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SPECIAL ARTICLE

Biospecimens and Molecular and Cellular Biomarkers in Aneurysmal Subarachnoid Hemorrhage Studies: Common Data Elements and Standard Reporting Recommendations

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Abstract

Introduction: Development of clinical biomarkers to guide therapy is an important unmet need in aneurysmal subarachnoid hemorrhage (SAH). A wide spectrum of plausible biomarkers has been reported for SAH, but none have been validated due to significant variabilities in study design, methodology, laboratory techniques, and outcome endpoints.

Methods: A systematic review of SAH biomarkers was performed per the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The panel's recommendations focused on harmonization of (1) target cellular and molecular biomarkers for future investigation in SAH, (2) standardization of best-practice procedures in biospecimen and biomarker studies, and (3) experimental method reporting requirements to facilitate meta-analyses and future validation of putative biomarkers.

Results: No cellular or molecular biomarker has been validated for inclusion as "core" recommendation. Fifty-four studies met inclusion criteria and generated 33 supplemental and emerging biomarker targets. Core recommendations include best-practice protocols for biospecimen collection and handling as well as standardized reporting guidelines to capture the heterogeneity and variabilities in experimental methodologies and biomarker analyses platforms.

Conclusion: Significant variabilities in study design, methodology, laboratory techniques, and outcome endpoints exist in SAH biomarker studies and present significant barriers toward validation and translation of putative biomarkers to clinical use. Adaptation of common data elements, recommended biospecimen protocols, and reporting guidelines will reduce heterogeneity and facilitate future meta-analyses and development of validated clinical biomarkers in SAH.

Keywords: Biomarkers, Biospecimens, Cerebrospinal fluid, Subarachnoid hemorrhage, S100β, Tumor necrosis factor alpha, interleukin-6, metalloproteinase-9, Plasma-type gelsolin, Apolipoprotein E, Cardiac troponin I, B-type natriuretic peptide, common data elements, Standard operating procedure

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Unruptured Intracranial Aneurysms and SAH CDE Project Investigators are listed in Appendix.

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Introduction

Biospecimens and biomarkers are becoming increasingly important in the era of personalized and precision medicine where the goal is to deliver the right treatment for the right disease to the right patient at the right time. Biomarker discoveries such as the Philadelphia chromosome in chronic myeloid leukemia and HER2 in breast cancer have led to major medical breakthroughs by identifying a novel therapeutic target and by using the biomarker to select the patient subpopulation that would respond to a therapy.

There is a need in neurocritical care to develop reliable clinical biomarkers that may help monitor disease progression, predict imminent complications, guide goal-directed therapy, select patient subgroups for therapy, and determine prognosis. To date, numerous studies have identified a wide spectrum of plausible biomarkers, but none have been validated and translated into clinical use [1]. One of the leading causes for lack of translation in biomarker discovery is the high degree of variabilities in the following fundamental aspects of biomarker studies: (1) Different anatomical source (e.g., ventricular versus lumbar cerebral spinal fluid) may significantly affect biomarker concentrations. (2) Biomarker concentrations are likely dynamic and may change over time during disease course. Lack of precision and standardization in biospecimen collection time points leads to significant biomarker measurement variabilities and predictive values. (3) Different biospecimen collection, processing, and preservation methods may significantly alter biomarker measurement results. (4) The variable choice of normal controls to establish reference biomarker values significantly impacts interpretation of study results. And (5) the source of origin of a target biomarker molecule (e.g., primarily from the central nervous system (CNS) versus extra-CNS or mixed origins) and how it moves across the blood brain barrier in subarachnoid hemorrhage (SAH) significantly impacts interpretation and implication of biomarker study results.

The scope of this review and recommendation includes molecular and cellular biomarkers that can be measured from biospecimens. The utility of non-molecular/cellular biomarkers, such as radiologic and electrophysiologic biomarkers, is addressed in other manuscripts prepared by other working groups (WG) of the Unruptured Cerebral Aneurysms and SAH Common Data Elements (CDE) project [84–86].

Common Data Elements Overview

Summary

The aim of the National Institute of Health (NIH)/National Institute of Neurological Disorders and Stroke (NINDS) Unruptured Aneurysms and SAH CDE project has been to provide a common structure for future unruptured intracranial aneurysm and SAH research. This paper describes the recommendations from the SAH Biospecimens and Biomarkers WG.

Process for Selecting CDEs

The biospecimens and biomarkers WG consisted of an international and multidisciplinary (neurosurgery, neurology, neurocritical care) ad hoc panel of experts. All members were trained by NIH/NINDS instruction materials how to use the CDEs. The committee focused on the following two components of CDE recommendations:

1. Standard Operating Procedure in Biospecimen and Biomarker experimental methodology and Standard Reporting Recommendations.
2. Molecular and Cellular Biomarker CDEs for SAH studies.

Important considerations in biomarker development include: [2] (1) *Analytical validity*—whether the biomarker test reliably and accurately measures the analyte of interest in the appropriate specimen, (2) *Clinical validity*—whether the biomarker test accurately and reliably identifies a clinically or biologically defined disorder or separates patient populations into groups with distinct clinical or biological outcome differences, and (3) *Clinical utility*—whether the use of the biomarker test to guide clinical decisions results in improved outcome. We conducted literature review and developed recommendations with reference to these three important considerations.

A critical element in our ability to determine validity and utility of a biomarker is the quality of reporting. This is a universal concern encountered across biomarker studies in medicine. Several disciplines of medicine such as oncology have moved toward standardized reporting requirements. The Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK) is required by many oncology journals for new biomarker studies [3], and these recommendations can be generalized and applied to biomarker studies outside of oncology. In addition to REMARK, the Biospecimen Reporting for Improved Study Quality (BRISQ) criteria also provide guidance and recommendations related to pre-analytical factors that may substantially influence reproducibility of the biomarker assay [4]. Recognizing that the field of biomarkers is undergoing rapid evolution with emergence of novel biomarker assay technologies as well as monitoring techniques, it is more important than ever that we establish standardizations in reporting of experimental

methodologies for emerging SAH biomarkers. We addressed this important consideration and generated specific recommendations for biospecimen methodology and data elements reporting.

1. *Standard Operating Procedure in Biospecimen and Biomarker Experimental Methodology and Standard Reporting Recommendations.*

For harmonization and prioritization of standard techniques and reporting recommendations, we systematically reviewed common procedures in current SAH biomarkers literature as well as existing CDEs from traumatic brain injury, stroke, and other neurological diseases [5, 6]. A preliminary list of recommendations was created and discussed via in-person and telephone conferences to generate consensus-based recommendations from the WG. Table 1 summarizes these recommendations. Data elements that were considered essential in determining a biomarker's analytical and clinical validity were selected as "core" reporting elements that should be reported for all studies. Data elements that was not considered essential but highly encouraged are listed as "supplemental" reporting elements. Emerging data element recommendations provide general guidance for more advanced studies using novel and emerging technologies.

A more detailed sample biospecimens standard operating procedure (SOP) guideline is provided in Appendix 2 of the biospecimens and biomarkers section of the NINDS Unruptured Aneurysms and SAH CDE project (https://www.commondataelements.ninds.nih.gov/Doc/SAH/F2428_Appendix_2_Biospecimen_SOP_Guidelines.pdf). This SOP includes general recommendations on biospecimen processing and handling with emphasis on blood and cerebrospinal fluid (CSF) biospecimens and may serve as a template for SAH studies involving biospecimen collection. Additional recommendations within this SOP discuss important biospecimen study design considerations and common pitfalls. For example, repeated freeze and thawing cycles and storage at temperatures above −80 °C may significantly affect protein concentrations in biospecimens [7, 8], and storage in polystyrene or glass collection tubes may significantly impact measurements of certain analytes [9]. A particular consideration in studying SAH CSF samples is the presence of significant amount of blood content within the CSF. If the CSF samples do not undergo centrifugation to separate out cellular and subcellular content before being stored frozen, the cellular and subcellular contents may lyse during the freeze–thaw cycle and release intracellular contents into the aqueous CSF, and this may significantly affect the measurement of certain analytes. Specific to the design of CSF biomarker studies, it is important to consider ventricular versus lumbar source of CSF may have significant differences in protein concentrations and comparison of CSF from different anatomical sources must account for bias introduced by different CSF compositions at different anatomical sites [10]. Furthermore, continuous versus intermittent CSF drainage methods may also significantly alter CSF protein concentration [11]. Study designs that avoid these common pitfalls and standardized biospecimen and biomarker study method reporting will reduce inter-study variabilities and promote future meta-analyses of SAH biomarker study results.

2. *Molecular and Cellular Biomarker CDEs for SAH Studies*

For a description of the Unruptured Aneurysms and SAH CDE project, we refer to the main article [86]. A systematic review was performed following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [12]. Using the PICOS approach outlined below, the first (S.C.) and last author (E.K.) independently searched PUBMED database for English language articles using the terms "subarachnoid hemorrhage" and "biomarker" or "subarachnoid hemorrhage" and "microdialysis" from January 1990 to May 2018. Articles were selected based on the following criteria:

a. *Patient population*: Adult patients ≥ 18 years of age with spontaneous SAH.
b. *Intervention*: Cellular or molecular biomarkers from biological fluids such as serum, plasma, CSF, urine, and cerebral microdialysate.
c. *Controls*: Patients without SAH.
d. *Outcome endpoints*: Primary outcomes of interest are mortality and long-term neurological outcome. Secondary outcomes of interest are prediction of cerebral vasospasm and delayed cerebral ischemia (DCI).

Congress presentations, abstracts, review articles, case reports or retrospective case series, studies with sample size ≤ 10 patients for CSF biomarkers and studies with sample size ≤ 30 for blood biomarkers and cerebral microdialysis, pediatric intensive care unit (ICU) studies, and studies not conducted on ICU patients were excluded.

Specific review elements included the adequacy of study design in relation to the intended use of each biomarker (risk categorization, screening for new disease/

Table 1 Biospecimen protocol and methodology reporting recommendations

Data element	Guidelines and examples
Core data element recommendations	
Biological tissue sample source	CSF, serum, plasma, urine, microdialysate, saliva, etc.
Conditions included/excluded	Certain conditions may alter biomarker composition. For example: Infections of the nervous system or target organ from which samples are collected Neoplastic and paraneoplastic conditions Demyelinating conditions Inflammatory conditions such as vasculitis and nervous system involvement of systemic autoimmune disorders Renal failure Liver failure Any intrathecal or intraventricular drug administration
Baseline/time-zero specimen	If samples are collected repeatedly over time, define and collect baseline biospecimen
Site and method of sample acquisition	Blood/serum/plasma: arterial versus venous blood source, peripheral venipuncture versus central vascular access CSF: report location of acquisition (e.g., ventricular, lumbar) and method of acquisition (e.g., through external ventricular catheter or lumbar drain catheter versus via lumbar puncture) Paired sampling (simultaneous collection of biological samples from different sites, e.g., blood and CSF) may provide additional insight into biomarker distribution across different anatomical compartments
Timing of biospecimen collection	Report biospecimen collection time relative to disease/event onset
Type of collection tube	Blood collection: EDTA, Na heparin, citrate, serum collection tubes, or other Other fluid types: polystyrene vs. polypropylene vs. other collection tubes
Method of biospecimen processing	Report biospecimen processing protocol. For fluid samples such as CSF, centrifugation to separate supernatant from cellular debris and separate storage is recommended Was centrifugation used? If so, report centrifugation methods (speed, temperature, duration) Time lapse between sample collection and processing. For RNA, protein, metabolite target biomarkers, samples should be immediately processed and stored frozen
Method of biospecimen storage	Report method of storage including: Temperature of biospecimen/aliquot storage. Storage at or below −80 °C is recommended for RNA, protein, metabolite targets Number of freeze/thaw cycles. Recommend minimization of freeze/thaw cycles
Supplemental data element recommendations	
Control biospecimens	Biospecimens from comparable, non-diseased individuals should be collected to establish normal level of target biomarker
Convalescent biospecimens	Collection of convalescent biospecimens from study subjects may provide additional insight into dynamic changes in target biomarker following acute illness and recovery
Serial biospecimen collection	Serial biospecimen collection over time can provide additional information of target biomarker kinetics and dynamic biomarker change over time If serial collections acquired, recommend use consistent method of acquisition including site of acquisition to minimize variance in biospecimen and biomarker analyses
Biospecimen storage	Recommend storage in small aliquots and automated bar-code inventory system with date and time stamps to minimize errors in storage/inventory
Biomarker analysis	Report number of freeze/thaw cycles the biospecimen went through before final biomarker analyses Recommend standardized number of freeze/thaw cycles of biospecimens in a single study as additional freeze/thaw cycles may introduce variance in analyses results
Selective inhibitors use	Selective inhibitors may be used to optimize biospecimen collection for a specific target biomarker Example: protease inhibitors, RNAase inhibitors
Biospecimen transport and shipping	If biospecimens undergo transport/shipping prior to final biomarker analysis, report conditions of shipping Recommend biospecimens be shipped frozen with abundant amount of dry ice to maintain temperature at or below −80 °C Document temperature excursions during shipping and transport

Table 1 (continued)

Data element	Guidelines and examples
Cerebral microdialysis biospecimens	For studies involving extracellular fluid collected via cerebral microdialysis, report the following data elements: Probe placement in at risk but viable tissue. Avoid placement in hematoma or infarcted tissue Report location of probe placement and number of probes. Recommendation: use concentric configuration commercially available probes Report probe molecular weight cutoff, membrane length, manufacturer, and model Report time from ictus to monitoring and then time to sample collection. Report the composition and source of the microdialysate Recommendation: microdialysate flow rate should be 0.3 µL/min over 1 h Recommendation: First hour microdialysate after probe placement should not be used Report 4 basic essential analytes (glucose, pyruvate, lactate, and the calculated L/P ratio) in addition to any novel target analytes Recommendation: Stored samples may be assayed using the batch analysis systems. Avoid sample evaporation. If low volume samples remain in the analyzer for extended period prior to analysis, unacceptable evaporation may occur. Calibration samples should be interspersed in the batch to detect a systematic elevation in analyte levels due to evaporative loss
Emerging data element recommendations	
Novel multiplex platforms	For novel multiplex platforms such as proteomics, metabolomics, lipidomics, and transcriptomics: Report if and what normalization techniques are used and any presence of batch effect Report statistical methodology to address multiple comparisons in hypothesis testing

CSF cerebrospinal fluid

complications, aid differential diagnosis, determine prognosis, predict response to therapy, or monitor disease status). We also considered the specific stage of biomarker development: discovery, replication, and clinical/epidemiologic characterization (T1), determining validity and utility for evidence-based recommendations of biomarker use (T2), determining best approaches for implementation and disseminations into clinical practice (T3), and population impact, cost effectiveness of biomarker in real-world settings (T4).

Classification into Core, Supplemental, and Exploratory

The created list of biomarkers was reviewed by the WG members. All SAH biomarkers published to date are in the T1 developmental stage with the majority being biomarker discovery and epidemiologic characterization. No SAH biomarker studies to date have assessed biomarker clinical utility. The WG defined "Core" CDE biomarkers as biomarkers with analytical and clinical validity that have been replicated/validated in independent SAH patient populations. We defined "Supplemental" CDEs as those biomarkers with evidence suggestive of analytical or clinical validity but have not yet been replicated and validated. "Exploratory" biomarkers are biomarkers that have demonstrated plausible association in SAH, but more data are needed to suggest analytical and/or clinical validity.

Biomarker CDE Recommendations

None of the biomarkers identified were classified as "Core," since no molecular biomarker has been validated in SAH using large prospective studies and across different populations. Supplemental and Exploratory SAH biomarkers to date are generally categorized into (1) biomarkers of cell death and recovery (molecules of CNS origin), (2) biomarkers of inflammation and vascular function, and (3) genetic, non-CNS, and extracellular biomarkers.

Biomarkers of Cell Death and recovery (Molecules of CNS origin) (Table 2)

Supplemental Data Elements

We identified a total of nine publications that met search criteria, five of which focused on S100β, a calcium-binding protein. Across studies with sample sizes ranging from 18 to 102, serum S100β appears consistently elevated after SAH and elevations are consistently associated with worse Hunt and Hess and Fisher grades, mortality, and 6-month outcome [13–16]. However, serum S100β cutoff thresholds for short-term survival and poor outcome prediction varied significantly between studies and the largest study included both SAH and traumatic brain injury (TBI) which limits interpretation [14–16]. One small study examined serum and CSF S100β and found no associations with angiographic vasospasm, but the strength of conclusion was severely limited by sample size [17]. While these results suggest possible clinical validity, the analytical validity of S100β remains in question as different assay platforms were used across studies with wide standard deviations within S100β measurements. Furthermore, S100β is not specific to SAH and is associated with multiple types of brain disorders such

Table 2 Biomarkers of cell death and recovery

Biomarker	Sample source	Observation
S100β	Serum, CSF	Serum S100β is higher at 24 h and 3 and 7 days post-SAH [13] Higher-serum S100β predicts unfavorable 6-month outcome [13, 15, 16] and is an independent predictor of short-term [14, 15] and 1-year survival [60] Serum S100β concentrations > 0.7 μg/dl correlate with 100% mortality in SAH and TBI [16] High-serum S100β levels correlate with worse HH and Fisher grades [13, 14] CSF and serum S100β are not associated with angiographic cerebral vasospasm [17] CSF S100β correlates with GOS [16]
Neuron-specific enolase (NSE)	Serum	Serum NSE is not associated with outcome [15]
Creatine kinase-BB isoenzyme (CKBB)	CSF	Higher CSF CKBB levels are associated with higher HH grade at hospital admission CSF CKBB levels > 40 μg/L is associated with poor outcome at hospital discharge [81]
Neurofilament subunit NF-H (pNF-H)	Serum, CSF	Higher-serum pNF-H levels in the first few days (samples every 6 h until day 8 or 12) after bleeding are strongly predictive for unfavorable outcome. Patients with angiographic vasospasm have significantly higher pNF-H levels in blood and CSF compared to those without [20]
Extracellular mitochondria	CSF	Extracellular mitochondria detected in CSF Higher mitochondrial membrane potentials JC1 in the CSF correlate with good clinical recovery at 3 months after SAH onset [21]
Neurofilament light (NFL) and total tau (T-tau)	CSF	Increased CSF tau ratio at day 10 versus day 4 post-SAH is significantly associated with DCI [19]

CSF cerebrospinal fluid, *DCI* delayed cerebral ischemia, *GOS* Glasgow Outcome Score, HH Hunt and Hess grade, *SAH* subarachnoid hemorrhage, *TBI* traumatic brain injury

as TBI, hydrocephalus, and CNS infections, and even extracranial injuries in the absence of brain injury [18]. These considerations limit the potential clinical validity and utility of S100β as a SAH biomarker, and it was categorized as a supplemental rather than core CDE.

Emerging Data Elements

Other biomarkers of cell death and recovery consist of single studies of molecules of CNS origin from CSF analyses. Target molecules generally include various types of neurofilaments [19, 20]. The largest CSF study described the presence of extracellular mitochondria in CSF and demonstrated that higher CSF JC1, a measure of mitochondrial membrane potential, is associated with better SAH outcome, which suggests this is a potential biomarker not of cell death but of potential recovery [21].

Biomarkers of Inflammation and Vascular Function (Table 3)

SAH triggers a complex pattern of inflammatory response both inside and outside of the CNS [22]. There is significant preclinical evidence that inflammatory mediators may play key roles in SAH-associated brain injury, and to date, the largest body of SAH human biomarkers studies involves markers of inflammation and of vascular function.

Supplemental Data Elements

The inflammatory mediator biomarkers that have shown relatively more consistent signals across multiple independent studies include blood leukocyte count, tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), plasma-type gelsolin (pGSN), and metalloproteinase-9 (MMP-9). One of the earliest observations of inflammation in SAH was the association of leukocyte elevation with mortality and symptomatic vasospasm in retrospective cohorts, where it was unclear whether the leukocytosis was a result of overall disease severity [23–25]. Subsequent prospective study with repeated measures observed that leukocyte and neutrophil elevations began early in SAH course prior to the onset of vasospasm and were independently associated with vasospasm and poor outcome after adjustment for SAH severity [26]. Leukocytosis is a very non-specific biomarker, and subsequent studies seeking more specific, downstream inflammatory mediators found elevated blood TNF-α concentrations predicted poor outcome in SAH [27], while CSF TNF-α levels showed inconsistent associations with SAH outcome [28, 29]. IL-6 appears to be consistently elevated after SAH, is higher in CSF compared to serum in SAH, and is higher in severe SAH grades but also elevated in SAH-associated complications such as pneumonia, delayed ischemic neurological deficit (DIND), and chronic hydrocephalus [25, 29–32]. The association between IL-6 and SAH outcome and vasospasm is inconsistent across studies [25, 27, 30, 31]. Leukocytes secrete MMP-9 and early elevations of MMP-9 in blood and CSF showed association with Hunt and Hess grade, vasospasm, and SAH outcome [26, 33], while its association with DCI is inconsistent [34]. MMP-9 is a protease that can

Table 3 Biomarkers of inflammation and vascular function

Biomarker	Sample source	Observations
Tumor necrosis factor alpha (TNF-α)	Serum	Elevated TNF-α throughout post-SAH days 0–14 is independently associated with poor long-term outcome [27] Serum TNF-α is not associated with angiographic vasospasm [27] Neither TNF-α nor TNF-α genotype is associated with DCI or poor outcome [82]
	CSF	Elevated CSF TNF-α on post-SAH days 4–10 is associated with poor outcome [28] CSF TNF-α does not show statistical association with outcome [29]
Leukocyte and neutrophil counts	Blood	Elevated blood leukocyte throughout post-SAH days 0–14 is associated with angiographic vasospasm [26] Elevated blood neutrophil count on post-SAH days 2–3 is associated with poor SAH 3-month outcome [26] Two retrospective studies show elevated leukocyte counts on admission are associated with mortality and elevated leukocyte counts during the course of SAH are associated with symptomatic vasospasm [23–25]
Interleukin 6 (IL-6)	Serum	IL-6 is not associated with SAH outcome or angiographic vasospasm [27] Higher IL-6 levels in the early phase (days 3–7) are associated with DIND and unfavorable outcome (GOS 1-3) [25, 30] IL-6 levels elevated over 2 weeks after bleeding, correlate with SAH severity grade [30–32] and vasospasm [30] IL-6 levels are increased with increasing age, intraventricular and intracerebral hemorrhage, seizures, vasospasm, DIND, chronic hydrocephalus, and pneumonia [31] IL-6 on admission is elevated in SAH compared to unruptured aneurysm [31] Higher IL-6 is associated with higher Fisher grade, severe EBI, susceptibility to pneumonia [31]
	CSF	IL-6 is higher in CSF than in serum in SAH [29] CSF IL-6 post-SAH day 5 is elevated in poor outcome group [29]
Plasma-type gelsolin (pGSN)	CSF, serum	Serum and CSF pGSN are decreased in SAH patients compared to controls [35, 36] Blood pGSN is negatively associated with WFNS and Fischer grade and is an independent predictor of poor functional outcome [36]
Metalloproteinase-9 (MMP-9)	CSF, plasma	Elevated MMP-9 in CSF post-SAH days 2–3 is associated with poor 3-month outcome [26] Plasma and CSF MMP9 within 48 h of admission are associated with DCI [33] Elevated serum MMP9 is associated with higher HH grade, vasospasm, and poor outcome [30] One study found no association between MMP9 on post-SAH days 1, 4, 7, 10, and 14 and DCI [34]
Soluble intercellular adhesion molecule-1 (sICAM1)	Serum	sICAM1 is elevated in SAH patients compared to controls Patients with poor outcome (mRS 4–6) have higher sICAM1 levels over first 2 weeks post-SAH compared to patients with good outcome (mRS 0–3) [37] No association between time course of sICAM1 and DCI [34]
C-reactive protein (CRP)	Serum, CSF	No association between blood and CSF CRP time course and DCI in SAH [34] CRP levels are significantly higher after a SAH [38] Higher CRP is associated with EBI and Hunt and Hess grades and increased susceptibility to pneumonia [32, 38] Higher CRP is associated with death or severe disability at 3 months
Interleukin 10 (IL-10)	Serum	Higher Fisher grade on admission results in higher IL-10 Higher IL-10 and CRP are associated with severe EBI and increased susceptibility to pneumonia [38]
Interleukin 1β (IL-1β)	CSF	IL-1β is higher in CSF than in serum in SAH patients [38]
Interleukin 1R antagonist (IL-1Ra)	CSF	IL-1Ra is higher in patients with poor SAH (HH grades 3–4) [28] Elevated IL-1Ra on post-SAH days 4–10 is associated with poor outcome [28]
Monocyte chemoattractant protein-1 (MCP-1)	Serum, CSF	Serum MCP-1 concentrations correlate with poor outcome, but not with angiographic vasospasm [41] CSF MCP-1 is significantly higher in patients with angiographically demonstrated vasospasm [41]

Table 3 (continued)

Biomarker	Sample source	Observations
Toll-like receptor 4 (TLR4) expression	Plasma	Patients with SAH show significantly higher TLR4 levels on days 1, 3, and 7 after admission compared to healthy controls [42] Patients with DCI show significantly higher TLR4 levels than those without DCI [42] Admission (day 1) TLR4 level is as an independent factor to predict patients at risk of DCI and/or 3-month poor clinical outcome [42]
Thioredoxin (Trx)	Serum	Trx is a potent antioxidant that modulates inflammation. Elevated plasma Trx levels at admission (within 24 h after initial bleeding) correlate with severity grade (WFNS, Fisher) and poor prognosis after 6 months [45]
Inflammatory cytokines	Serum	Serum samples analyzed at: < 24 h, 24–48 h, and 3–5 and 6–8 days after SAH [43] Systemic inflammatory activity peaked at 24–48 h post-SAH [43] Platelet-derived growth factors (PDGF)-AA, PDGF-AB/BB, soluble CD-40 ligand, TNF-α increased overtime [43] Participants with higher clinical severity had increased levels of pro and anti-inflammatory cytokines: IL-6, CC chemokines CCL2, CCL11, colony-stimulating factor (CSF) 3, IL-8, IL-10, CX chemokines CX3CL1, and TNF-α compared to lower-severity patients [43]
Galectin-3	Plasma	Gal-3 plays a role in macrophage activation, angiogenesis, cell–cell adhesion, cell–matrix interactions, and metastasis. Gal-3 is an independent determinant for poor outcome [47] Gal-3 levels on days 1–3 correlate with DCI and infarction, but not with angiographic vasospasm and chronic hydrocephalus [47] ROC indicates cutoff value of 3.30 predicting DCI development (specificity, 62.5%; sensitivity, 90.9%) [47]
Osteopontin (OPN)	Plasma	OPN binds to integrin receptors expressed by leukocytes and induces immune cell adhesion, migration, and survival. OPN levels measured on days 1–3, 4–6, 7–9, and 10–12 correlated with 90-day poor outcome [44] Based on ROC curves, OPN levels at days 10–12 are most useful to predict poor outcome [44] Levels higher at certain days are associated with delayed cerebral ischemia, cerebral infarctions, and hydrocephalus [44]
Von Willebrand factor (vWF)	Serum	vWF levels > 94.5 nmol/L are independently associated with poor 3-month outcome [48] Plasma vWF antigen and activity were higher in SAH patients versus controls. No significant difference between survivors and non-survivors in SAH [49]
Asymmetric and symmetric dimethylarginine (ADMA and SDMA)	Plasma, serum, CSF	ADMA and SDMA inhibit nitric oxide production from L-arginine. ADMA was significantly lower and peak arginine/ADMA ratio was higher in patients with HH 1–2 compared to HH 3–5 [32] Baseline plasma arginine/ADMA ratio is significantly lower in patients with DCI [51] CSF ADMA is associated with DCI and CSF SDMA with 30-day poor neurological outcome [51]
Platelet activation	Plasma	Patients with DCI have more platelet activation compared to those without DCI within 72 h of ictus. At 3 months: death or severe disability is more likely with higher platelet activation [83]
ADAMTS13	Plasma	A zinc-containing metalloprotease that cleaves vWF Plasma ADAMTS13 activity post-bleed days 0, 1, 3, 5, 7, and 10 significantly is lower in SAH than in healthy controls [49]
Neuropeptide Y (NPY)	CSF	NPY is a potent vasoconstrictor that regulates cerebral vascular diameter and cerebral blood flow CSF NPY is significantly higher in SAH compared to controls and CSF NPY from post-SAH days 4–10 is higher in patients with vasospasm [52]

CSF cerebrospinal fluid, *DCI* delayed cerebral ischemia, *DIND* delayed ischemic neurological deficit, *EBI* early brain injury, *GOS* Glasgow Outcome Score, *HH* Hunt and Hess grade, *SAH* subarachnoid hemorrhage, *WFNS* World Federation of Neurosurgical Societies

cleave plasma-type gelsolin (pGSN), which is decreased in both plasma and CSF after SAH, and decreased blood pGSN is an independent predictor of poor SAH outcome [35, 36]. Soluble intercellular adhesion molecule-1 (sICAM1) and C-reactive protein (CRP) are non-specific inflammatory markers that are elevated in SAH and shown association with SAH outcome but inconsistent association with DCI [32, 34, 37, 38].

Emerging Data Elements

Inflammatory cytokines and chemokines are involved in the pathophysiological cascade associated with delayed cerebral ischemia [25, 27, 31, 39, 40]. Studies have examined individual or combinations of cytokines in serum and CSF after SAH [28, 38, 41–43]. Cytokine and chemokines in SAH and other conditions such as sepsis show high inter-individual and inter-study variability and this limits biomarker specificity, which subsequently limits their utilities as clinical biomarkers [34]. Emerging multiplex and precision medicine techniques have not been sufficiently studied in SAH. Additional emerging biomarkers implicated in immune function and inflammation include osteopontin, thioredoxin, and galectin-3 (Gal-3) [44–47]. As Gal-3 is involved in microglia activation and proliferation in response to ischemic injury it might be a specific indicator for secondary ischemic complications after SAH [47].

SAH-associated inflammatory response is also associated with the release of vasoactive molecules that mediate vascular function and are emerging as putative SAH biomarkers. In systemic circulation, von Willebrand factor (vWF) elevation is independently associated with SAH outcome, though vWF activity showed no association with SAH mortality [48, 49]. Blood ADAMTS 13, a protease that cleaves vWF, is significantly lower after SAH [49]. Endothelial production of nitric oxide is an important regulator of vascular tone in compromised SAH and asymmetric and symmetric dimethylarginines (ADMA and SDMA) [50]. Emerging data showed blood and CSF ADMA arginine/ADMA ratio are associated with Hunt and Hess grade and DCI, while SDMA is associated with outcome in SAH [32, 51]. Other emerging SAH biomarkers of vascular functions include neuropeptide Y which is associated with unfavorable outcome as well as Doppler sonographic vasospasm and subsequent cerebral ischemia [52].

Genetic, Systemic, and Extracellular Biomarkers (Table 4)

Supplemental Data Elements

Several genetic case control studies showed association between ApoE4 genotype with DIND, cerebral infarction, and unfavorable outcome after SAH, though the mechanism of this association remains insufficiently understood [53–56]. Haptoglobin (Hp) genetic heterogeneity, and specifically the Hp2-2 phenotype, shows association with worse SAH outcome, mortality, and cerebral vasospasm following SAH [57, 58].

Cardiac troponin I (cTI) and B-type natriuretic peptide (BNP) originate outside the central nervous system, but increased circulating levels of these two molecules have shown consistent associations with post-SAH mortality [59–63]. While a meta-analysis of 12 studies showed cTI to be associated with an increased risk of DCI [64], poor outcome, and death after SAH, a multicenter study of 301 subjects failed to show independent association between cTI and SAH outcome [62]. Pro-inflammatory cytokines, including TNF-α and IL-1β, selectively promote BNP synthesis and BNP elevation also correlated with cerebral infarction particularly in patients without angiographic vasospasm, suggesting that BNP might be a marker for microcirculatory dysfunction [59, 65, 66].

Emerging Data Elements

The variability in genotypes might be one explanation of the heterogeneity of the expression of biomarkers relevant in the pathogenesis of cerebral vasospasm and DCI. Ryanodine receptors (RYRs) are calcium release channels in vascular smooth muscle cells and may play an important role in cerebral vascular tone. Three specific RYR1 genetic variants appear to confer higher risk of symptomatic vasospasm [67]. MicroRNAs (miRNAs) are endogenously expressed noncoding RNAs that regulate gene expression at the posttranscriptional level [68]. Recent findings suggest that a combination of circulating miRNAs may differentiate SAH patients with or without DCI [69].

Due to limited standardization in methodology, current recommendations for core and supplemental CDE do not include parameters obtained by cerebral microdialysis. Cerebral microdialysis, however, has future potential as it allows near real-time monitoring of extracellular interstitial fluid chemical composition in patients [70]. Anatomical precision of cerebral microdialysis has the added benefit of being able to clearly identify the origin of molecular biomarkers while minimizing confounding by systemic factors [39]. Using catheters with the standard 20-kDa nominal molecular weight cutoff membrane, available clinical analyzers can measure concentrations of small molecules such as glucose, pyruvate, lactate, and glutamate at the bedside. Use of 100-kDa microdialysis membranes allows measurements of larger molecules including cytokines [71, 72]. Microdialysis is especially suitable as early warning system of DCI in SAH [73–75]. The lactate/pyruvate ratio (L/P ratio) is a well-established marker of aerobic versus anaerobic metabolism [76]. In several case series, it has been shown that lactate values and L/P ratio correlate with the outcome after severe SAH [77–79]. In order to further expand the scientific and clinical validity of microdialysis with larger data sets, future studies utilizing cerebral microdialysis must be standardized according to current guidelines per "Recommendations from the 2014 International Forum on Microdialysis—the consensus statement" [80].

Table 4 Genetic, non-CNS, and Extracellular Biomarkers

Biomarker	Sample source	Observations
Apolipoprotein E (ApoE4)	Blood	Presence of ApoE4 is associated with DIND [53] and unfavorable outcome [53–55] ApoE ε2- or ε4-containing genotypes are not associated with SAH outcome or cerebral infarction [56]
Haptoglobin (Hp) genotype	Blood	Presence of Hp2-2 phenotype is an independent risk factor for focal and global cerebral vasospasm and for poor functional outcome and mortality following SAH [57, 58]
Ryanodine receptor 1 gene (RYR1)	Blood	RYR1 genotype is associated with increased risk of symptomatic vasospasm [67]
Circulating microRNA	Serum	miR-4532, miR-4463, miR-1290, and miR-4793 differentiate SAH patients with DCI from those without DCI [69]
Cardiac Troponin I (cTI)	Serum	Elevated cTI is associated with death [59, 60] Peak cTI is independently predictive of death or severe disability at hospital discharge but not predictive of 3-month mRS [61, 62] Peak cTI and GCS on presentation independently predict in-hospital mortality [63]
B-type Natriuretic Peptide (BNP)	Plasma	BNP > 600 pg/mL is associated with death [59] BNP measured once after SAH (mean 5.5 ± 3.0 days) Top quartile is associated with increased odds of cerebral infarction, particularly in patients without angiographic vasospasm [66]
Glucose, lactate, lactate/pyruvate ratio (L/P ratio), glycerol	Extracellular fluid (ECF)	Lactate, L/P, and glycerol are higher in high-grade SAH [79] L/P independently predict 6- or 12-month outcome [73, 79] High L/P and low glucose are associated with neurological deficits [73] and cerebral infarction [77] "Ischemic pattern" (lactate/glucose and L/P > 20%, 20% increase in glycerol) is present in 17 of 18 patients with DIND [74] Lactate > 4 mM and glutamate > 3 mM for at least 6 consecutive hours are associated with neurological deterioration/DIND [75]

DCI delayed cerebral ischemia, *DIND* delayed ischemic neurological deficit, *ECF* extracellular fluid, *GCS* Glasgow Coma Scale, *SAH* subarachnoid hemorrhage

Conclusions and Future Directions

Biomarkers can facilitate risk prediction and optimization of treatment for individual patients and are essential tools and bridge toward a future of precision medicine in SAH. Biomarkers may enable preventive actions and a proactive approach, which is extremely important in neurocritical care, as secondary brain injuries may occur within minutes leading to death or severe disability. To date, however, no molecular biomarker has been validated in SAH using large prospective studies and across different populations. The very high variability of individual values from different anatomical compartments of biospecimen origin, irregular schedules for biospecimen acquisition, and lack of standardization in biospecimen storage and processing pose significant barriers toward biomarker validation and translation into clinical practice. It is crucial that investigators working toward development of clinical biomarkers in SAH begin to harmonize and standardize research methodology and biospecimen collection and processing protocols to synergize the large body of research efforts and combine results into larger data sets to facilitate biomarker validation and translation.

Decades of research has clearly demonstrated that SAH-associated brain injury involves dynamic and numerous inter-related pathophysiological cascades. The complexity of such system may not be adequately captured by a single biomarker. The future of SAH biomarkers development may require simultaneous examination of a combination of biomarker panels adapted to the dynamic course of the disease over an extended period. Emerging high-efficiency multiplex technologies and advanced statistical and bioinformatics methods present a great opportunity for a novel approach to the quest for biomarkers and precision medicine in SAH.

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Authors' Contributions

SHYC, RLM., and EK were involved in protocol development and wrote and edited the manuscript. The corresponding author confirms that authorship requirements have been met, that the final manuscript was approved by ALL authors, and that this manuscript has not been published elsewhere and is not under consideration by another journal. The UIA and SAH CDEs project adhered to ethical guidelines.

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Compliance with Ethical Standards

Conflicts of interest
Dr. Macdonald reports personal fees from Edge Therapeutics and grants from Brain Aneurysm Foundation, outside the submitted work. Dr. Chou reports research grants from National Institute of Health/NINDS (K23-NS0738060, the University of Pittsburgh Physicians, and the University of Pittsburgh School of Medicine. Outside the submitted work, Dr. Chou received fees from Edge Therapeutics (site principal investigator, consulting), non-financial support from Merck, consulting fees from the National Institute of Health. Dr. Chou is a co-inventor on a patent MITOCHONDRIAL BIOMARKERS OF, AND THERAPEUTICS AQ15 FOR, CNS INJURY AND DISEASE filed in the United States Patent and Trademark Office as Application 62/3817.

Ethical approval and Informed consent
This work did not involve human or animal participants.

Appendix: SAH WORKING GROUP MEMBERS

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