Commentary

Clinical utility of degradomics as predictors of complications and clinical outcome in aneurysmal subarachnoid hemorrhage

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Most of the debilitating conditions following aneurysmal subarachnoid hemorrhage result from symptomatic cerebral vasospasm and delayed cerebral ischemia. Several scales are being used, but they still lack objectivity and fail to quantify complications considered essential for prognostication routine use of biomarkers to predict complications and outcomes after aneurysmal rupture is still experimental. Degradomics were studied extensively in traumatic brain injury, but there is no discussion of these biomarkers related to aneurysmal subarachnoid hemorrhage. Degradomics involve the activation of proteases that target specific substrates and generate specific protein fragments called degradomes. While the proteolytic activities constitute the pillar of development, growth, and regeneration of tissues, dysregulated proteolysis resulting from pathological conditions like aneurysmal subarachnoid hemorrhage ends up in apoptotic processes and necrosis. To our knowledge, this is the first overview that lists a panel of degradomics with cut-off values in serum and cerebrospinal fluid, where specificity and sensitivity are only found in Kallikrein 6, Ubiquitin C Terminal Hydrolase 1 and Alpha-II-Spectrin.

Keywords

Degradomics; Degradomes; Proteases; Aneurysm; Subarachnoid hemorrhage; Substrates; Blood-brain barrier

1. Introduction

Among the variety of etiologies of strokes, aneurysmal subarachnoid hemorrhage (aSAH) constitutes 2–5% of all stroke cases [1]. Delayed cerebral ischemia (DCI) is the main predictor of clinical outcomes. It is a multifactorial phenomenon with various contributing factors such as cortical spreading depression, disrupted/altered cerebral autoregulation, micro-thrombosis, and inflammation [2, 3]. The most used scales to assess the extent of rupture of the aneurysm and the severity of the clinical outcome are the Hunt and Hess and the World Federation of Neurological Surgeons (WFNS) [4]. But these tests lack objectivity regarding the prediction of complications and defining the outcome, especially when it comes to patients whose neurological examination yield very little information about their current neurological status.

Microdialysis has led to a breakthrough in serum biomarkers involving proteomics, glycomics, lipidomics, metabolomics, and degradomics [5]. Temporal correlations of clinical complications with specific biomarkers have been confirmed as early as 15 minutes after subarachnoid hemorrhage [6]. Change in the expression of these molecular fingerprints, especially before clinical signs occur, presents an early opportunity to identify biomarkers to predict an outcome.

Many recent articles have studied the correlation between specific degradomes and aSAH complications, as mediators of the disease and predictors of outcome. Still, none have gained widespread clinical use due to the small number of patients involved and lack of review articles that set definite values and timing that would most benefit patients' prognosis. To our knowledge, no overview englobes degradomics involved in aSAH. This article is a narrative review of the degradomic profile of aSAH in serum and CSF in the latest literature that helps identify potentially helpful biomarkers that can predict the clinical outcome of patients with aSAH, guide therapeutic approach, and serve as new therapeutic targets in the future. According to the date of publications, relevant articles were selected from the PubMed database and using keywords: 'Aneurysms'; 'Subarachnoid Hemorrhage'; and specific keywords for each protease/degradome.

2. Degradomics

Degradomics is applying of genomic and proteomic approaches to identify the biologically relevant proteolytic substrates called degradomes [7]. It refers to post-translational modification of proteins after activating proteases in a specific disease state, leading to hydrolysis of peptide bonds. The smaller peptides generated from the target protein are called breakdown products (BDPs) [8]. Proteases have functional

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roles in various physiological events, including digestion, fertilization, cellular proliferation, differentiation, cell signaling and migration, wound healing, apoptosis, angiogenesis, and inflammatory responses [9]. During acute cellular injury due to ischemia or excitotoxicity, calcium influx leads to the activation of proteases. Uncontrolled up-regulation of proteases such as calpains, caspases, cathepsins, and matrix metalloproteinases (MMPs) constitute one of the essential protease-substrate repertoires alteration pathways that are related to the central nervous system (CNS) injury and neurodegeneration (Fig. 1) [10]. Their implication in the cellular signaling pathway is crucial for neuronal functioning, synaptogenesis, memory formation and neuro-hemostasis [11]. It is mainly achieved by controlling neuron lifespan and promoting inflammation. Proteases constitute a significant cause of blood-brain barrier (BBB) disruption with the extravasation of inflammatory cells and molecules, contributing to various inflammation's impacts upon aneurysmal rupture [12]. For example, MMP-2 and MMP-9 degrade components of the basal lamina in capillaries, including collagen type IV, laminin, fibronectin, and gelatine. Hence, it may play critical roles in facilitating BBB permeability and extravasation of T lymphocytes [13]. Other proteases are crucial for formation and degradation of myelin, and many of them contribute to multiple sclerosis [9]. Calpains have an established role in axon damage in several models of axon injury, such as stretch, crush, and ischemia [14]. BDPs were extensively linked to several states of disease, and they serve as encryptic neoproteins that can be used to diagnose, assess, and treat their pathophysiologic implication [15]. For example, during hippocampal excitotoxicity, intense stimulation of N-methyl-D-aspartate (NMDA) receptors leads to BDP formation after protease activation, playing a significant role in the neurodegenerative process of Alzheimer's disease [16]. The evolving field of mass spectrometry-based proteomics has made degradomics a more approachable domain [17]. Proteases have long been implicated with aSAH. Work in [18] showed how proteases overactivation after SAH was the leading cause of disability, as the incorporation of calpain inhibitor in rats in which SAH was induced have improved performance regarding behavior and cognitive abilities, as well as a reduction in BBB opening, with a significant anti-inflammatory effect. The link between degradomics and aSAH lies in the various cellular injury processes that lead to the rupture of aneurysms where vascular wall injury initiates the proteolytic cascade. A pool of BDPs will be poured into the blood significantly when BBB is disrupted. This process generates a list of potentially measurable proteases and resultant substrates and breakdown products that can be used to assess a patient's condition and predict his outcome. In this commentary, we discuss different degradomics implicated in predicting complications and outcomes after aSAH. We will be dividing degradomics into two entities, proteases and degradomes. The latter include both substrates and BDPs (Fig. 2). This will give us an insight about the mechanism of cellular injury that initiates the activation of proteases, with the resultant degradomes that act as evidence of the brain insult affecting different cellular pathways. Table 1 (Ref. [19–29]) summarizes the degradomic profile of aSAH regarding the prediction of severity and clinical outcome. Degradomics related to vasospasm are summarized in Table 2 (Ref. [25, 26, 30–33]).

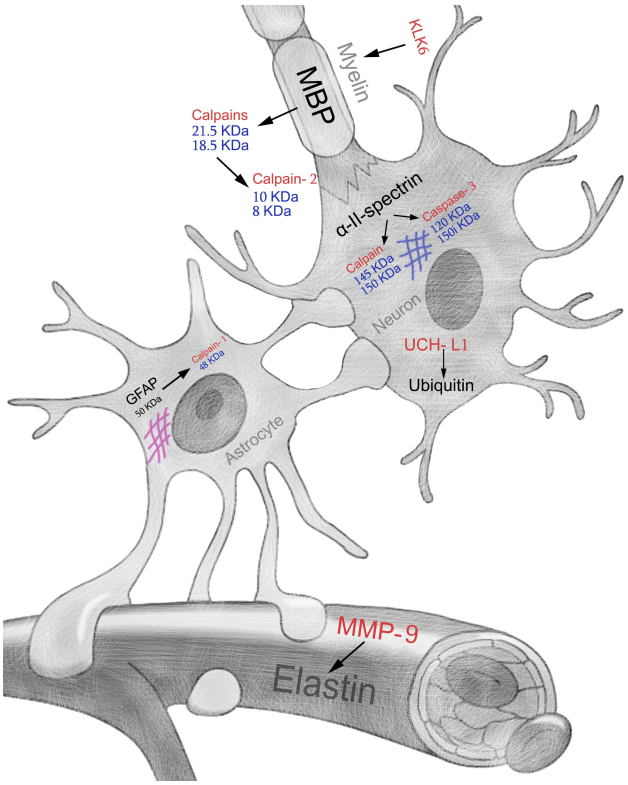

Fig. 1. Showing different proteases and degradomes in neurons, astrocytes, and blood vessels during aneurysmal subarachnoid hemorrhage. MMP-9, Matrix Metalloproteinase 9; GFAP, Glial Fibrillary Acidic Protein; KLK6, Kallikrein-6; MBP, Myelin Basic Protein; UCH-L1, Ubiquitin C Terminal Hydrolase 1; KDa, Kilodalton.

3. Proteases

Five hundred proteases are responsible for the hydrolysis of peptide bonds in the human body. They are encoded by approximately 2% of the genome [34, 35]. Proteolysis is classified as endopeptidases or exopeptidases according to the site of cleavage [36]. Different biological processes ensue protease activation through the proteolysis of bioactive molecules such as hormones, receptors, cytokines, or precursors [36]. Inappropriate activation of proteolytic enzymes during aSAH can negatively impact various functions in the damaged tissues, by disrupting transcription factors, cytoskeletal proteins, signaling enzymes, and cell cycle regulatory proteins [37]. The extent of cellular damage with the resultant activation of proteases contributes to the clinical outcome, as it reflects the ischemic process taking place after aSAH (Fig. 2). In addition, the calcium influx after cel-

lular injury generates a proteolytic cascade involved in apoptosis, which proved to be the foundation of vasospasm following aneurysmal rupture [10]. The proteases that will be discussed are caspase-3, Ubiquitin C Terminal Hydrolase 1, matrix metalloproteinases, and kallikrein-6.

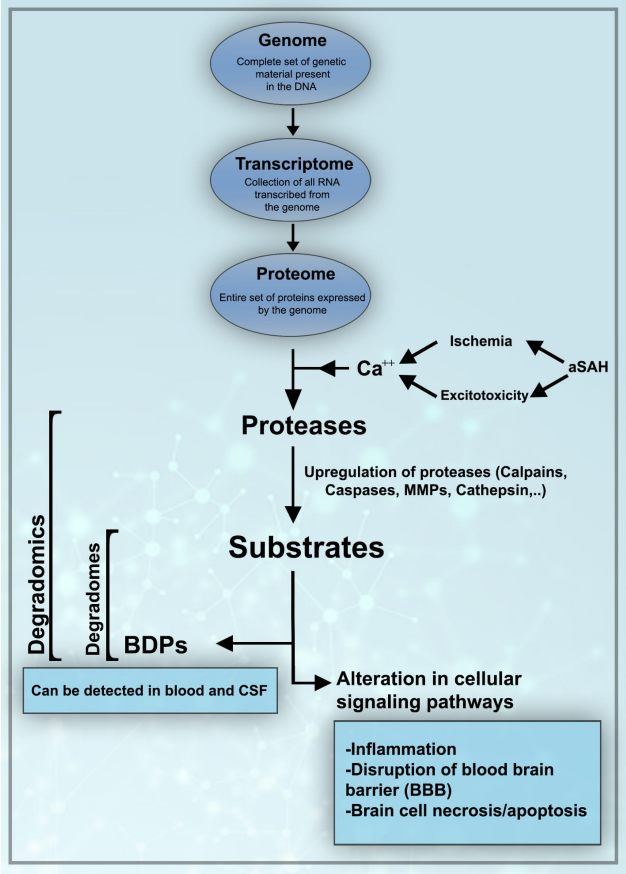

Fig. 2. Showing degradomics and proteases repertories during cellular injury after aneurysmal subarachnoid hemorrhage. BDPs, Breakdown products; aSAH, aneurysmal subarachnoid hemorrhage; MMPs, Matrix Metalloproteinase.

3.1 Caspase-3 (Cas-3)

Caspases are aspartate-specific cysteine proteases that autoactivate from their zymogen procaspase form. There are 14 caspases identified till now [38]. They are associated with inflammation and apoptotic signaling, both intrinsic and extrinsic pathway [39]. Apoptosis activator caspases 2-8-10 activate apoptosis executioner caspases 3-6-7, initiating mitochondrial apoptotic pathway [40]. During ischemic brain injury, increased calcium levels activate m-calpain, activating procaspases [41]. Cas-3 was evident even 10 minutes after SAH induction in rat brains. This implies that cell death starts earlier than was previously known [42].

In [30] involved 20 patients with aSAH and 15 controls with normal pressure hydrocephalus without any other central nervous system disease, found that the mean concentration of serum Cas-3 in the 3rd, on the 5th and 7th day were 127.03 $\pm$ 25.54, 123.50 $\pm$ 29.32, and 118.05 $\pm$ 30.07 μM pNA, respectively, compared with 28.03 $\pm$ 1.89 μM pNA in the control group. This increase towards the 7th day after aSAH was concomitant with the highest risk of symptomatic vasospasm, suggesting a major role of Cas-3 (Table 2).

In [19] involved 118 patients with aSAH and 118 healthy controls, found that serum Cas-3 highly correlated with the severity of aSAH as measured by WFNS scores and modified Fisher scores, reflecting the extent of neuronal damage. On day 3 of the presentation, Cas-3 could highly predict six months' mortality and unfavorable outcome in aSAH as measured by the Glasgow Outcome Scale (GOS), with a cutoff value of 0.65 ng/mL (Table 1). Although these two studies were significantly informative, they still had minimal patients to transform Cas-3 testing into clinical practice. Still, a serum value of 0.65 ng/mL may be a good start for future prospective studies to base their clinical outcome.

Caspase inhibitors showed promising results in decreasing apoptosis and attenuating cerebral vasospasm [43]. The treatment of TMP (tetramethylpyrazine) resulted in the reduction of apoptotic cells and mitochondrial injury in rat brain cells, which lead them to perform better in the neurobehavioral outcome compared to those treated with saline [44].

3.2 Ubiquitin Carboxy-Terminal Hydrolase L1 (UCH-L1)

UCH-L1 is a stable protease released in considerable amounts into the CSF of patients having aSAH. It was formerly known as Pgp9.5, detectable only in neurons and neuroendocrine cells. It is mainly located in perikarya and dendrites [45]. In [29] involved 30 aSAH patients whose CSF samples were taken for ten days post aneurysmal rupture, a cutoff value of 9.5 ng/mL (positive predictive value of 0.64, sensitivity = 90%, specificity = 50%) was able to discriminate patients with excellent or bad GOS grades at six months, with increasing levels correlating with higher grades and worse outcome (Table 1).

In [25] included 25 patients with aSAH and measured their UCHL-1 levels in CSF from day three until day ten post-onset. A positive correlation was found between higher peaks of UCH-L1 and Hunt and Hess score. Mean value of 2.8 u in grade 2 (complete nuchal rigidity, moderate to severe headache, cranial nerve palsy) and grade 3 (confusion with the mild focal neurological deficit) was found, compared with 7.0 u in grade 4 (stupor with severe deficits) and grade 5 (coma). It was also correlated with a higher infarction rate, with a mean of 6.3 u in infarcted group, compared with 0.6 u with no infarcts. The mean value was 12.9 u in patients with moderate to severe symptomatic vasospasm, compared to 3.2 u in patients with mild or without vasospasm, as detected by angiographic luminal reduction (Table 2). CSF UCH-L1 was also significantly elevated about the outcome at discharge with the value of 9.8 u in patients dead or sent to a nursing home, compared to 2.4 u in those when he was sent home or to rehabilitation (Table 1).

A cut-off value of 9.5 ng/mL [29] may be used for future

studies to implement early and aggressive treatment for those with a bad outcome. Whereas Siman *et al.* [25] compared means of patients without having an exact value to be considered a reference to predict complications although significant results were found. This will need further prospective clinical studies to confirm the exact value for each complication.

Both studies [25, 29] have found that CSF levels of UCH-L1 differ in the first ten days. This may be sufficient for six months' outcome prediction but may be considered less valuable to predict vasospasm as it may be too late to implement aggressive treatment and prevent further damage.

3.3 Matrix Metalloproteinase (MMP)

MMPs are a family of extracellular and membrane bound proteases [46], secreted by macrophages, leukocytes, smooth muscle cells, endothelial cells, astroglia and microglia in response to inflammatory markers and tissue growth factors [32]. MMP-3 and MMP-9 were both increased during inflammation and related to apoptosis of neurons, glial cells, and endothelial cells [47, 48]. Tissue remodeling and regeneration depend highly on MMPs. MMPs-2, -7, -9 and -12 degrade insoluble elastin, the origin of arterial wall elasticity [49]. Activated MMPs play a significant role in neuronal development, function and differentiation [50]. Knowing that ruptured and unruptured aneurysms involve increased extracellular matrix remodeling [51], aneurysm rupture was associated with higher MMP-7 levels but not MMP-10 and 12 [52]. MMP-1 peaked at 30 minutes post aSAH and was concomitant with narrowing the vessels in rat models [53].

In [31] involved 45 patients with SAH, and found that serum MMP-9 levels of more than 700 ng/mL increased the chance of ensuing symptomatic vasospasm by 25-fold (Table 2).

In [32] it was proved that MMP-9 levels correlated well with aSAH and ultrasound detected vasospasm, yet failed to correlate with Hunt and Hess scores. aSAH patients had MMP-9 levels ranging from 13,720 to 106,800 (log scale of the area under the curve) compared to healthy controls with a range of 2806 to 22,420 using zymography (Table 2). On the other hand, there was a significant correlation between higher Hunt and Hess scores and higher levels of MMP-9 in serum, with levels reaching $\approx$ 600 ng/mL in grades 4–5 compared with $\approx$ 200 ng/mL in healthy controls (Table 1) [26]. This discordance between the Hunt and Hess score in the two previous studies may be due to the smaller number of patients in [32] Three days after aSAH, there's a significant reduction in vasospasm, following the administration of gelatinase inhibitor with K_i (SB-3CT)—an MMP-9 inhibitor—which may become a promising therapeutic tool for vasospasm (Table 2) [54]. Others have found a significant reduction in cerebral ischemia in animal models using MMP inhibitors (BB-94, BB-1101, and KB-R7785) [55]. BB-3103 is another MMP inhibitor that decreased endothelial gap formation and the ability of monocytes to traverse the endothelium [56]. This may constitute a bridge towards newer therapeutic approaches in aSAH, and further work should be done to translate them into clinical practice.

3.4 Kallikrein 6

Known as Neurosin or Kallikrein-related peptidase 6 (KLK6), it is a protease expressed mainly in the brain and spinal cord. It is shown that this protein plays a significant role in remyelination after injury of the central nervous system, and it is highly expressed by oligodendrocytes [57]. It is assumed that the expression of this protein is reduced as a result of the death of oligodendrocytes or their migration to the site of injury to promote rapid re-myelination [27].

The reference interval for serum KLK6 was established to be 1.04 to 3.93 ng/mL [27]. Serum KLK6 levels were studied during the first 56 hours in 13 patients with aSAH. It was proved more informative than the results obtained after 56 hours of the onset of aSAH [27]. The mean serum levels of KLK6 in patients with worse outcome (0.47 ng/mL) was significantly lower than the lowest reference limit of 1.04 ng/mL. The lowest levels were found in patients who died or survived with significant neurocognitive deficits, perhaps reflecting the extent of the initial brain damage suffered (Table 1) [27].

In [23] enrolled 92 patients with aSAH and 92 healthy controls. KLK6 was found significantly decreased in patients with higher WFNS and modified Fisher scores. Blood was collected between 1.2–24 h after aSAH. KLK6 values in low WFNS and modified Fisher scores were between 0.1 and 0.2 mg/L, whereas in higher grades, serum values were below 0.1 mg/L (Table 1). An optimal serum level of 0.109 mg/L predicted the occurrence of DCI with 74.2% sensitivity and 80.3% specificity. KLK6 concentrations had a predictive ability of DCI equivalent to those of WFNS and modified Fisher scores. Thus, it can be a potential biomarker to evaluate aSAH patients at risk of DCI and assessing hemorrhagic severity of aSAH.

Both of these studies [23, 27] have shown promising results regarding the potential use of KLK6 to predict clinical outcomes and guide the therapeutic approach. Due to the small number of patients involved in both studies, more prospective studies involving a more significant number of patients should be conducted to better assess its performance.

4. Degradomes

It is a term that describes the substrates that the proteases act upon, as well as the BDPs produced after the proteolytic process (Fig. 2). Degradomes of aSAH provide early insight into the pathophysiological processes underlying the devastating complications of this condition. Degradomes are the repertoire of proteases, and their interaction regulates the biological behavior of cells [15, 16]. Many cytological substrates subject to degradation by proteases carry crucial structural role and functional cellular behavior, including cytoskeleton, signaling pathways, enzymes, and mitotic or apoptotic properties [35]. This can lead to a subsequent cellular injury following the disruption of these critical cellular proteins. Brain tissue damage following aSAH activates

Table 1. Biomarkers that correlate with severity and poor prognosis of aneurysmal subarachnoid hemorrhage.

Severity	Biomarker	Study	Level of biomarker/Cut-off	Controls	Time of sampling	Number of patients
WFNS	Cas-3 (serum)	Wang *et al.* (2016) [19]	>0.65 ng/mL	<0.65 ng/mL	Day 3	118 patients with aSAH, 118 controls
	Alpha-II-spectrin SBDPs (CSF)	Papa L *et al.* (2018) [20]	Mean 60–120 ADU	Mean 20–60 ADU	Between 4th and 10th day	20 patients with aSAH, 20 controls
	MBP (serum and CSF)	Hirashima *et al.* (2001) (CSF) [21]	94.3 ng/mL (grade 3–4)	25.1 ng/mL (grade 1–2)	4–9 days	28 patients with aSAH, 25 controls
	GFAP (serum)	Nylén *et al.* (2007) [22]	>0.15 µg/L	<0.15 µg/L	Day 3	116 patients with aSAH
	KLK6 (serum)	Bian *et al.* (2019) [23]	<0.1 mg/mL (higher grades)	Between 0.1 and 0.2 mg/mL (grade 1 and 2)	Admission	92 patients with aSAH, 92 controls
GCS	GFAP (serum)	Vos *et al.* (2006) [24]	1.17 µg/L (GCS <8)	0.73 µg/L (GCS 9–15)	Admission	67 patients with aSAH
	Alpha-II-spectrin SBDPs (CSF)	Papa L *et al.* (2018) [20]	Mean 60–120 ADU	Mean 20–60 ADU	Between 4th and 10th day	20 patients with aSAH, 20 controls
Hunt and Hess	UCH-L1 (CSF)	Siman *et al.* (2011) [25]	Mean 7.0 u in grade 4 and 5	Mean 2.8 u in grade 2 and 3	Between 3rd and 10th day	25 patients with aSAH
	MMP-9 (serum)	Wang *et al.* (2018) [26]	500–600 ng/mL (grade 3–4–5)	400–500 ng/mL (grade 1–2)	Most significant at 4th day	23 patients with aSAH, 43 controls
Worse outcome	KLK6 (serum)	Martínez-Morillo *et al.* (2013) [27]	Mean 0.47 ng/mL	>1.04 ng/mL	First 56 hours	13 patients with aSAH, 136 controls
	GFAP (serum)	Nylén *et al.* (2007) [22]	>0.15 µg/L	<0.15 µg/L	Day 3	116 patients with aSAH
	MBP (serum and CSF)	Wąsik *et al.* (2019) (serum) [28]	744 pg/mL (Mortality) 406.5 pg/mL (GOS 1–3)	344.1 pg/mL -	First 3 days	104 patients with aSAH
		Hirashima *et al.* (2001) (CSF) [21]	94.3 ng/mL	25.1 ng/mL	4–9 days	28 patients with aSAH, 25 controls
	Alpha-II-spectrin SBDPs (CSF)	Papa L *et al.* (2018) [20]	Mean 60–120 ADU	Mean 20–60 ADU	Between 4th and 10th day	20 patients with aSAH, 20 controls
	MMP-9 (serum)	Wang *et al.* (2018) [26]	466.92 ng/mL (poor prognosis)	392.88 ng/mL (good prognosis)	Most significant at 4th day	23 patients with aSAH, 43 controls
	UCH-L1 (CSF)	Lewis *et al.* (2010) [29]	>9.5 ng/mL	<9.5 ng/mL	First 10 days	30 patients with aSAH
	Cas-3 (serum)	Wang *et al.* (2016) [19]	>0.65 ng/mL	<0.65 ng/mL	Day 3	WFNS, modified Fisher scores, GOS, and 6 months mortality

UCH-L1, Ubiquitin C Terminal Hydrolase 1; MMP-9, Matrix Metalloproteinase 9; SBDPs, Spectrin breakdown products; MBP, Myelin Basic Protein; GFAP, Glial Fibrillary Acidic Protein; KLK6, Kallikrein-6; WFNS, World Federation of Neurological surgeons.

Table 2. Biomarkers that correlate with vasospasm after aneurysmal subarachnoid hemorrhage.

Biomarker	Study author/Year	Level of biomarker/Cut-off	Controls	Time of sampling	Correlation	Number of patients
Cas-3 (serum)	kacira *et al.* (2007) [30]	127.03 ± 25.54 µM 123.50 ± 29.32 µM, and 118.05 ± 30.07 µM	28.03 ± 1.89 µM	3rd, 5th and 7th day respectively	Higher in patients with aSAH, concomitant rise with symptomatic vasospasm on 7th day	20 patients with aSAH, 15 controls
UCH-L1 (CSF)	Siman *et al.* (2011) [25]	Mean 12.9 u	Mean 3.2 u	Between 3rd and 10th day	Symptomatic Vasospasm	25 patients with aSAH
MMP-9 (serum)	McGirt *et al.* (2002) [31]	>700 ng/mL	<700 ng/mL	Mean peak within 4 to 11 days or until onset of vasospasm	Symptomatic Vasospasm	45 patients with aSAH, 42 patients with stroke unrelated to SAH
	Horstmann *et al.* (2006) [32]	13,720 to 106,800 (log of the area under the curve)	2806 to 22,420	Day 1 to day 12	Presence of aSAH, symptomatic vasospasm	11 patients with aSAH, 20 controls
	Wang *et al.* (2018) [26]	437.43 ng/mL	382.37 ng/mL	most significant at 4th day	Symptomatic Vasospasm	23 patients with aSAH, 43 controls
Alpha-II-spectrin	Lewis *et al.* (2007) [33]	50–70 ADU	20–35 ADU	12 hours before onset of vasospasm	Symptomatic Vasospasm SBDPs (CSF)	20 patients with aSAH, 10 controls

UCH-L1, Ubiquitin C Terminal Hydrolase 1; MMP-9, Matrix Metalloproteinase 9; SBDPs, Spectrin breakdown products; Cas-3, Caspase 3.

these cascades, and a wide variety of degradomes are produced. Some of them can be measured and quantified to predict complications and outcomes after an aneurysmal bleed.

4.1 Alpha-II-spectrin

Alpha-II-spectrin protein is a cytoskeletal component of the cortical membrane of presynaptic terminals and axons. When cells undergo apoptosis, this protein is subject to cleavage by proteases like calpain-2 and Cas-3 [58], producing SBDP150, SBDP145, and SBDP120 studied as markers of TBI severity and vasospasm [59].

In [20] enrolled 20 patients with aSAH and 20 controls and found a cutoff level of 2.0 ADU (arbitrary densitometric units) of SBDP150 and a 0.5 ADU of SBDP145 to distinguish between the two groups with sensitivity and specificity of 100%. A cutoff value of 0.5 ADU of SBDP120 was 90% sensitive and 100% specific. There was also a significant relation with the severity of SAH when comparing the peak of SBDPs in the 3rd day between survivors and nonsurvivors. Between the 4th and the 10th day, survivors had steady elevations with mean levels between 20 and 60 ADU. Meanwhile, nonsurvivors had significant elevations with multiple peaks and troughs from day 4 to day 10 with mean levels between 60 and 120 ADU. In addition, higher levels of SBDP150, SBDP145, and SBDP120 levels were correlated with increasing levels of WFNS grades of severity and Glasgow coma scale (GCS) (Table 1).

Despite the limited informative GCS regarding neurological cues, it is still a widely used scale in the emergency department for mental status. SBDP150, SBDP145, and SBDP120 levels were higher in subgroups with a GCS score of 15 and WFNS Grade 1 compared to the control group with no aSAH. This can prevent the false assumption of normality and delayed neurological investigations for this subgroup, increasing the risk of delayed neurological damage [20]. In addition, the breakdown products level in those presenting with a GCS of 15 was lower than any other GCS scores [20].

Twenty patients were involved with Fisher grade 3 (localized clots and/or layers of blood >1 mm in thickness), demonstrated a significant increase in CSF values of SBDP 12 hours before the clinical onset of symptomatic vasospasm, with emphasis on SBDP150 and SBDP145, reaching 50–70 ADU compared with 20–35 ADU with patients without symptomatic vasospasm, suggesting that the primary mechanism of symptomatic vasospasm is a necrotic process (Table 2) [33].

Despite the minimal number of patients used, these only two articles were highly informative regarding the correlation between SBDP and the prediction of aSAH complications. The timing and the values mentioned can be used for future studies to help predict cerebral vasospasm and outcome and guide treatment using anti-apoptotic agents. The apoptotic process is deemed a significant cause of morbidity after aneurysm rupture.

4.2 Myelin Basic Protein (MBP)

MBP is the second most abundant structural protein of myelin sheath, and it provides adhesions of cytosolic surface to the layers of myelin provided by oligodendrocytes. It also mediates extracellular signals by binding to the cytoskeleton [60]. It is acutely cleaved into 21.5 and 18.5 kDa by calpains in rats subjected to the experimental CCI model and found abundantly in CSF of multiple sclerosis patients [61, 62]. The 21.5 and 18.5 kDa MBP isoforms are themselves degraded by calpain-2 into smaller BDPs of molecular weights 10 and 8 kDa [63]. MBP was considered part of degradomics due to the relevance of its BDP in traumatic brain injury. Until now, no article has mentioned MBP BDP during aSAH. Two articles have studied the relevance of MBP measurement to predict the outcome of aSAH and came up with highly significant results. The first one enrolled 104 patients with aSAH, serum MBP levels on days 0–3 post aSAH were higher among poor outcome patients as defined by GOS 1–3 (406.5 pg/mL), nonsurvivors (744 vs. 344.1 pg/mL), intracerebral hemorrhage (462.8 vs. 284.2 pg/mL), 3-month GOS, intracerebral hemorrhage volume, WFNS, Hunt and Hess, GCS and modified fisher score (Table 1) [28].

Similarly, [21] involved 28 patients having subarachnoid hemorrhage and found a correlation between CSF MBP levels on days 4–9 post-SAH and WFNS clinical grades on admission with grade 4 to 5 reaching 94.3 ng/mL vs. 25.1 ng/mL in patients with grade 1 to 2, when taking 0.51–2.61 ng/mL as normal ranges of MBP. This study had also found a correlation between high MBP and higher Fisher grade, cerebral infarction, and worse outcomes (Table 1).

MBP was suggested to be released into CSF before entering the blood. The necrotic process is believed to release higher amounts of MBP into CSF than serum. These MBP will be subject to proteolysis, and BDP levels can be measured in future studies to prove the relation between MBP and DCI. Hence, MBP constitutes a potential objective test to detect early ischemic changes.

4.3 Glial Fibrillary Acidic Protein (GFAP)

GFAP (55 kDa) is an intermediate cytoskeletal filament located in astrocytes, and it is a CNS-specific protein [64]. Lately, many studies have mentioned that during glial cells injury, GFAP is degraded by calpain-1, resulting in calpain-specific BDP of 48 kDa when put in a media resembling traumatic brain injury, which is acidic [65]. GFAP and GFAP-BDP were extensively studied during traumatic brain injury [10]. GFAP was mentioned during aSAH, but GFAP-BDP was not included so far concerning aSAH. GFAP was increased in Sprague-Dawley rats during induced endovascular perforation, causing SAH, which implied DNA damage in astrocytes and oligodendroglia [66]. BBB breakdown leads to the pouring of GFAP into the blood [67]. All of this leads to a broader subject called reactive gliosis. Glial cells respond well to reactive oxygen species that forms from oxyhemoglobin in the extravasated blood [68]. They can react to their surroundings by forming a barrier between the healthy brain tis-

sue and the injured area [69]. The change in expressivity can beneficially or detrimentally affect the brain's function, as the hemorrhage progresses [70]. It influences neuronal synaptic communication, microcirculatory blood flow [71, 72], and an increase in GFAP expression [73]. Reactive gliosis is more evident at the site of the hemorrhage, but it is also found elsewhere in the brain due to the generalized reduction in cerebral blood flow that proceeds SAH [73].

In [24] described a link between the severity of the initial brain damage after SAH and serum GFAP measured on admission. They included 67 patients with aSAH, and showed a significant relation between higher serum GFAP levels and lower GCS, higher WFNS, and higher Fischer grades. Patients with GCS <8 had a mean of 1.17 μg/L compared to 0.73 μg/L in GCS 9–15 (Table 1).

In [22] it was considered 0.15 μg/L a cut-off value for serum GFAP in 116 patients with aSAH. Higher readings were significantly correlated with higher Fisher and WFNS scores when taken on day three. They were found at least as good as WFNS and fisher grading on hospital admission to predict the long-term outcome (Table 1). GFAP can be a useful tool to assess patients in the neurology intensive care unit when recurrent imaging is not a feasible tool. Neurological examination of the mental status is difficult because of sedating medications and fading consciousness.

5. Conclusions

aSAH can be a debilitating condition with a devastating outcome. Highly complex molecular pathways are involved in the pathophysiology of the complications that ensue subarachnoid hemorrhage. An array of biomarkers that are produced in aSAH is discussed in this paper and can be used to predict complications and clinical outcomes. It can also serve as a therapeutic target for future clinical trials.

Caspase-3, UCH-L1, MMP-9, and alpha-II-spectrin SBDPs correlated with vasospasm's clinical outcome and severity in patients with aneurysmal SAH. MBP and GFAP correlated only with the clinical outcome. KLK6 has predicted both DCI and poor outcomes in patients with aSAH. More prospective trials should focus on the effectiveness of degradomics in the prediction of complications and clinical outcomes. It might be a good idea to target a higher number of patients with a battery of these potentially valuable biomarkers to validate their performance.

Abbreviations

aSAH, Aneurysmal Subarachnoid Hemorrhage; BBB, Blood Brain Barrier; BDPs, Breakdown Products; Caspase-3, Cas-3; CCI, Controlled Cortical Impact; CNS, Central Nervous System; DCI, Delayed Cerebral Ischemia; GCS, Glasgow Coma Scale; GFAP, Glial Fibrillary Acidic Protein; GOS, Glasgow Outcome Scale; KLK6, Kallikrein 6; MBP, Myelin Basic Protein; MMPs, Matrix Metalloproteinases; NMDA, N-methyl-D-aspartate; SB-3CT, gelatinase inhibitor with K_i; SBDPs, Spectrin Breakdown Products; TMP, Tetramethylpyrazine; UCH-L1, Ubiquitin C Terminal Hydrolase; WFNS, World Federation of Neurological Surgeons.

Author contributions

SB and HC wrote the paper and set the methodology; AB, SA, AM and MNEH gathered the articles and did the tables. Dr Ibrahim Omeis reviewed the paper and edited it.

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Conflict of interest

The authors declare no conflict of interest.

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