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

Yujie Chen,[a,b]** Ian Galea,[c] R. Loch Macdonald,[d] George Kwok Chu Wong,[e] and John H. Zhang [f,g]*

[a]Department of Neurosurgery and State Key Laboratory of Trauma, Burn and Combined Injury, Southwest Hospital, Third Military Medical University (Army Medical University), Chongqing 400038, China
[b]CAS Key Laboratory of Separation Science for Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China
[c]Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton SO16 6YD, United Kingdom
[d]Community Neurosciences Institutes, Community Regional Medical Center, Fresno, CA 93701, USA
[e]Division of Neurosurgery, Department of Surgery, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong 999077, China
[f]Neuroscience Research Center, Loma Linda University School of Medicine, Loma Linda, CA 92350, USA
[g]Department of Physiology and Pharmacology, Loma Linda University School of Medicine, Loma Linda, CA 92354, USA

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.

eBioMedicine 2022;83: 104223
Published online xxx
https://doi.org/10.1016/j.ebiom.2022.104223

Keywords: Subarachnoid haemorrhage; Early brain injury; Cerebrospinal fluid; Microcirculation; Metabolomic; Biomarker

Introduction

Subarachnoid haemorrhage (SAH) is an acute cerebrovascular disorders and is mainly caused by ruptured 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.[1] 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.[2] 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.[2,3]

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. These two initial changes lead to

*Corresponding author at: Neuroscience Research Center, Department of Neurosurgery and Physiology, Loma Linda University School of Medicine, Loma Linda, CA 92350, USA.
**Corresponding author at: Department of Neurosurgery and State Key Laboratory of Trauma, Burn and Combined Injury, Southwest Hospital, Third Military Medical University (Army Medical University), 29 Gaotanyan Street, Shapingba District, Chongqing, 400038, China.
E-mail addresses: chenyj@tmmu.edu.cn (Y. Chen), jhzhang@llu.edu (J.H. Zhang).

the stimulus of early brain injury [exogenous materials in subarachnoid space and cerebrospinal fluid (CSF)] and the goal of neurocritical care management (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 subarachnoid haemorrhage. In addition, these two factors intersect in several ways and create a vicious cycle for SAH patients. Therefore, monitoring and intervention for 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 pathophysiology 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 as exogenous stimuli for brain haemostasis. Several decades of experimental and clinical data indicated that CSF haemoglobin induced secondary brain injury after SAH,[4] as we speculated before. Targeting CSF haemoglobin reduces delayed cerebral ischaemia and improve neurological outcomes for SAH in animal models and has potential for clinical application in patients.[5]

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.[6] Elevated CSF lipocalin-2 mediates neuroinflammation and iron homeostasis and is reported to be a biomarker for unfavourable outcomes of SAH patients[7] and a potential therapeutic target for long-term neurological rehabilitation.[8]

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 initiation 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[9] 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.[10]

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.[11]

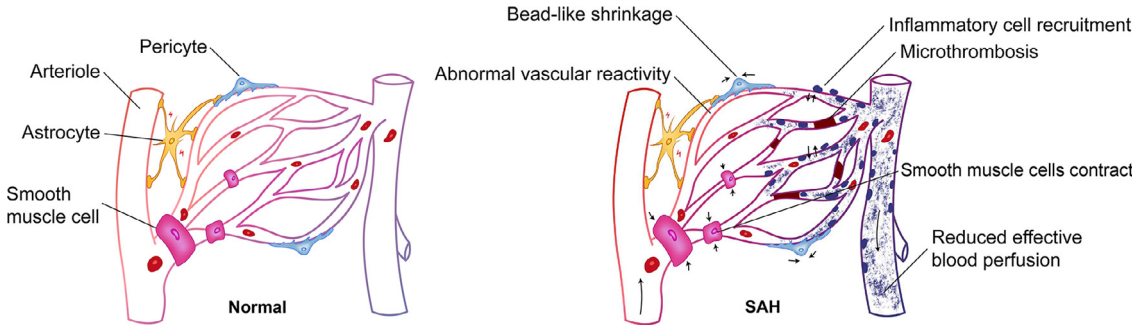

Figure 1. Schematic diagram of the microcirculation dysfunction induced by initial stimulation after SAH.

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.[12] 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 in traditional understanding,[13] 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.[14] 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.[15] 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.[16] In addition, free iron (Fe^{3+}) released from haemolytic red blood cells is also involved in arteriolar and capillary microvasospasms after SAH in mice,[17] while iron deposits in the cortex have been reported to be associated with cognitive outcomes in SAH patients.[18]

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

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 reported cohorts with poor-grade ruptured aneurysms, only approximately 21% of patients suffer from cerebral infarction.[28] 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.[29] Cerebral microdialysis and multimodal neuromonitoring strategies have found that brain tissue hypoxia ($PbtO_2$<20 mmHg) exists in more than 60% of patients within the first 24 h after SAH.[30]

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

HIF-1α 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 under 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.[34,35] 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.[36] The underlying mechanism of lactate-mediated pathophysiological processes might be much more complicated than previously thought, which is not just metabolomic production under hypoxia but acts as a pathogenic mediator, as it can regulate downstream gene expression by lactylation of histones[37] and of nonhistone proteins,[38] 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.[39] It has been demonstrated that lysine lactylation in brain macrophages is regulated by neural excitation and social stress, with parallel changes in lactate levels.[40] Histone lactylation might occur separately from interleukin-6-induced inflammatory metabolic adaptation.[41] Thus,

targeting lactylation may be a potential therapeutic approach for cancer,[42] inflammation,[38] Alzheimer's disease[43] 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.[44] In this cohort, no patients exhibited signs of severe ischaemia but they commonly had a disturbed energy metabolic pattern, possibly reflecting mitochondrial dysfunction[45] in addition to improved microcirculation. Extracellular mitochondria in CSF may provide additional information on brain integrity and recovery after SAH.[46,47] Thus, mitochondrial dysfunction could be a potential therapeutic target for SAH management,[48] 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 superimposes the patient's own susceptibility factors, resulting in multiple interwoven pathophysiological changes in 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.[49] 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 will be a technical roadmap for future SAH research, referring to the five-phase framework for developing epileptogenic biomarkers.[50] 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.[51]

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.[52] 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 transgenic mouse model of Alzheimer's disease and

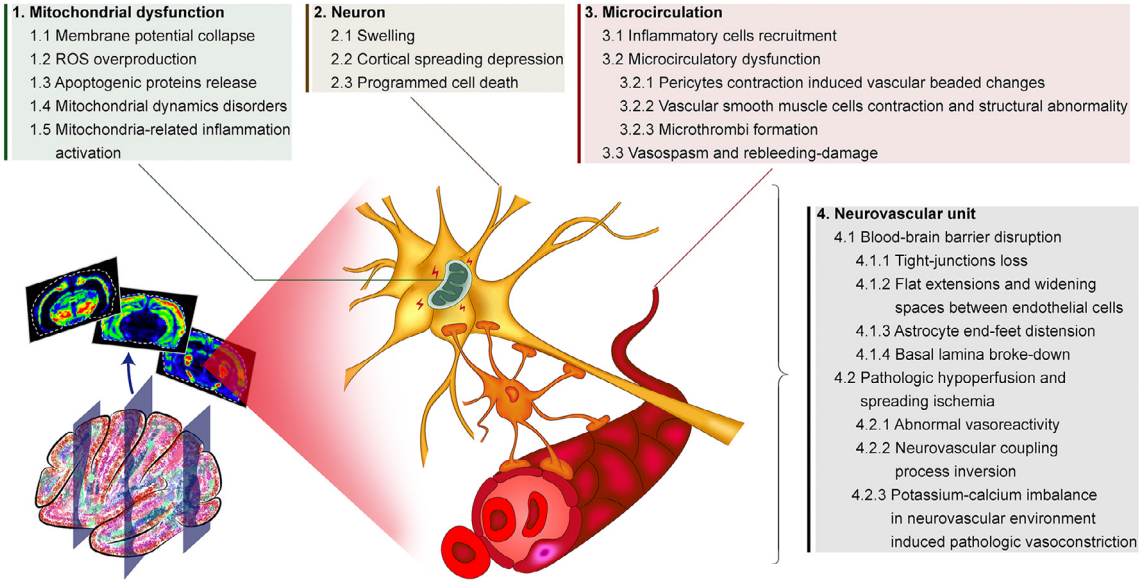

Figure 2. Schematic diagram of the different levels of metabolic changes in the brain parenchyma after SAH.

found 93 metabolites within different brain regions for the etiopathogenesis of Alzheimer's disease.[53] This newly adopted technology might have applications in scenarios lacking reliable immunohistochemical and diagnostic biomarkers[54] for real-time metabolic changes in the brain parenchyma. In addition, extended algorithms, such as false discovery rate (FDR)-controlled metabolite annotation[55] or the spatial single nuclear metabolomics (SEAM) platform,[56] 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.[57–59] 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;[60] this is valuable resource 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,[61,62] 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.[29,63]

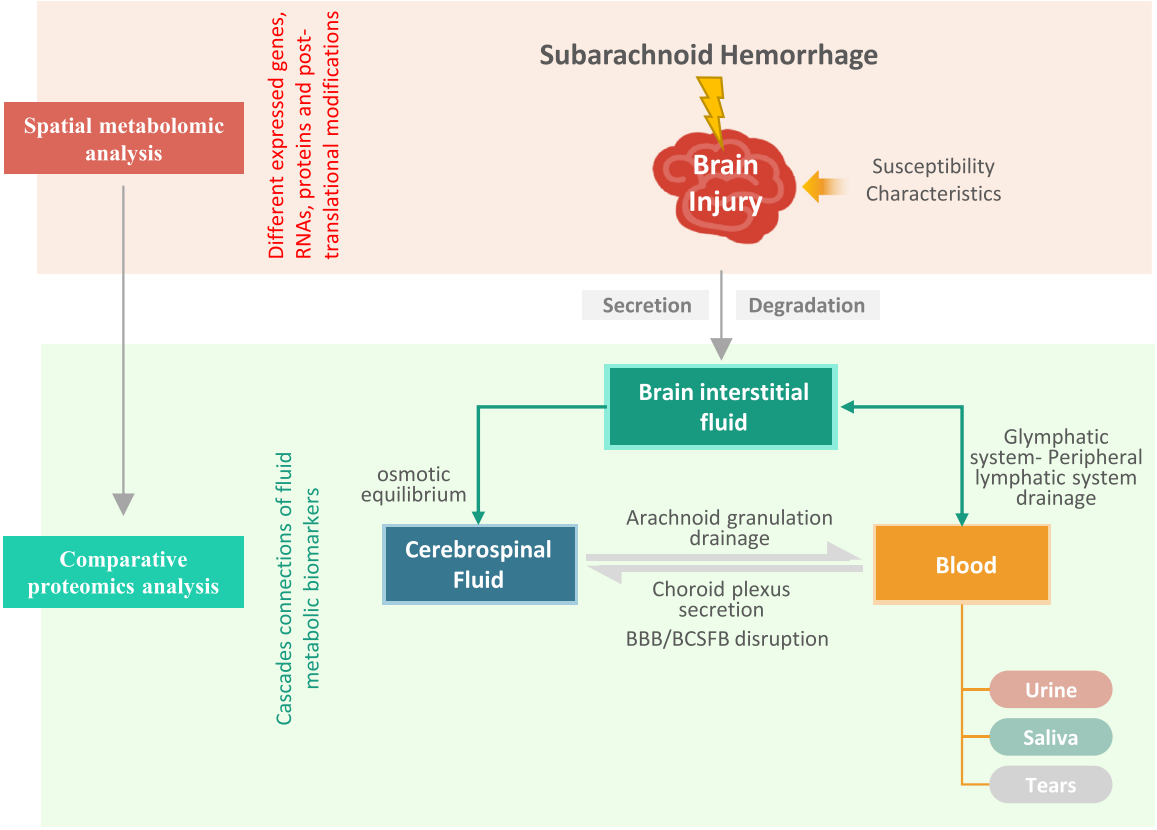

Figure 3. Cascade connections of various biomarkers to monitor early brain injury after SAH.

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 S100β and norepinephrine can be monitored in urine, according to a large systematic review.[64] 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 to blood, to 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[65] 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.[50]

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 form blood clots in the adjacent cistern and block the normal flow of CSF, further increasing the intracranial pressure. The balance between CSF pressure and precapillary and venous pressure is unbalanced in favour of higher CSF, and the volume of fluid in the brain is further increased.[66] 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[23] because a recent finding of CSF flow block did not correlate with the size of haemorrhage,[67] while CSF macrophage CD163, a haemoglobin scavenger receptor for blood clearance, was significantly associated with hydrocephalus and outcome after SAH in patients.[68] 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.[69] Despite the effectiveness of CSF external drainage strategies, the average output of CSF after SAH is unveiled as a risk factor for vasospasm and chronic hydrocephalus,[70] and CSF volume based on algorithm-assisted segmentation of computed tomography images might be a surrogate for vasospasm after SAH.[71] 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 for 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,[72] 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.[73] Many recent experiments have discovered many pathophysiological mechanisms that deteriorate the BBB after SAH, and alleviating these cascades might be decisive in inhibiting the negative impact of bleeding in the subarachnoid space.[74] 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.[75] Thus, endogenous recovery mechanisms and barriers should be considered protected and restored to 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 studies are still 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

Metabolic biomarkers	Intrinsic function	Sample types	Time	Main outcome	References
Blood hemolytic-related					
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 late elevated HO-1 expression associates with delayed cerebral ischemia	Neurocrit Care. 2022;36(1):279-91.
Oxyhemoglobin (oxyHb)	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.
Haptoglobin (Hp) 2-2	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.
Histidine-rich glycoprotein (HRG)	Regulation of coagulation and fibrinolysis.	CSF	<11 days after SAH	Early predictor of cerebral vasospasm	Acta Med Okayama. 2019;73(1):29-39.
Neuroglobin (Ngb)	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.
Neuroinflammation-related					
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.
		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.
Lipocalin-2 (LCN-2)	Neuro-inflammation and iron homeostasis	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.
Fatty acid-binding protein 3 (FABP3) CXC-chemokine ligand 16 (CXCL-16)	Vascular inflammation and cellular death	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):106068.
Tumor necrosis factor superfamily 14 (LIGHT/TNFSF14)	Immune response	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.
Soluble growth stimulation expressed gene 2 (sST2)	Immune response to neurovascular injury	Plasma	<14 days after SAH	Plasma sST2 predicts new epileptiform abnormalities, delayed cerebral ischemia, 90day 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.
Ficolin-1 Mannose-binding lectin (MBL)	Lectin complement pathway	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. Mol Neurobiol. 2017;54(8):6572-80.
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.	Brain Behav. 2020;10(2):e01517.
M-ficolin		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)	Pro-inflammatory chemokine	CSF Serum	<7 days after SAH	CCL5 levels correlated with clinical outcome. 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.	Cytokine. 2020;133:155142.
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. Significant correlation between CSF clusterin level 5-7 days after SAH and Glasgow Outcome Scale at 3 months.	World Neurosurg. 2017;107:424-8.
Soluble triggering receptor expressed on myeloid cells-1 (sTREM-1)	Inflammatory	CSF	<7 days after SAH	Correlations of early CSF sTREM-1 levels to patients' severity and prognosis,	J Clin Neurosci. 2017;35:139-43.
Migration inhibitory factor (MIF)	Proinflammatory cytokine	Serum	On admission	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
Vascular-related					
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/Orn < 2.71 at SAH onset predicted cerebral vasospasm with a sensitivity of 86.7% and specificity of 72.2%. Arg/Orn >/= 2.71 predicted excellent functional outcome.	Transl Stroke Res. 2022;13(3):382-90. Clinical Trial No. DRKS00015293
Asymmetric Dimethylarginine (ADMA) Symmetric Dimethylarginine (SDMA) Dimethylguanidine valeric acid (DMGV) Ornithine	Vasoactive molecules linked to the nitric oxide pathway	CSF	/	SDMA, DMGV, and Ornithine associate with poor mRS at discharge and at 90d after SAH ADMA and the L-arginine/ADMA ratio are associated with the incidence of delayed cerebral ischemia after SAH SDMA was associated with initial neuronal damage and poor neurological outcome after SAH	Neurosurgery. 2021;88(5):1003-11. Neurocrit Care. 2018;29(1):84-93.
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.
Hypoxia-related					
Lactate Lactate dehydrogenase (LDH)	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.
Pyruvate	Hypoxia	CSF	/	CSF pyruvate level was significantly associated with WFNS grading scale	ACS Chem Neurosci. 2019;10(3):1660-7.
2-hydroxyglutarate	Marker of tissue hypoxia	CSF	On admission and the anticipated vasospasm timeframe.	Correlated with GOS at 1 year post-SAH independent of vasospasm status.	Br J Neurosurg. 2018;32(6):637-41.
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.
Neuronal Injury-related					
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. Higher NfL levels during early brain injury are associated with poor functional outcome on day 30 after ictus and increased mortality rate.	Transl Stroke Res. 2020;11(4):671-7.
		CSF Serum	<3 days after SAH	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	Brain. 2021;144(3):761-8.
tTau/pTau Abeta40/Abeta42	Neuron injury	CSF	<7 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.	Neurosurg Rev. 2018;41(2):605-14.
Sortilin	Enriched in neurons and is likely involved in neurodegenerative diseases.	CSF (Human) 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.	Neuroscience. 2021;470:23-36.
S100A8/A9(calprotectin)	Neuron injury	Serum	<2 days after SAH	Correlated with the clinical severity and the poor prognosis at 3 months in SAH patients	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	CSF	<1 days after SAH	Critical role of neuronal damage process	Neurochem Int. 2019;128:14-20.
Netrin-1	Axon guidance molecule	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
Other Metabolites					
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 Osteopontin Galectin-3	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.
Galectin-3		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.
Periostin		Plasma	<3 days after SAH	Associates with higher incidence of delayed cerebral ischemia, but not angiographic vasospasm.	Neurotherapeutics. 2019;16(2):480-90.
		Serum	<3 days after SAH	Higher level of periostin than patients with good outcome at 12 months.	J Clin Lab Anal. 2018;32(5):e22389.
Osteopontin		Plasma CSF	<8 days after SAH	Independent predictor of poor outcome	Cells. 2019;8(7):695
		Plasma	<3 days after SAH		Mol Neurobiol. 2018;55(8):6841-9.
Cleaved receptor for advanced glycation end-products (cRAGE)	Proteolytic cleavage of full-length cell surface receptor RAGE protein	Plasma	On admission	Highly associated with the severity and 6-month poor outcome	Clin Chim Acta. 2018;486:335-40.
Alpha-II spectrin breakdown products (SBDP150, SBDP145, and SBDP120)	Cytoskeletal protein	CSF	<14 days after SAH	Potential biomarkers for early recognition and severity of SAH	Sci Rep. 2018;8(1):13308.
Soluble Platelet-derived growth factor beta (sPDGFβ)	Shed from pericytes to CSF is related to the injury of pericytes, PDGF-BB/PDGFR signaling	CSF	<10 days after SAH	CSF sPDGFβ 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.
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.**

SAH. Herein, we have reviewed the current understanding of the original 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 subarachnoid hemorrhage, neurological researchers still discuss mainly early brain injury and delayed cerebral ischemia as contributors to the neurological outcomes after SAH. But the real-time metabolic changes induced by subarachnoid hemorrhage initiates are not fully unveiled. Current advances in spatial metabolomics and comparable proteomics might provide more precise spatial and cascade information or therapeutic target 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-1α", "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.

Acknowledgements

This work was supported by National Natural Science Foundation of China (82030036), National Institutes of Health (R01 NS117179) and State Key Laboratory of Trauma, Burn and Combined Injury (SKLYQ202002). The funders have not roles in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

We would like to thank Dr. Jiru Zhou, Dr. Junhan Li, Dr. Jie Lin and Miss. Lin Gao for their invaluable help in designing figures in this manuscript.

References

1 Wahood W, Rizvi AA, Alexander AY, et al. Trends in admissions and outcomes for treatment of aneurysmal subarachnoid hemorrhage in the United States. *Neurocrit Care*. 2022;37:209–218.
2 Neifert SN, Chapman EK, Martini ML, et al. Aneurysmal subarachnoid hemorrhage: the last decade. *Transl Stroke Res*. 2021;12(3):428–446.
3 Andersen CR, Presseau J, Saigle V, et al. Core outcomes for subarachnoid haemorrhage. *Lancet Neurol*. 2019;18(12):1075–1076.
4 Akeret K, Buzzi RM, Schaer CA, et al. Cerebrospinal fluid hemoglobin drives subarachnoid hemorrhage-related secondary brain injury. *J Cereb Blood Flow Metab*. 2021;41(11):3000–3015.
5 Zeineddine HA, Honarpisheh P, McBride D, et al. Targeting hemoglobin to reduce delayed cerebral ischemia after subarachnoid hemorrhage. *Transl Stroke Res*. 2022. https://doi.org/10.1007/s12975-022-00995-9. In press.
6 Fang Y, Wang X, Lu J, et al. Inhibition of caspase-1-mediated inflammasome activation reduced blood coagulation in cerebrospinal fluid after subarachnoid haemorrhage. *EBioMedicine*. 2022;76:103843.
7 Yu F, Saand A, Xing C, et al. CSF lipocalin-2 increases early in subarachnoid hemorrhage are associated with neuroinflammation and unfavorable outcome. *J Cereb Blood Flow Metab*. 2021;41(10):2524–2533.
8 Li Q, Ru X, Yang Y, et al. Lipocalin-2-mediated insufficient oligodendrocyte progenitor cells remyelination for white matter injury after subarachnoid hemorrhage via SCL22A17 receptor/early growth response protein 1 signaling. *Neurosci Bull*. 2022. https://doi.org/10.1007/s12264-022-00906-w. In press.
9 Ho WM, Gorke AS, Glodny B, et al. Time course of metabolomic alterations in cerebrospinal fluid after aneurysmal subarachnoid hemorrhage. *Front Neurol*. 2020;11:589.
10 Wang KC, Tang SC, Lee JE, et al. Cerebrospinal fluid high mobility group box 1 is associated with neuronal death in subarachnoid hemorrhage. *J Cereb Blood Flow Metab*. 2017;37(2):435–443.
11 Koch M, Acharjee A, Ament Z, et al. Machine learning-driven metabolomic evaluation of cerebrospinal fluid: insights into poor outcomes after aneurysmal subarachnoid hemorrhage. *Neurosurgery*. 2021;88(5):1003–1011.
12 Uhl E, Lehmberg J, Steiger HJ, Messmer K. Intraoperative detection of early microvasospasm in patients with subarachnoid hemorrhage by using orthogonal polarization spectral imaging. *Neurosurgery*. 2003;52(6):1307–1315. disacussion 15-7.
13 Naraoka M, Matsuda N, Shimamura N, Ohkuma H. Role of microcirculatory impairment in delayed cerebral ischemia and outcome after aneurysmal subarachnoid hemorrhage. *J Cereb Blood Flow Metab*. 2022;42(1):186–196.
14 Liu H, Dienel A, Scholler K, et al. Microvasospasms after experimental subarachnoid hemorrhage do not depend on Endothelin a receptors. *Stroke*. 2018;49(3):693–699.
15 Li Q, Chen Y, Li B, et al. Hemoglobin induced NO/cGMP suppression deteriorate microcirculation via pericyte phenotype transformation after subarachnoid hemorrhage in rats. *Sci Rep*. 2016;6:22070.
16 Joerk A, Ritter M, Langguth N, et al. Propentdyopents as heme degradation intermediates constrict mouse cerebral arterioles and are present in the cerebrospinal fluid of patients with subarachnoid hemorrhage. *Circ Res*. 2019;124(12):e101–ee14.
17 Liu H, Schwarting J, Terpolilli NA, Nehrkorn K, Plesnila N. Scavenging free iron reduces arteriolar microvasospasms after experimental subarachnoid hemorrhage. *Stroke*. 2021;52(12):4033–4042.
18 Galea I, Durnford A, Glazier J, et al. Iron deposition in the brain after aneurysmal subarachnoid hemorrhage. *Stroke*. 2022;53(5):1633–1642.

19 Zhou J, Guo P, Guo Z, Sun X, Chen Y, Feng H. Fluid metabolic pathways after subarachnoid hemorrhage. *J Neurochem*. 2022;160 (1):13–33.
20 Boluijt J, Meijers JC, Rinkel GJ, Vergouwen MD. Hemostasis and fibrinolysis in delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage: a systematic review. *J Cereb Blood Flow Metab*. 2015;35(5):724–733.
21 Ishikawa M, Kajimura M, Morikawa T, et al. Leukocyte plugging and cortical capillary flow after subarachnoid hemorrhage. *Acta Neurochir*. 2016;158(6):1057–1067.
22 Dienel A, Kumar TP, Blackburn SL, McBride DW. Role of platelets in the pathogenesis of delayed injury after subarachnoid hemorrhage. *J Cereb Blood Flow Metab*. 2021;41(11):2820–2830.
23 Fang Y, Huang L, Wang X, et al. A new perspective on cerebrospinal fluid dynamics after subarachnoid hemorrhage: from normal physiology to pathophysiological changes. *J Cereb Blood Flow Metab*. 2022;42(4):543–558.
24 Zeng H, Fu X, Cai J, et al. Neutrophil extracellular traps may be a potential target for treating early brain injury in subarachnoid hemorrhage. *Transl Stroke Res*. 2022;13(1):112–131.
25 Zhou J, Guo P, Hao X, Sun X, Feng H, Chen Z. Neutrophil extracellular traps (NETs): a new therapeutic target for neuroinflammation and microthrombosis after subarachnoid hemorrhage? *Transl Stroke Res*. 2022. https://doi.org/10.1007/s12975-022-01039-y. In press.
26 Hanhai Z, Bin Q, Shengjun Z, et al. Neutrophil extracellular traps, released from neutrophil, promote microglia inflammation and contribute to poor outcome in subarachnoid hemorrhage. *Aging*. 2021;13(9):13108–13123.
27 Wu X, Zeng H, Xu C, et al. TREM1 Regulates neuroinflammatory injury by modulate proinflammatory subtype transition of microglia and formation of neutrophil extracellular traps via interaction with SYK in experimental subarachnoid hemorrhage. *Front Immunol*. 2021;12:766178.
28 Taran S, Trivedi V, Singh JM, English SW, McCredie VA. The use of standardized management protocols for critically ill patients with non-traumatic subarachnoid hemorrhage: a systematic review. *Neurocrit Care*. 2020;32(3):858–874.
29 Lu AY, Damisah EC, Winkler EA, Grant RA, Eid T, Bulsara KR. Cerebrospinal fluid untargeted metabolomic profiling of aneurysmal subarachnoid hemorrhage: an exploratory study. *Br J Neurosurg*. 2018;32(6):637–641.
30 Helbok R, Schiefecker AJ, Beer R, et al. Early brain injury after aneurysmal subarachnoid hemorrhage: a multimodal neuromonitoring study. *Crit Care*. 2015;19:75.
31 Ostrowski RP, Zhang JH. The insights into molecular pathways of hypoxia-inducible factor in the brain. *J Neurosci Res*. 2020;98 (1):57–76.
32 Dong Y, Li Y, Feng D, et al. Protective effect of HIF-1alpha against hippocampal apoptosis and cognitive dysfunction in an experimental rat model of subarachnoid hemorrhage. *Brain Res*. 2013;1517:114–121.
33 Hu Q, Du Q, Yu W, Dong X. 2-Methoxyestradiol alleviates neuroinflammation and brain edema in early brain injury after subarachnoid hemorrhage in rats. *Front Cell Neurosci*. 2022;16:869546.
34 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(5):104765.
35 Renfrow JJ, Frey CD, Arnel M, Wolfe SQ, McLouth C, Datar S. Utility of cerebrospinal fluid lactate in aneurysmal subarachnoid hemorrhage. *Surg Neurol Int*. 2018;9:155.
36 Winberg J, Holm I, Cederberg D, Rundgren M, Kronvall E, Marklund N. Cerebral microdialysis-based interventions targeting delayed cerebral ischemia following aneurysmal subarachnoid hemorrhage. *Neurocrit Care*. 2022;37:255–266.
37 Zhang D, Tang Z, Huang H, et al. Metabolic regulation of gene expression by histone lactylation. *Nature*. 2019;574(7779):575–580.
38 Yang K, Fan M, Wang X, et al. Lactate promotes macrophage HMGB1 lactylation, acetylation, and exosomal release in polymicrobial sepsis. *Cell Death Differ*. 2022;29(1):133–146.
39 Brooks GA. Lactate as a fulcrum of metabolism. *Redox Biol*. 2020;35:101454.
40 Hagihara H, Shoji H, Otabi H, et al. Protein lactylation induced by neural excitation. *Cell Rep*. 2021;37(2):109820.
41 Dichtl S, Lindenthal L, Zeitler L, et al. Lactate and IL6 define separable paths of inflammatory metabolic adaptation. *Sci Adv*. 2021;7 (26).
42 Pan L, Feng F, Wu J, et al. Demethylzeylasteral targets lactate by inhibiting histone lactylation to suppress the tumorigenicity of liver cancer stem cells. *Pharmacol Res*. 2022;181:106270.
43 Pan RY, He L, Zhang J, et al. Positive feedback regulation of microglial glucose metabolism by histone H4 lysine 12 lactylation in Alzheimer's disease. *Cell Metab*. 2022;34(4). 634-48 e6.
44 Engquist H, Lewen A, Hillered L, et al. CBF changes and cerebral energy metabolism during hypervolemia, hemodilution, and hypertension therapy in patients with poor-grade subarachnoid hemorrhage. *J Neurosurg*. 2020;134(2):555–564.
45 Wagner M, Jurcoane A, Hildebrand C, et al. Metabolic changes in patients with aneurysmal subarachnoid hemorrhage apart from perfusion deficits: neuronal mitochondrial injury? *AJNR Am J Neuroradiol*. 2013;34(8):1535–1541.
46 Chou SH, Lan J, Esposito E, et al. Extracellular mitochondria in cerebrospinal fluid and neurological recovery after subarachnoid hemorrhage. *Stroke*. 2017;48(8):2231–2237.
47 Youn DH, Kim BJ, Kim Y, Jeon JP. Extracellular mitochondrial dysfunction in cerebrospinal fluid of patients with delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage. *Neurocrit Care*. 2020;33(2):422–428.
48 Zhang Z, Zhang A, Liu Y, et al. New mechanisms and targets of subarachnoid hemorrhage: a focus on mitochondria. *Curr Neuropharmacol*. 2022;20(7):1278–1296.
49 Chen Y, Chen Q, Sun J, Zhang L, Tan L, Feng H. Multimodal monitoring technologies for pathophysiology and management of traumatic brain injury. *J Transl Critic Care Med*. 2019;1(1):12–19.
50 Simonato M, Agoston DV, Brooks-Kayal A, et al. Identification of clinically relevant biomarkers of epileptogenesis - a strategic roadmap. *Nat Rev Neurol*. 2021;17(4):231–242.
51 Whitehouse DP, Monteiro M, Czeiter E, et al. Relationship of admission blood proteomic biomarkers levels to lesion type and lesion burden in traumatic brain injury: a CENTER-TBI study. *EBioMedicine*. 2022;75:103777.
52 Seeley EH, Caprioli RM. Molecular imaging of proteins in tissues by mass spectrometry. *Proc Natl Acad Sci USA*. 2008;105 (47):18126–18131.
53 Chen Y, Hu D, Zhao L, Tang W, Li B. Unraveling metabolic alterations in transgenic mouse model of Alzheimer's disease using MALDI MS imaging with 4-aminocinnoline-3-carboxamide matrix. *Anal Chim Acta*. 2022;1192:339337.
54 Neumann JM, Niehaus K, Neumann N, et al. A new technological approach in diagnostic pathology: mass spectrometry imaging-based metabolomics for biomarker detection in urachal cancer. *Lab Invest*. 2021;101(9):1281–1288.
55 Palmer A, Phapale P, Chernyavsky I, et al. FDR-controlled metabolite annotation for high-resolution imaging mass spectrometry. *Nat Methods*. 2017;14(1):57–60.
56 Yuan Z, Zhou Q, Cai L, et al. SEAM is a spatial single nuclear metabolomics method for dissecting tissue microenvironment. *Nat Methods*. 2021;18(10):1223–1232.
57 Katsanos AH, Makris K, Stefani D, et al. Plasma glial fibrillary acidic protein in the differential diagnosis of intracerebral hemorrhage. *Stroke*. 2017;48(9):2586–2588.
58 Garland P, Morton M, Zolnourian A, et al. Neurofilament light predicts neurological outcome after subarachnoid haemorrhage. *Brain*. 2021;144(3):761–768.
59 Helbok R, Schiefecker A, Delazer M, et al. Cerebral tau is elevated after aneurysmal subarachnoid haemorrhage and associated with brain metabolic distress and poor functional and cognitive long-term outcome. *J Neurol Neurosurg Psychiatry*. 2015;86(1):79–86.
60 O'Connell GC, Alder ML, Smothers CG, Chang JHC. Large-scale informatic analysis to algorithmically identify blood biomarkers of neurological damage. *Proc Natl Acad Sci USA*. 2020;117 (34):20764–20775.
61 Illes S. More than a drainage fluid: the role of CSF in signaling in the brain and other effects on brain tissue. *Handb Clin Neurol*. 2017;146:33–46.
62 Plog BA, Dashnaw ML, Hitomi E, et al. Biomarkers of traumatic injury are transported from brain to blood via the glymphatic system. *J Neurosci*. 2015;35(2):518–526.
63 Santacruz CA, Vincent JL, Bader A, et al. Association of cerebrospinal fluid protein biomarkers with outcomes in patients with traumatic and non-traumatic acute brain injury: systematic review of the literature. *Crit Care*. 2021;25(1):278.
64 Edalatfar M, Piri SM, Mehrabinejad MM, et al. Biofluid biomarkers in traumatic brain injury: a systematic scoping review. *Neurocrit Care*. 2021;35(2):559–572.

65 Shin MK, Vazquez-Rosa E, Koh Y, et al. Reducing acetylated tau is neuroprotective in brain injury. *Cell*. 2021;184(10). 2715-32 e23.
66 Goulay R, Flament J, Gauberti M, et al. Subarachnoid hemorrhage severely impairs brain parenchymal cerebrospinal fluid circulation in nonhuman primate. *Stroke*. 2017;48(8):2301–2305.
67 Golanov EV, Bovshik EI, Wong KK, et al. Subarachnoid hemorrhage - induced block of cerebrospinal fluid flow: Role of brain coagulation factor III (tissue factor). *J Cereb Blood Flow Metab*. 2018;38(5):793–808.
68 Thomas AJ, Ogilvy CS, Griessenauer CJ, Hanafy KA. Macrophage CD163 expression in cerebrospinal fluid: association with subarachnoid hemorrhage outcome. *J Neurosurg*. 2018;131(1):47–53.
69 Chen J, Wang L, Xu H, et al. Meningeal lymphatics clear erythrocytes that arise from subarachnoid hemorrhage. *Nat Commun*. 2020;11(1):3159.
70 Garcia-Armengol R, Puyalto P, Misis M, et al. Cerebrospinal fluid output as a risk factor of chronic hydrocephalus after aneurysmal subarachnoid hemorrhage. *World Neurosurg*. 2021;154:e572–e5e9.
71 Scherer M, Jung JO, Cordes J, et al. Association of cerebrospinal fluid volume with cerebral vasospasm after aneurysmal subarachnoid hemorrhage: a retrospective volumetric analysis. *Neurocrit Care*. 2020;33(1):152–164.
72 Ivanidze J, Ferraro RA, Giambrone AE, Segal AZ, Gupta A, Sanelli PC. Blood-brain barrier permeability in aneurysmal subarachnoid hemorrhage: correlation with clinical outcomes. *AJR Am J Roentgenol*. 2018;211(4):891–895.
73 Lublinsky S, Major S, Kola V, et al. Early blood-brain barrier dysfunction predicts neurological outcome following aneurysmal subarachnoid hemorrhage. *EBioMedicine*. 2019;43:460–472.
74 Solar P, Zamani A, Lakatosova K, Joukal M. The blood-brain barrier and the neurovascular unit in subarachnoid hemorrhage: molecular events and potential treatments. *Fluids Barriers CNS*. 2022;19(1):29.
75 Garland P, Durnford AJ, Okemefuna AI, et al. Heme-hemopexin scavenging is active in the brain and associates with outcome after subarachnoid hemorrhage. *Stroke*. 2016;47(3):872–876.