasome-derived caspase-1 activity, which is tightly associated with blood coagulation in CSF and poor prognosis of SAH patients.<sup>6</sup> Elevated CSF lipocalin-2 mediates neuroinflammation and iron homeostasis and is reported to be a biomarker for unfavourable outcomes of SAH patients<sup>7</sup> and a potential therapeutic target for long-term neurological rehabilitation.<sup>8</sup>

Another set of biomarkers comprises the brain metabolites released into CSF, which could serve as biomarkers of brain injury by exogenous stimuli and, more importantly, as the initiators of secondary brain injury that eventually leads to irreversible pathophysiological cascades. Ho WM *et al.* employed a validated kit to identify and quantify 186 endogenous metabolites in the CSF sample of SAH patients<sup>9</sup> and surprisingly found no significant changes in glutamate, creatinine and several other metabolomics in CSF within 24 hrs after SAH, but taurine levels significantly decreased, and 17 amino acids, including glutamate and creatinine, substantially increased and reached a maximum 1 week after SAH. Wang KC *et al.* reported that high mobility group box 1 (HMGB1) released by injured neurons into the CSF also participates in subsequent immune responses and serves as a prognostic indicator for SAH patients.<sup>10</sup>

Therefore, time-dependent alterations in CSF metabolites and compounds may elucidate pathophysiological processes after SAH, and machine learning-driven metabolomic evaluation of CSF might provide more in-depth information on the pathologic underpinnings of secondary brain injury after SAH.<sup>11</sup>



**Figure 1.** Schematic diagram of the microcirculation dysfunction induced by initial stimulation after SAH.

Left: Schematic diagram of the normal cerebral microcirculation. Right: Schematic diagram of microcirculation disturbance after SAH. After microvasospasms in the compensation period, platelet aggregation and leukocyte plugging in capillaries cause microcirculation dysfunction in the decompensation phase. When the microthrombi fill microvessels and the glymphatic drainage system, the whole microcirculation collapses without blood flow, and microcirculation dysfunction moves into an irreversible phase.

During aneurysm clipping surgery, Uhl *et al.* observed monosegmental and pearl-string multisegmental microvasospasms in arterioles for the first time, with a 75% reduction in cortical microcirculation after SAH.<sup>12</sup> A recent digital subtraction angiography (DSA) study found that the intracerebral circulation time within 24 hrs after ictus was significantly correlated with the occurrence of delayed cerebral ischaemia in SAH patients as an independent factor in addition to angiographic vasospasm,<sup>13</sup> which suggests that microcirculatory dysfunction in the ultra-early and subacute phases can be a causative factor for delayed cerebral ischaemia development and poor outcome.

More importantly, these microvasospasms in arterioles are not mediated by endothelin A receptors, suggesting distinct mechanisms in large and small cerebral vessels, which might be an explanation for the unfavorable results of CONSCIOUS trials.<sup>14</sup> Our previous study demonstrated that haemoglobin induced acute pericapillary pericyte contraction, resulting in vivid and similar pearl-string-like microvasospasms for microcirculation dysfunction after SAH in mice.<sup>15</sup> Haem degradation intermediate bilirubin oxidation end products (BOXes) and propentdyopents (PDPs) are significantly abundant in the CSF of patients with SAH and exhibit vasoconstrictive effects contributing to neurological deficits.<sup>16</sup> In addition, free iron ( $\text{Fe}^{3+}$ ) released from haemolytic red blood cells is also involved in arteriolar and capillary microvasospasms after SAH in mice,<sup>17</sup> while iron deposits in the cortex have been reported to be associated with cognitive outcomes in SAH patients.<sup>18</sup>

As we previously proposed and reviewed,<sup>19</sup> there are three steps of microcirculation disturbance after SAH: microcirculation compensation, decompensation and irreversible phases. After microvasospasms in the compensation period, platelet aggregation<sup>20</sup> and leukocyte plugging<sup>21</sup> in capillaries cause microcirculatory dysfunction in the decompensation phase. When the microthrombi fill microvessels<sup>22</sup> and impair the glymphatic drainage system,<sup>23</sup> the whole microcirculation collapses without blood flow, and microcirculatory dysfunction moves into an irreversible phase. Recent findings involving neutrophil extracellular traps (NETs)<sup>24</sup> released from neutrophils after SAH might represent important intersections amongst platelet aggregation, microthrombosis, and microglia-mediated inflammation,<sup>25</sup> further supporting the pathophysiological characteristics of the irreversible phase of microcirculation.<sup>26,27</sup>

### Hypoxia aggravates pathophysiological changes after bleeding into the CSF

It is well known that the vast majority of patients lack ischaemic lesions in brain imaging at the early phase of SAH; even in limited cohorts with poor-grade ruptured aneurysms, only approximately 21% of patients suffer

from cerebral infarction.<sup>28</sup> However, the metabolomic profiling of CSF indicates that 2-hydroxyglutarate, a known marker of tissue hypoxia, correlates with the long-term outcomes of SAH patients independent of vasospasm status.<sup>29</sup> Cerebral microdialysis and multimodal neuromonitoring strategies have found that brain tissue hypoxia ( $\text{PbtO}_2 < 20$  mmHg) exists in more than 60% of patients within the first 24 h after SAH.<sup>30</sup>

During brain hypoxia, hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) is traditionally considered to be the core of pathophysiological processes. Accumulated evidence has established the dual nature of HIF-1 $\alpha$  as an adaptive factor and cell death mediator, which depends on pathophysiological conditions, developmental phase, comorbidities, and administered medications.<sup>31</sup> Dong Y *et al.* inhibited HIF-1 $\alpha$  using YC-1 and found increased hippocampal apoptosis and cognitive dysfunction,<sup>32</sup> while 2-methoxyestradiol, acting as a HIF-1 $\alpha$  inhibitor, ameliorated early brain injury and cerebral vasospasm in SAH rats.<sup>33</sup> There are two principal ways by which HIF-1 $\alpha$  is activated: an oxygen-dependent reaction and an oxygen-independent reaction. Hypoxia and oxidizing redox status are considered established enhancers of HIF-1 $\alpha$  protein stabilization, while nonhypoxic activators appear to include only HIF-1 $\alpha$  activity and not protein stabilization. Normoxia and several protein modifications decrease HIF-1 $\alpha$  expression and activity.

HIF-1 $\alpha$  not only regulates the expression of target genes and thereby influences resultant protein levels but also contributes to epigenetic changes that may reciprocally provide feedback regulation loops. On this basis, we wondered whether there are more comprehensive pathophysiological changes underlying brain hypoxia after SAH. Lactate in CSF, as the intermediate product of the tricarboxylic acid cycle and energy metabolism, has been demonstrated to be a biomarker for early brain injury and tightly correlated with neurological outcomes in SAH patients.<sup>34,35</sup> Therefore, assessment of the lactate/pyruvate ratio in dialysate obtained as part of multimodal monitoring during early brain injury might be informative and beneficial for SAH patients.<sup>36</sup> The underlying mechanism of lactate-mediated pathophysiological processes might be much more complicated than previously thought. In addition to acting as a biomarker secondary to metabolomic production under hypoxia, lactate may be a pathogenic mediator, as it can regulate downstream gene expression by lactylation of histones<sup>37</sup> and of nonhistone proteins,<sup>38</sup> a newly reported posttranslational modification. Despite limited evidence of the role and mechanism of lactylation, repeated lactate exposure has major effects on the expression of regulatory enzymes of glycolysis and mitochondrial respiration.<sup>39</sup> It has been demonstrated that lysine lactylation in brain macrophages is regulated by neural excitation and social stress, with parallel changes in lactate levels.<sup>40</sup> Histone lactylation might occur separately from interleukin-6-

induced inflammatory metabolic adaptation.<sup>41</sup> Thus, targeting lactylation may be a potential therapeutic approach for cancer,<sup>42</sup> inflammation,<sup>38</sup> Alzheimer's disease<sup>43</sup> and other brain disorders.

### Advances in monitoring metabolomic changes associated with early brain injury after SAH

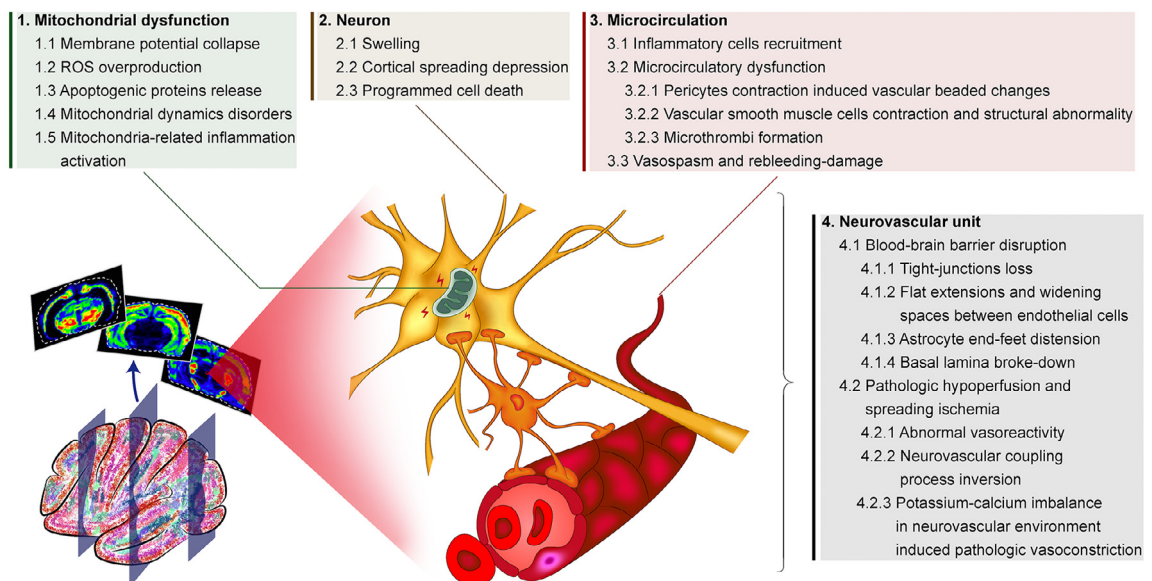
Ideally, the goal of neurocritical care management for SAH and other acute brain injuries is to restore effective oxygen and glucose delivery to the brain, which typically requires adequate intracranial vascular perfusion; thus, therapeutic hypervolaemia, haemodilution, and hypertension (HHH) therapy has been used for SAH patients for a long time. A recent study indicated that although global and regional cerebral blood flow improved with HHH therapy, metabolites in microdialysis fluid were statistically unchanged in SAH patients.<sup>44</sup> In this cohort, no patients exhibited signs of severe ischaemia but they commonly had a disturbed energy metabolic pattern, possibly reflecting mitochondrial dysfunction<sup>45</sup> in addition to improved microcirculation. Extracellular mitochondria in CSF may provide additional information on brain integrity and recovery after SAH.<sup>46,47</sup> Thus, mitochondrial dysfunction could be a potential therapeutic target for SAH management,<sup>48</sup> but further studies are needed to precisely evaluate the spatial variations and characterize the biological cascade.

### Spatial metabolomics to assess real-time metabolic changes (Figure 2)

Although SAH usually has a specific causative factor, complex primary injury is superimposed on the

patient's own susceptibility factors, resulting in multiple interwoven pathophysiological changes along the time sequence. A large amount of biological information and biomarkers can be used in clinical diagnosis and treatment. In addition to neuroimaging and electrophysiological monitoring, metabolic biomarker measurement reflects the real-time changes in brain injury lesions on another level, especially the occurrence, development and outcome of secondary brain injury after SAH; together, these three techniques constitute a multimodal monitoring system to capture the larger picture of SAH pathophysiology.<sup>49</sup> Thus, in contrast to the previous monitoring and clinical validation of a single indicator, multiple indicators, or even a spectrum of biomarker expression, in the search for and systematic study of biomarkers closely related to the pathophysiological mechanism of brain injury could be a technical roadmap for future SAH research, similar to the five-phase framework for developing epileptogenic biomarkers.<sup>50</sup> Interestingly, recent analysis indicates that biomarkers within 24 hrs after acute brain injury are related to the severity of injury rather than the pathoanatomical type of injury, including SAH.<sup>51</sup>

Unlike traditional proteomic and metabolomic analyses of biological samples harvested from human patients or experimental animal models, high-spatial-resolution imaging mass spectrometry-based spatial metabolomics can visualize the composition, abundance, and spatial distribution of molecules in tissues or cells and has been widely used in life science research.<sup>52</sup> Recently, Chen Y *et al.* employed matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) to visualize the spatial-chemical changes in metabolite concentrations in an APP/PS1



**Figure 2.** Schematic diagram of the different levels of metabolic changes in the brain parenchyma after SAH.

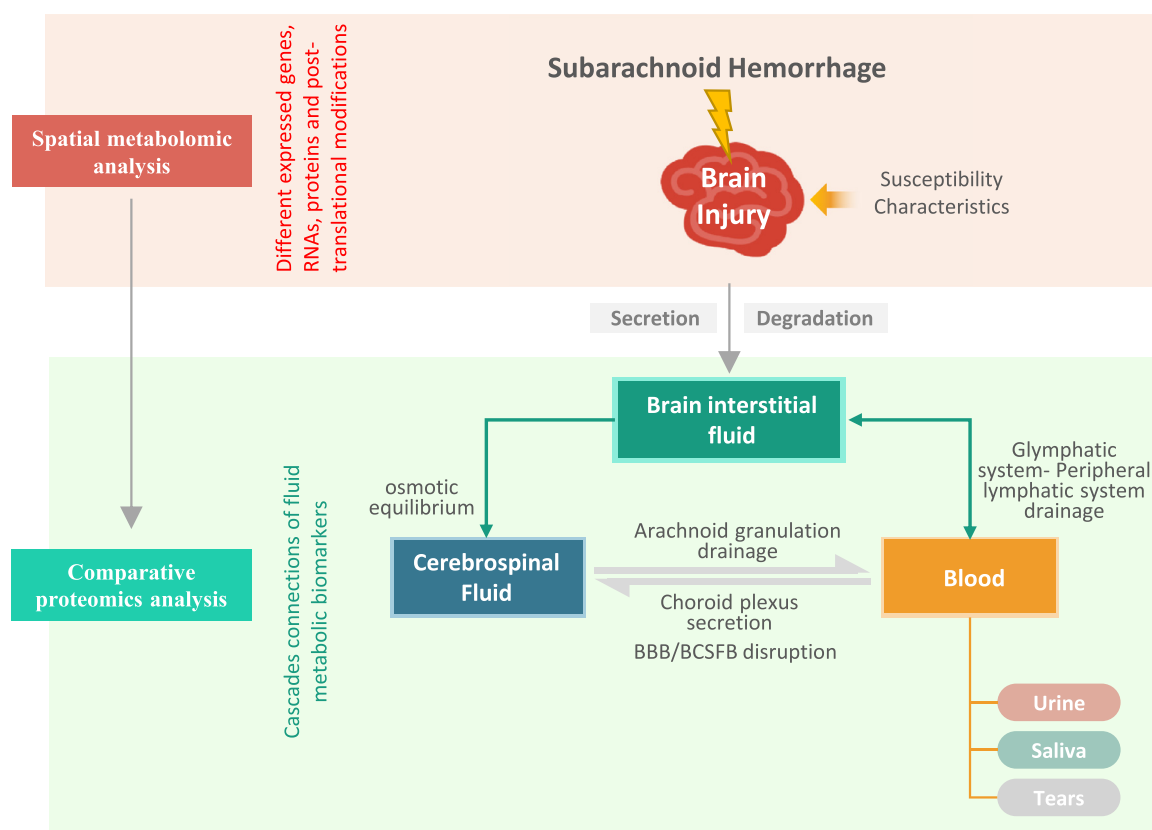
transgenic mouse model of Alzheimer's disease and found 93 metabolites within different brain regions linked to the etiopathogenesis of Alzheimer's disease.<sup>53</sup> This newly adopted technology might have applications in scenarios lacking reliable immunohistochemical and diagnostic biomarkers<sup>54</sup> for real-time metabolic changes in the brain parenchyma. In addition, extended algorithms, such as false discovery rate (FDR)-controlled metabolite annotation<sup>55</sup> or the spatial single nuclear metabolomics (SEAM) platform,<sup>56</sup> could explore the spatial metabolic profile and tissue histology at the single-cell level, leading to a deeper understanding of tissue metabolic organization.

### Cascade biomarkers and comparative proteomics analysis of early brain injury in SAH patients (Figure 3)

Blood circulation runs through all tissues and organs of the body, and therefore clinical biomarkers are commonly assayed in blood. The correlation of peripheral blood NF-L, GFAP, Tau and other biomarkers with brain injury and prognosis after SAH has been widely recognized.<sup>57–59</sup> Grant C. O'Connell's team enrolled nearly 12,000 human specimens to evaluate over 17,000 protein-coding genes and developed algorithms to investigate their potential to produce blood

biomarkers for neurological damage based on their expression profiles both across the body and within the brain;<sup>60</sup> this valuable resource is expected to be applied for advancing clinical diagnosis and treatment of acute and chronic central nervous system diseases such as traumatic brain injury, ischaemic or haemorrhagic stroke, Alzheimer's disease and multiple sclerosis.

CSF, as one of the intrinsic components of the intracranial system, is wrapped around and supports the whole brain and spinal cord and plays a role in supporting and removing metabolic products, which contain a variety of signals that participate in regulating the activities of the central nervous system, as well as brain tissue metabolites and the exchange of materials with the blood. Although CSF is connected with the blood circulation through the interstitial perivascular drainage pathway, the glymphatic system and peripheral lymphatic vessels in the brain,<sup>61,62</sup> it is a relatively closed system. Compared with blood biomarkers, CSF biomarkers can directly reflect brain tissue injury and metabolism after traumatic brain injury. Since the beginning of the 21st century, CSF biomarkers associated with secondary brain injury after SAH have gradually gained attention, especially low abundance proteins specific to CSF, making important progress in the diagnosis and monitoring of early brain injury and prognostication.<sup>29,63</sup>



**Figure 3.** Cascade connections of various biomarkers to monitor early brain injury after SAH.



In addition, easily accessible body fluids such as urine, saliva and tears may have unimaginable practical value in clinical service environments where there are no monitoring facilities for brain injury. Cortisol remains the only biomarker that can be detected in blood, CSF, urine and saliva. MicroRNA and Apo-lipoprotein E are salivary biomarkers, and *Stoob* and norepinephrine can be monitored in urine, according to a large systematic review.<sup>64</sup> The development of biomarkers based on the biological metabolic cascade system of brain injury, where metabolite changes in the brain or subarachnoid space are reflected in the CSF, and then in blood, urine and saliva, has potential for enable a future breakthrough in the field of rapid, non-invasive, on-site diagnostics for brain injury.

Comparative proteomics evaluates metabolic changes or differences in all protein expression levels in suborganellar targets, cells, tissues, and biofluids under different conditions. Thus, multiple biological samples from SAH patients and animal models might provide a more systematic understanding of secondary brain injury after SAH (Table 1). Recently, Andrew A. Pieper's research team<sup>65</sup> analysed ac-Tau from four different angles: ac-Tau production induced by brain injury, plasma ac-Tau marker monitoring, neuroprotective mechanism of ac-Tau inhibition, and ac-Tau-induced encephalopathy after brain injury. This study provided an in-depth view of the role and mechanism of ac-Tau in the pathophysiological process of brain trauma and its prospects for application as a biomarker, which could serve as an excellent example for the development of biomarkers for brain trauma and other central nervous system diseases and for further translational clinical practice.<sup>50</sup>

## Implications for the mechanism and therapeutic strategies of SAH

### Restoring the balance of CSF circulation

As summarized above, exogenous stimuli in the CSF induce cerebral circulation dysfunction after SAH, while brain metabolites are secreted into the interstitial fluid and CSF, acting as both biomarkers and pathogenic factors. The uncleared visible components of the blood appear as blood clots in the sulci and cisterns, and block the normal flow of CSF, further increasing the intracranial pressure. The balance between CSF pressure and precapillary and venous pressure is unbalanced and the volume of fluid in the brain is further increased.<sup>66</sup> In addition, hindrance of CSF circulation along glymphatic pathways is another mechanism that may interrupt the drainage of damaging substances from the subarachnoid space and parenchyma<sup>23</sup> because a recent finding of CSF flow block did not correlate with the size of haemorrhage,<sup>67</sup> while CSF macrophage CD163, a haemoglobin scavenger receptor for

blood clearance, was significantly associated with hydrocephalus and outcome after SAH in patients.<sup>68</sup> Moreover, meningeal lymphatics also participate in clearing red blood cells from the CSF to cervical lymph nodes without significant lymphangiogenesis and lymphangiectasia in SAH mice.<sup>69</sup> Despite the effectiveness of CSF external drainage strategies, the average output of CSF after SAH is a risk factor for vasospasm and chronic hydrocephalus,<sup>70</sup> and CSF volume based on algorithm-assisted segmentation of computed tomography images might be a surrogate for vasospasm after SAH.<sup>71</sup> Therefore, restoring CSF circulation balance after SAH is beneficial not only for the overall clinical outcome of SAH patients but also for obtaining more precise information on both destructive stimuli and metabolites from microdialysis fluid and CSF to enable advances in vascular neural network protection and neurological recovery.

### Reconstruction of intracranial barrier structures

The blood-brain barrier (BBB) is an endogenous protective structure for brain haemostasis and normal metabolism. However, SAH induces serious alterations in component cells, leading to brain homeostasis disruption. It was reported that most SAH patients lack imaging lesions of brain parenchymal injury but have significant BBB disruption,<sup>72</sup> which occurs immediately after SAH but has long-lasting consequences. Early detection of BBB dysfunction could effectively predict neurological outcomes after SAH, with an area under the receiver operating characteristic curve of 0.829, in combination with the Rosen-Macdonald score.<sup>73</sup> Recent experiments have discovered several pathophysiological mechanisms that effect the BBB after SAH, and alleviating these cascades might be decisive in inhibiting the negative impact of bleeding in the subarachnoid space.<sup>74</sup> Free haem, released through haemolysis, is bound by haemopexin and rapidly scavenged by CD91. It was reported that CSF haemopexin significantly increased and was associated with iron deposition in brain tissue and poorer neurological outcome in SAH patients, and more interestingly, the serum haemopexin level was lower when the BBB was compromised after SAH.<sup>75</sup> Thus, recovery of endogenous recovery mechanisms and barriers can break the vicious cycle, maintain brain metabolic haemostasis and eventually alleviate early brain injury after SAH. However, due to limited studies in this research area, further work is needed.

## Conclusion

Over the last two decades, neurological researchers have uncovered many pathophysiological mechanisms, including early brain injury and delayed cerebral ischaemia, which may determine neurological outcomes after SAH. Herein, we have reviewed the current

Metabolic biomarkers	Intrinsic function	Sample types	Time	Main outcome	References
<b>Blood hemolytic-related</b> Hemoxygenase-1 (HO-1)	Dependent on intracranial hematoma burden	CSF	<14 days after SAH	Unable to predict functional outcome. early low HO-1 expression associates with vasospasm	Neurocrit Care. 2022;36(1):279-91.
	From lysing erythrocytes	CSF	<14 days after SAH	Patients with delayed ischemic neurologic deficits had a significantly higher cumulative oxyHb exposure within the first week after bleeding.	World Neurosurg. 2018;120:e660-e6.
	Binds to and facilitates clearance of heme	Blood	<8 days after SAH	HP 2-2 genotype leads to increased proinflammatory cytokine levels compared with HP 1-1/1-2 genotypes	Curr Neurovasc Res. 2020;17(5):652-9.
	Regulation of coagulation and fibrinolysis.	CSF	<11 days after SAH	Early predictor of cerebral vasospasm	Acta Med Okayama. 2019;73(1):29-39.
	Hemoprotein specific to the brain, and its production is specifically increased during hypoxic-ischemic brain damage.	Serum	<7 days after SAH	Serum Ngb level was significantly elevated in delayed cerebral ischemia patients, with a strong association with the cerebral microdialysis parameters and 12-month functional outcome in severe SAH patients	Brain Behav. 2020;10(3):e01547. Stroke. 2019;50(7):1887-90. World Neurosurg. 2018;116:e258-e65.
<b>Neuroinflammation-related</b>					
High-mobility group box-1 (HMGB1)	Prothrombotic and proinflammatory	Serum	on admission	Independent biomarker predictive of delayed cerebral ischemia, but not reflect initial insult	Neurosurg Rev. 2022;45(1):807-17.
Lipocalin-2 (LCN-2)	Neuro-inflammation and iron homeostasis	Serum	<14 days after SAH	Elevated early after SAH (day 1) and remained significantly high until day 13 in patients who developed cerebral vasospasm.	Crit Care Med. 2018;46(11):e1023-e8.
Fatty acid-binding protein 3 (FABP3)	Vascular inflammation and cellular death	CSF	<5 days after SAH	Higher CSF LCN2 throughout post-SAH days 1-5 was associated with unfavorable outcome at 3 and 6 months. Higher plasma LCN-2 levels over time were associated with worse 6-month outcome.	J Cereb Blood Flow Metab. 2021;41(10):2524-33.
Tumor necrosis factor superfamily 14 (LIGHT/TNFSF14)	Immune response	Serum	24 h after SAH	Early FABP3 and CXCL-16 levels are significantly associated with poor 30-day outcome	J Stroke Cerebrovasc Dis. 2021;30(11):10608.
Soluble growth stimulation expressed gene 2 (sST2)	Immune response to neurovascular injury	Serum	24 h after SAH	LIGHT/TNFSF14 was found to be independently associated with 30-day mortality, but not with delayed cerebral ischemia	Acta Neurol Scand. 2021;143(5):530-7.
Ficolin-1	Lectin complement pathway	Plasma	<14 days after SAH	Plasma sST2 predicts new epileptiform abnormalities, delayed cerebral ischemia, 90-day outcome, and mortality after SAH, independent of clinical and radiographic markers.	Ann Neurol. 2019;86(3):384-94. Stroke. 2020;51(4):1128-34. Stroke. 2021;52(8):e494-e6.
Mannose-binding lectin (MBL)		CSF	<7 days after SAH	Increased CSF levels of ficolin-1 and MBL were associated with a poor functional outcome	J Neuroinflammation. 2020;17(1):338.
Soluble lectin-like oxidized low-density lipoprotein receptor-1 (sLOX-1)		Serum	/	Positive association with hemorrhagic severity, appears to have the potential to become a promising predictor of delayed cerebral ischemia after SAH.	Mol Neurobiol. 2017;54(8):6572-80. Brain Behav. 2020;10(2):e01517.
M-ficolin	Pro-inflammatory chemokine	Plasma	<1 days after SAH	Correlated to Hunt-Hess score at admission.	Mol Neurobiol. 2019;56(1):78-87.
Chemokine C-C motif ligand 5 (CCL5)		CSF	<7 days after SAH	CCL5 levels correlated with clinical outcome.	Cytokine. 2020;133:155142.
		Serum		CSF CCL5 levels increased on day 1 after SAH in patients developing chronic hydrocephalus, delayed ischemic neurological deficits and intraventricular hemorrhage.	
				Serum CCL5 levels were significantly lower on day 7 after aSAH in patients developing chronic hydrocephalus, pneumonia infection and who have additional intracerebral bleeding.	
RhoA	RhoA/Rho-kinase in peripheral blood mononuclear cells	Peripheral blood mononuclear cells	<5 days after SAH	RhoA expression and activity in peripheral blood mononuclear cells might be related with SAH severity and cerebral vasospasm	Stroke. 2018;49(6):1507-10.
Interleukin-33 (IL-33)	Inflammatory cytokine	Serum	On admission	High serum IL-33 concentrations have close relation to the inflammation, severity and poor outcome in aSAH	Clin Chim Acta. 2018;486:214-8.
Clusterin	Inflammatory cytokine	CSF	<14 days after SAH	Higher levels of CSF clusterin were found 5-7 days after SAH in patients with good outcome.	World Neurosurg. 2017;107:424-8.
Soluble triggering receptor expressed on myeloid cells-1 (sTREM-1)	Inflammatory	CSF	<7 days after SAH	Significant correlation between CSF clusterin level 5-7 days after SAH and Glasgow Outcome Scale at 3 months.	J Clin Neurosci. 2017;35:139-43.
Migration inhibitory factor (MIF)	Proinflammatory cytokine	Serum	On admission	Correlations of early CSF sTREM-1 levels to patients' severity and prognosis. Serum MIF provides information about inflammation, brain injury severity and outcome after aSAH	Clin Chim Acta. 2017;473:60-4.

Table 1 (Continued)

Metabolic biomarkers	Intrinsic function	Sample types	Time	Main outcome	References
<b>Vascular-related</b>					
L-arginine to L-ornithine ratio	Enzyme arginase-1 is released into CSF during red blood cell lysis and contributes to dysregulated metabolism of the nitric oxide precursor L-arginine	CSF	<22 days after SAH	Arg/Om < 2.71 at SAH onset predicted cerebral vasospasm with a sensitivity of 86.7% and specificity of 72.2%. Arg/Om > 2.71 predicted excellent functional outcome.	Transl Stroke Res. 2022;13(3):382-90. Clinical Trial No. DRKS00015293
Asymmetric Dimethylarginine (ADMA)	Vasoreactive molecules linked to the nitric oxide pathway	CSF	/	SDMA, DMGV, and Ornithine associate with poor mRS at discharge and at 90d after SAH	Neurosurgery. 2021;188(5):1003-11. Neurocrit Care. 2018;29(1):84-93.
Symmetric Dimethylarginine (SDMA)				ADMA and the L-arginine/ADMA ratio are associated with the incidence of delayed cerebral ischemia after SAH	
Dimethylguanidine valeric acid (DMGV)				SDMA was associated with initial neuronal damage and poor neurological outcome after SAH	
Ornithine					
Calcitonin gene-related peptide (CGRP)	Vasodilatory	CSF	<10 days after SAH	Negative short-term impact on health-related quality of life and emotional health like anxiety and depression, protective against vasospasm-associated cerebral ischemia after SAH	Neurosurg Rev. 2021;44(3):1479-92.
Soluble vascular endothelial-cadherin (sVE-cadherin)	Released from injured endothelium	CSF	<8 days after SAH	Develop worse functional outcome at 3 months after SAH	Neurology. 2020;94(12):e1281-e93.
Tissue kallikrein (TK)	Serine proteinases, component of the kallikrein-kinin system	Serum	/	Highly correlated with hemorrhagic severity. Independent predictor for delayed cerebral ischemia.	Clin Chim Acta. 2020;502:148-52.
Lipoprotein-associated phospholipase A2 (Lp-PLA2)	Chronic vascular inflammation	Serum	<24 h after SAH	Elevated Lp-PLA2 level have higher risk of vasospasm and 6-month mortality	Neurosurgery. 2020;86(1):122-31.
<b>Hypoxia-related</b>					
Lactate	Hypoxia	CSF	<14 days after SAH	Reflect the early brain injury and represent predictive biomarkers of delayed cerebral ischemia following SAH	J Stroke Cerebrovasc Dis. 2020;29(5):104765.
Lactate dehydrogenase (LDH)	Hypoxia	CSF	/	CSF pyruvate level was significantly associated with WFNS grading scale	ACS Chem Neurosci. 2019;10(3):1666-7.
Pyruvate				Correlated with GOS at 1 year post-SAH independent of vasospasm status.	Br J Neurosurg. 2018;32(6):637-41.
2-hydroxyglutarate	Marker of tissue hypoxia	CSF	On admission and the anticipated vasospasm timeframe.		
8-iso-Prostaglandin F2alpha (8-iso-PGF2alpha)	Oxidative stress	Plasma	On admission	Plasma 8-iso-PGF2alpha elevation associates with the severity and poor outcome after SAH	Clin Chim Acta. 2017;469:166-70.
Extracellular mitochondria	Intracellular metabolic integrity	CSF	<3 days after SAH	Extracellular mitochondria may provide a biomarker-like glimpse into brain integrity and recovery after injury	Stroke. 2017;48(8):2231-7.
<b>Neuronal Injury-related</b>					
Neurofilament Light Chain (NFL)	Neuron injury	Plasma	<2 days after SAH	NFL levels are associated with disease severity during the early brain injury phase of SAH.	Transl Stroke Res. 2020;11(4):671-7.
tTau/pTau				Higher NFL levels during early brain injury are associated with poor functional outcome on day 30 after ictus and increased mortality rate.	Brain. 2021;144(3):761-8.
Abeta40/Abeta42				CSF and serum NFL on Days 1-3 post-SAH strongly predicted modified Rankin score at 6 months, independent of World Federation of Neurosurgical Societies (WFNS) score	Neurosurg Rev. 2018;41(2):605-14.
Sortilin	Enriched in neurons and is likely involved in neurodegenerative diseases.	CSF (Human)	<3 days after SAH	Higher tTau/pTau and lower Abeta40/Abeta42 CSF levels predict unfavorable long-term functional and health-related quality of life outcomes. Neuropsychological deficits correlate with increased CSF tTau and pTau concentrations.	Neuroscience. 2021;470:23-36.
S100A8/A9/calprotectin)	Neuron injury	Cortical lysates (Rats)	<3 days after SAH	Sortilin ~100 kDa was detectable in the CSF of the SAH, but not sham rats. Levels of sortilin ~100 kDa and fragments ~40 kDa in cortical lysates were elevated in the SAH relative to control rats.	J Stroke Cerebrovasc Dis. 2020;29(5):104770.
Regulated in development and DNA damage responses 1 (REDD1)	Highly conserved stress-response protein and can be induced by hypoxia/ischemia and DNA damage	Serum	<2 days after SAH	Correlated with the clinical severity and the poor prognosis at 3 months in SAH patients	Neurochem Int. 2019;128:14-20.
Netrin-1	Axon guidance molecule	CSF	<1 days after SAH	Critical role of neuronal damage process	
		Serum	/	Reflects severity, inflammation and prognosis of human aneurysmal SAH.	Clin Chim Acta. 2019;495:294-300.

Table 1 (Continued)



Metabolic biomarkers	Intrinsic function	Sample types	Time	Main outcome	References
<b>Other Metabolites</b>					
F2-Isoprostanes (F2-IsoPs) Isofurans (IsoF)	Specific markers of lipid peroxidation	CSF	<8 days after SAH	Specific biomarker predicting delayed cerebral ischemia	Neurocrit Care. 2022;36(1):202-7.
F2-IsoPs		Urinary	<3 days after SAH	Increased F2-IsoP levels on day 3 after SAH were associated with development of cerebral vasospasm and worse neurologic performance after 1 month and 4 months	World Neurosurg. 2017;107:185-93.
Adipocyte fatty acid-binding protein (FABP4)	Adipokine	Serum	<2 days after SAH	Elevated serum levels of FABP4 were related to poor 3 months outcome and mortality in a cohort of patients with SAH	J Neuroinflammation. 2020;17(1):66.
Periostin	Matricellular proteins (MCPs)	Plasma	<3 days after SAH	One-time early-stage measurement of plasma MCPs served for reliable prediction of delayed cerebral ischemia development	Mol Neurobiol. 2019;56(10):7128-35.
Osteopontin		Plasma	<3 days after SAH	Biomarker for predicting subsequent development of delayed cerebral infarction and eventual poor outcome without vasospasm.	Transl Stroke Res. 2018;9(2):110-9.
Galectin-3		Plasma	<3 days after SAH	Associates with higher incidence of delayed cerebral ischemia, but not angiographic vasospasm.	Neurotherapeutics. 2019;16(2):480-90.
Galectin-3		Plasma	<3 days after SAH	Higher level of periostin than patients with good outcome at 12 months.	J Clin Lab Anal. 2018;32(5):e22389.
Periostin		Plasma	<3 days after SAH	Independent predictor of poor outcome	Cells. 2019;8(7):695
Osteopontin		Serum	<3 days after SAH		
		Plasma	<8 days after SAH		
		CSF			
		Plasma	<3 days after SAH		
		Plasma	On admission	Highly associated with the severity and 6-month poor outcome	Mol Neurobiol. 2018;55(8):6841-9. Clin Chim Acta. 2018;486:335-40.
Cleaved receptor for advanced glycation end-products (cRAGE)	Proteolytic cleavage of full-length cell surface receptor RAGE protein	CSF	<14 days after SAH	Potential biomarkers for early recognition and severity of SAH	Sci Rep. 2018;8(1):13308.
Alpha-II spectrin breakdown products (SBDP150, SBDP145, and SBDP120)	Cytoskeletal protein	CSF	<10 days after SAH	CSF sPDGF $\beta$ level increases after SAH and is higher in patients who developed cerebral vasospasm, but not for unfavorable outcome after SAH	Neurol Sci. 2018;39(6):1105-11.
Soluble Platelet-derived growth factor beta (sPDGF $\beta$ )	Shed from pericytes to CSF is related to the injury of pericytes, PDGF-BB/PDGFR signaling	CSF	<10 days after SAH		
Copeptin	Marker of endogenous vasopressin levels	Plasma	On admission	Significantly improved the predictive performance of WFNS scores	Clin Chim Acta. 2017;475:64-9.
Soluble Fms-like tyrosine kinase 1 (sFlt-1)	Alternatively spliced transcript of the full-length domain of the VEGF-R1	Serum	<7 days after SAH	Serum levels of sFlt-1 are increased in patients with aSAH who are at risk for severe vasospasm.	World Neurosurg. 2017;108:84-9.
Nesfatin-1	Involved in central cardiovascular homeostasis	Serum	/	mortality/poor outcome of the SAH with assessing serum nesfatin-1	Int J Neurosci. 2017;127(2):154-60.

**Table 1: Novel metabolic biomarkers for monitoring pathophysiological changes during early brain injury after SAH.**

understanding of the initial and hyperacute changes after SAH. A worthy research goal would be to monitor and analyse metabolic changes after SAH and identify those with potential therapeutic strategies for early brain injury and subsequently delayed neurological deterioration after SAH.

### Outstanding questions

Whereas much has been learned about the pathophysiological mechanisms of SAH, neurological researchers still consider mainly early brain injury and delayed cerebral ischemia as contributors to the neurological outcomes after SAH. But the real-time metabolic changes induced by SAH are not fully understood. Current advances in spatial metabolomics and comparable proteomics might provide more precise spatial and cascade information and therapeutic targets for best translational value.

### Search strategy and selection criteria

Data for this review were identified by searches of MEDLINE, Current Contents, PubMed, and references from relevant articles using the search terms “subarachnoid haemorrhage”, “early brain injury”, “delayed cerebral ischemia”, “metabolism”, “microcirculation”, “vasospasm”, “haemoglobin”, “haemolytic”, “secondary brain injury”, “neutrophil extracellular traps”, “hypoxia”, “hypoxia-inducible factor-1 $\alpha$ ”, “lactate”, “lactylation”, “mitochondria”, “metabolomic”, “proteomic”, “biomarker”, “glymphatic”, “blood-brain barrier”. Only articles published in English were included with a particular focus on the past 3 years.

### Contributors

Dr. Yujie Chen and Dr. John H. Zhang conceptualized and outlined the manuscript. Dr. Yujie Chen drafted the manuscript, Dr. Ian Galea, Dr. R. Loch Macdonald, Dr. George Kwok Chu Wong, and Dr. John H. Zhang discussed contents and revised the manuscript. All authors have read and approved the current version of the manuscript.

### Data sharing statement

Not Applicable.

### Declaration of interests

The authors declare that they have no conflicts of interests.

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