Cardiovascular Disease Prevention and Control

https://cdpc.cultechpub.com/cdpc

Cultech Publishing

Review

A Mini-Review on Molecular Pathways and Circulating Biomarkers in Hemorrhagic Cerebrovascular Disease

Aisha Siddiquah[1,*], Farooq Ahmed[2]

[1]Department of Biotechnology, Quaid-i-Azam University Islamabad, Islamabad, Pakistan

[2]Department of Health, Mardan Paramedical Institute, Mardan, Pakistan

Corresponding author: Aisha Siddiquah, Aisha_siddiquah@yahoo.com

Abstract

Hemorrhagic cerebrovascular disease, encompassing intracerebral hemorrhage and subarachnoid hemorrhage, represents one of the most severe forms of stroke and is associated with disproportionately high mortality, morbidity, and long-term neurological disability. Unlike ischemic stroke, effective disease-modifying therapies for hemorrhagic stroke remain limited, largely due to the complex and evolving nature of secondary brain injury following the initial vascular rupture. Accumulating evidence indicates that hemorrhage triggers a cascade of interrelated molecular mechanisms, including neuroinflammation, blood-brain barrier disruption, oxidative and metabolic stress, and dysregulated coagulation and thromboinflammatory signaling. These processes collectively drive hematoma expansion, perihematomal edema formation, neuronal and glial cell death, and delayed neurological deterioration. In recent years, substantial progress has been made in identifying circulating biomarkers that reflect these underlying pathological pathways. Proteins such as matrix metalloproteinases, inflammatory mediators, neuronal injury markers, and iron-handling proteins, along with regulatory microRNAs and metabolic by-products, provide dynamic and minimally invasive indicators of ongoing brain injury. These biomarkers not only correlate with disease severity and clinical outcome but also offer insight into individual pathophysiological profiles. This mini-review summarizes current knowledge on the key molecular pathways driving hemorrhagic cerebrovascular injury and critically examines circulating protein, microRNA, and metabolic biomarkers associated with hemorrhagic stroke. It highlights the potential of integrating biomarker signatures with genetic, epigenetic, and imaging data to enable precision medicine approaches, improve prognostication, and guide the development of targeted therapeutic strategies for hemorrhagic cerebrovascular disease.

Keywords

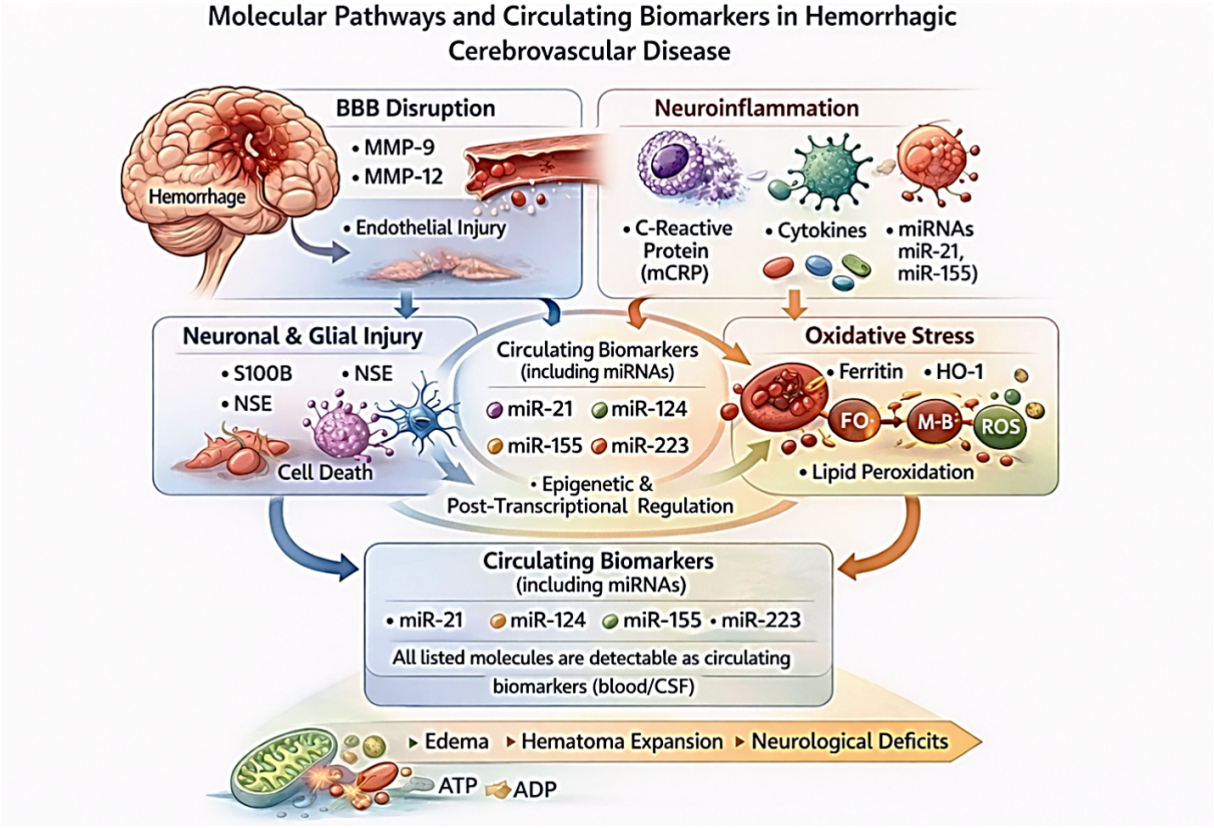
Hemorrhagic cerebrovascular disease, Molecular pathways, Circulating biomarkers, Precision medicine

Article History

Received: 04 February 2026 Revised: 20 April 2026

Accepted: 23 April 2026 Available Online: 29 April 2026

Graphical Abstract



1. Introduction

Hemorrhagic cerebrovascular disease (HCD) accounts for approximately 10%-20% of all stroke cases worldwide, yet it contributes disproportionately to stroke-related mortality, long-term disability, and healthcare burden [1]. Among its major subtypes, intracerebral hemorrhage (ICH) most commonly arises from rupture of small penetrating arteries weakened by chronic hypertension or cerebral amyloid angiopathy, leading to bleeding within the brain parenchyma. In contrast, subarachnoid hemorrhage (SAH) is typically caused by rupture of intracranial aneurysms or vascular malformations, resulting in blood accumulation within the subarachnoid space. Although these conditions differ in vascular origin and anatomical distribution, they share convergent pathophysiological cascades that extend well beyond the initial bleeding event and evolve dynamically over hours to days following hemorrhage [2].

Unlike ischemic stroke where reperfusion therapies, such as thrombolysis, and mechanical thrombectomy have fundamentally transformed clinical outcomes–therapeutic options for hemorrhagic stroke remain largely supportive, with limited disease-modifying interventions available [3-5]. This persistent therapeutic gap reflects the complex, multifactorial nature of secondary brain injury following hemorrhage. After vascular rupture, blood components trigger a cascade of pathological processes, including robust inflammatory activation, disruption of the blood-brain barrier (BBB), oxidative and metabolic stress, and dysregulation of coagulation and thrombotic pathways [6]. These mechanisms do not act in isolation; rather, they interact synergistically, creating self-amplifying feedback loops that exacerbate perihematomal edema, neuronal death, and neurological deterioration beyond the primary hemorrhagic insult [7,8]. Despite advances in understanding the pathophysiology of hemorrhagic stroke, several critical challenges remain unresolved. Clinically, there is a lack of reliable tools to predict hematoma expansion, perihematomal edema progression, and delayed neurological deterioration in the early stages of disease. Current imaging techniques provide structural information but are limited in capturing the dynamic molecular and cellular processes that drive secondary injury. Furthermore, there is a lack of validated biomarkers that can accurately reflect underlying pathophysiological mechanisms and guide individualized therapeutic strategies. This gap between mechanistic insight and clinical application represents a major barrier to improving outcomes in HCD. In contrast to ICH, SAH is characterized by distinct secondary injury mechanisms, including delayed cerebral ischemia, cerebral vasospasm, and cortical spreading depolarizations, which contribute substantially to neurological deterioration [9]. Aneurysm rupture triggers a pronounced inflammatory response within the subarachnoid space, involving endothelial dysfunction, oxidative stress, and microvascular dysregulation [10]. Biomarkers such as cerebrospinal fluid bilirubin, inflammatory cytokines, netrin-1, and specific microRNAs have been investigated in SAH cohorts, particularly in relation to vasospasm risk and delayed ischemic complications [11]. These processes highlight important pathophysiological and biomarker differences between SAH and ICH that warrant distinct consideration.

In recent years, increasing attention has been directed toward the identification of biomarkers that reflect these ongoing pathological processes in real time. Circulating biomarkers detected in blood or cerebrospinal fluid provide a minimally invasive means to interrogate molecular events occurring within the injured brain. Such biomarkers can capture dynamic changes in inflammation, BBB integrity, oxidative stress, and neuronal injury, offering insights that are not readily apparent through neuroimaging alone. When integrated with genetic and epigenetic information, circulating biomarkers hold promise for early risk stratification, prediction of hematoma expansion, and identification of patients most likely to benefit from targeted or pathway-specific therapeutic interventions. A comprehensive understanding of the molecular pathways that generate and regulate these biomarkers is essential for advancing translational research and precision medicine strategies in HCD [1,12]. This review aims to provide a concise yet integrative overview of the key molecular pathways driving secondary injury in HCD, including neuroinflammation, BBB dysfunction, oxidative stress, and thromboinflammatory signaling. In parallel, we systematically examine major classes of circulating biomarkers–proteins, microRNAs, and metabolic indicators–and their relevance to disease severity, progression, and clinical outcomes. Importantly, this review seeks to bridge the gap between mechanistic insights and clinical application by highlighting how biomarker signatures reflect underlying pathophysiology and can be leveraged for risk stratification and therapeutic targeting. Furthermore, we identify current limitations in biomarker research, including heterogeneity, lack of longitudinal data, and limited clinical translation, and discuss future directions emphasizing multi-omics integration and precision medicine approaches. Overall, this mini-review aims to synthesize current evidence linking molecular mechanisms with circulating biomarkers to support improved prognostication and the development of targeted, pathway-specific therapies in HCD.

To enhance methodological transparency, a structured literature search was conducted to identify relevant studies on molecular pathways and circulating biomarkers in HCD. Electronic databases including PubMed, Scopus, and Web of Science were systematically searched for articles published primarily within the last five years (2019-2025), with additional inclusion of seminal earlier studies where necessary. Search terms included combinations of "intracerebral hemorrhage," "subarachnoid hemorrhage," "biomarkers," "MMP-9," "neuron-specific enolase," "microRNA," "blood-brain barrier," and "oxidative stress." Studies were included if they investigated molecular mechanisms, circulating biomarkers, or clinical associations in hemorrhagic stroke. Exclusion criteria comprised studies unrelated to cerebrovascular disease, non-English publications, case reports, and articles lacking mechanistic or clinical relevance. Priority was given to clinical studies, systematic reviews, and high-impact experimental research to ensure the reliability and relevance of the included evidence. While this review is narrative in nature, efforts were made to ensure comprehensive coverage and minimize selection bias. While this review is narrative in nature, these structured search criteria were applied to improve comprehensiveness, reproducibility, and transparency of the literature selection process.

2. Molecular Pathways in Hemorrhagic Cerebrovascular Disease

2.1 Inflammatory Pathways

Inflammation is a central driver of secondary injury following cerebral hemorrhage. Blood extravasation into the brain parenchyma or subarachnoid space leads to rapid activation of resident microglia and recruitment of peripheral immune cells, particularly neutrophils and monocytes. These cells release pro-inflammatory cytokines, such as interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α), which correlate with neurological deterioration and poor outcome [13,14].

A growing body of evidence highlights the role of neutrophil extracellular traps (NETs) in hemorrhagic stroke. NETs, composed of DNA, histones, and granular enzymes, promote endothelial injury, exacerbate BBB disruption, and contribute to thromboinflammatory signaling [15,16]. Microglia-neutrophil crosstalk further amplifies inflammation, creating a sustained neurotoxic environment that promotes edema and neuronal death [17,18].

Beyond NETs, both innate and adaptive immune responses play critical roles in shaping secondary brain injury following hemorrhage. Innate immune activation is initiated rapidly after vascular rupture, with microglia adopting pro-inflammatory (M1-like) phenotypes that release cytokines, chemokines, and reactive oxygen species, thereby amplifying neuronal injury and BBB disruption [19]. In parallel, infiltration of peripheral immune cells–including neutrophils, monocytes, and macrophages–further sustains the inflammatory milieu through cytokine production and phagocytic activity. Adaptive immune responses are increasingly recognized as important contributors to hemorrhagic brain injury [11,20]. T lymphocytes, particularly $CD4^+$ and $CD8^+$ T cells, infiltrate the injured brain and modulate inflammation through cytokine secretion and cell-cell interactions. Regulatory T cells (Tregs) may exert protective effects by suppressing excessive inflammation and promoting tissue repair, whereas pro-inflammatory T cell subsets can exacerbate neuronal damage [21,22]. The balance between these immune populations is likely a key determinant of disease progression and recovery.

2.2 Blood-Brain Barrier Dysfunction

Blood-brain barrier breakdown is a hallmark of hemorrhagic brain injury and a major determinant of perihematomal edema. Mechanical stress from the hematoma, combined with inflammatory signaling, induces endothelial activation

and degradation of tight junction proteins. Matrix metalloproteinases (MMP-2, MMP-9, and MMP-12) play a key role in this process by cleaving extracellular matrix and basal lamina components.

Endothelial-derived mediators, such as vascular endothelial growth factor and angiopoietin-2 further increase vascular permeability. In parallel, endothelial-to-mesenchymal transition a process in which endothelial cells lose their characteristic junctional and barrier properties and acquire mesenchymal, fibroblast-like phenotypes with increased migratory and extracellular matrix-producing capacity which contributes to loss of vascular integrity and impaired repair mechanisms [23-25]. BBB dysfunction not only facilitates vasogenic edema but also allows peripheral immune cells and circulating toxins to access the injured brain, perpetuating secondary injury [6].

2.3 Oxidative Stress and Iron Toxicity

Oxidative stress is a defining feature of hemorrhagic stroke pathology. Following erythrocyte lysis, hemoglobin degradation releases heme and free iron, which catalyze the generation of reactive oxygen species through Fenton reactions. This oxidative burden damages lipids, proteins, and DNA, impairing mitochondrial function and accelerating neuronal death [26,27].

Upregulation of heme oxygenase-1 (HO-1) and accumulation of ferritin reflect cellular attempts to detoxify excess iron. However, these responses may be insufficient or, in some contexts, even maladaptive. Oxidative stress interacts closely with inflammatory signaling and promotes regulated cell death pathways, such as ferroptosis and necroptosis, further expanding tissue injury [28-33].

2.4 Thromboinflammatory and Hemostatic Dysregulation

Although hemorrhagic in origin, HCD is paradoxically associated with local thrombotic activity. Platelet activation, fibrin deposition, and microvascular thrombosis occur within perihematomal regions, impairing microcirculatory flow. NETs and damaged endothelium provide a scaffold for coagulation activation, linking inflammation and thrombosis [2,34]. Emerging evidence also highlights the role of platelet-derived microvesicles as mediators of thromboinflammatory signaling. These vesicles, released from activated platelets, carry procoagulant factors and inflammatory mediators that can amplify both coagulation cascades and vascular injury, thereby contributing to secondary brain damage [26,35]. In parallel, elevated D-dimer levels reflect ongoing fibrin degradation and have been associated with both hematoma expansion and thrombotic complications, suggesting their utility as dual biomarkers of bleeding and thrombosis risk.

This dysregulated hemostatic environment complicates therapeutic strategies, as interventions aimed at limiting bleeding may increase thrombotic risk, while anti-thrombotic approaches may exacerbate hemorrhage. Understanding this balance is critical for biomarker-guided therapy [36].

3. Circulating Biomarkers in Hemorrhagic Cerebrovascular Disease

3.1 Protein Biomarkers

Circulating biomarkers provide a minimally invasive means to monitor disease progression and may support early risk stratification and personalized therapeutic approaches . Elevated plasma or CSF levels of MMP-9 and MMP-12 reflect BBB disruption and are associated with edema formation and hematoma expansion. Quantitative analyses from clinical studies further support this association; for example, elevated MMP-9 levels have been linked to significantly increased risk of adverse outcomes, with reported odds ratios (ORs) reaching up to ~29.5 in patients with high circulating levels, and statistically significant associations ($p < 0.05$) after multivariate adjustment. C-reactive protein (CRP), particularly its monomeric form, serves as an indicator of inflammation, and metabolic dysfunction particularly impaired energy metabolism–further complements protein and RNA biomarkers (Figure 1) [37,38]. These metabolic signatures reflect the oxidative and bioenergetic collapse characteristic of hemorrhagic brain injury and may enhance multimodal biomarker panels for clinical use (Table 1) [39,40].

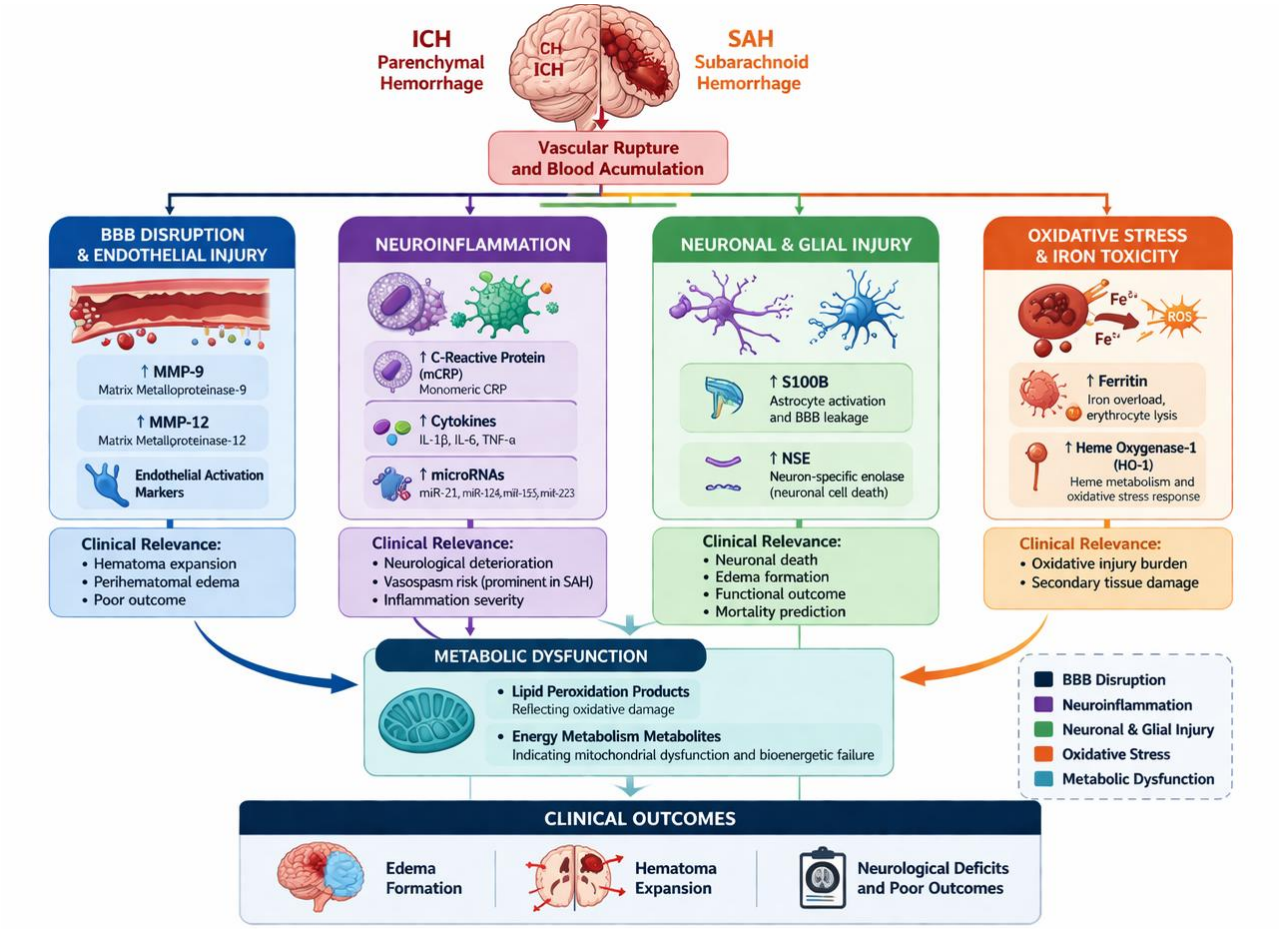


Figure 1. Circulating biomarkers reflecting secondary injury pathways in HCD. Schematic overview illustrating the major circulating biomarkers released following intracerebral or SAH and their association with key secondary injury pathways. Vascular rupture leads to BBB disruption, neuroinflammation, oxidative stress, and metabolic failure. BBB breakdown is reflected by elevated matrix metalloproteinases (MMP-9, MMP-12) and endothelial activation markers. Neuroinflammatory activation induces systemic and local release of CRP and cytokine-regulated microRNAs. Neuronal and glial injury results in increased circulating levels of S100B and neuron-specific enolase. Erythrocyte lysis and iron overload promote oxidative stress, reflected by ferritin and HO-1. Importantly, all listed biomolecules are detectable as circulating (fluid) biomarkers in blood or cerebrospinal fluid, linking molecular injury pathways to measurable clinical indicators. Circulating microRNAs (miR-21, miR-124, miR-155, miR-223) integrate inflammatory, oxidative, and BBB repair pathways, while metabolic biomarkers indicate mitochondrial dysfunction and bioenergetic collapse [41]. Together, these biomarkers provide a mechanistic link between molecular injury cascades and clinical outcomes in HCD.

Table 1. Circulating biomarkers in HCD: associated pathways, biological sources, and clinical relevance.

Biomarker Class	Biomarker	Primary Pathway Reflected	Biological Source	Clinical Relevance	AUC (reported range)	Key Ref.
BBB integrity	MMP-9	BBB disruption, extracellular matrix degradation	Activated leukocytes, endothelial cells	Associated with hematoma expansion, perihematomal edema, poor outcome	~0.70-0.85 (variable across studies)	[42]
	MMP-12	BBB damage, vascular remodeling	Macrophages, microglia	Predicts edema severity and secondary injury	Limited data / not consistently reported	[6]
Inflammation	C-reactive protein (mCRP)	Systemic and neuroinflammation	Liver, activated immune cells	Correlates with neurological deterioration and mortality	~0.65-0.75 (moderate predictive value)	[43]
Neuronal injury	Neuron-specific enolase	Neuronal cell death	Injured neurons	Early predictor of mortality and functional outcome	~0.70-0.81 (reported in SAH cohorts)	[44,45]
Glial injury	S100B	Astroglial activation and BBB leakage	Astrocytes	Associated with edema formation and long-term disability	~0.70-0.80 (variable across studies)	[46]
Oxidative stress / iron toxicity	Ferritin	Iron overload, oxidative stress	Macrophages, hepatocytes	Reflects erythrocyte lysis and secondary tissue damage	Limited quantitative AUC data available	[47,48]
	HO-1	Heme metabolism, oxidative stress response	Microglia, neurons	Indicates oxidative injury burden	Not well established	[49]
Regulatory RNAs	miR-21	Inflammation, endothelial survival	Circulating exosomes	Associated with disease severity and recovery	Emerging biomarker (AUC not standardized)	[41,50-52]
	miR-124	Neuroprotection, microglial polarization	Neurons	Differentiates hemorrhagic from ischemic stroke	Limited clinical AUC data	[14]
	miR-155	Pro-inflammatory signaling	Immune cells	Linked to BBB dysfunction and poor outcome	Variable / not consistently reported	[53]
	miR-223	Immune regulation, oxidative stress	Neutrophils, platelets	Modulates thromboinflammatory responses	Limited data available	[54]
Metabolic markers	Lipid peroxidation products	Oxidative damage	Injured brain tissue	Reflects mitochondrial dysfunction	Insufficient AUC data	[39,55]
	Energy metabolism metabolites	Bioenergetic failure	Neurons, glia	Enhances multimodal prognostic models	Emerging evidence (AUC not standardized)	[56]

3.2 Biomarker Profiles in Intracerebral Hemorrhage and Subarachnoid Hemorrhage

Biomarker profiles in HCD vary depending on the anatomical location and underlying pathophysiology of the hemorrhage. ICH, characterized by parenchymal bleeding and perihematomal injury, is more strongly associated with biomarkers reflecting BBB disruption, neuronal injury, and iron-mediated toxicity. These include matrix metalloproteinases (MMP-9), Neuron-specific enolase, S100B, ferritin, and HO-1, which correlate with hematoma expansion, edema formation, and neuronal damage [57]. In contrast, SAH is associated with blood accumulation in the subarachnoid space and a high risk of delayed cerebral ischemia and vasospasm. Accordingly, biomarkers related to vascular dysfunction and inflammation, such as inflammatory cytokines, CRP, and specific microRNAs–are particularly relevant in SAH, where they may reflect vasospasm risk and delayed neurological deterioration [58,59].

Several biomarkers, including MMPs, CRP, and circulating microRNAs, are shared between ICH and SAH, reflecting common secondary injury pathways, such as neuroinflammation, oxidative stress, and BBB disruption [14,60,61]. However, their relative clinical significance and temporal dynamics may differ between these conditions. This distinction highlights the importance of context-specific biomarker interpretation in advancing precision medicine approaches for hemorrhagic stroke.

4. Genetic and Epigenetic Markers in Hemorrhagic Cerebrovascular Disease

Genetic and epigenetic factors represent distinct but interconnected layers of biological regulation in HCD. While genetic variants influence baseline susceptibility to vascular rupture, epigenetic mechanisms dynamically regulate gene expression in response to injury. In addition, microRNAs serve as key post-transcriptional regulators linking genetic predisposition with environmental and pathological signals.

4.1 Genetic Susceptibility to Hemorrhagic Stroke

Genetic factors play a critical role in determining vascular integrity, susceptibility to vessel rupture, and the biological response to hemorrhagic injury. Unlike ischemic stroke, where polygenic risk scores are increasingly established, genetic determinants of HCD tend to cluster around pathways regulating vessel wall structure, amyloid deposition, and coagulation balance [62,63].

Among the most well-characterized genetic risk factors are APOE ε2 and ε4 alleles, which are strongly associated with lobar ICH and cerebral amyloid angiopathy. APOE ε2 is linked to vessel wall fragility and increased risk of hematoma expansion, whereas APOE ε4 promotes amyloid-β deposition and chronic vascular dysfunction [64,65]. These alleles not only influence hemorrhage risk but also affect recurrence rates and long-term cognitive outcomes.

Inherited mutations affecting vascular development and stability contribute to hemorrhage in younger populations [66-68]. Variants in genes involved in endothelial signaling, smooth muscle cell function, and extracellular matrix maintenance are implicated in arteriovenous malformations and cavernous malformations. In these conditions, genetic predisposition interacts with inflammatory and oxidative stress pathways, lowering the threshold for rupture. While genetic variants define baseline susceptibility, they do not fully explain the dynamic molecular responses observed after hemorrhagic injury, highlighting the importance of epigenetic regulation.

4.2 Epigenetic Regulation of Secondary Injury

Epigenetic mechanisms—including DNA methylation, histone modifications, and non-coding RNAs—have emerged as key regulators of gene expression following hemorrhagic stroke. Unlike genetic variants, epigenetic changes are dynamic and responsive to injury-related stimuli, such as inflammation, oxidative stress, and metabolic failure [69,70]. DNA methylation patterns in endothelial and immune cells influence BBB repair, immune cell polarization, and angiogenic responses after hemorrhage. Injury-induced hypomethylation of pro-inflammatory genes can sustain cytokine production, while hypermethylation of protective genes may impair recovery. Aging further modifies these epigenetic signatures, potentially explaining worse outcomes in elderly patients [71,72]. Although multiple studies consistently demonstrate the involvement of DNA methylation and histone modification in regulating inflammatory and vascular pathways, variability exists in the direction and magnitude of these changes, likely reflecting differences in disease stage, patient heterogeneity, and methodological approaches. Epigenetic regulation in HCD is inherently shaped by complex gene-environment interactions, in which inflammatory signaling, oxidative stress, and metabolic disturbances dynamically influence gene expression patterns. These interactions are highly nonlinear, with feedback loops that can amplify or attenuate injury responses depending on temporal and cellular context.

Histone acetylation and deacetylation regulate transcriptional responses involved in oxidative stress resistance and neuronal survival. Dysregulation of histone deacetylases has been linked to enhanced inflammation and neuronal death, positioning epigenetic modifiers as potential therapeutic targets. Among epigenetic mechanisms, microRNAs have emerged as particularly important regulators due to their dual role in gene expression control and their detectability as circulating biomarkers.

4.3 MicroRNAs as Epigenetic Regulators

As a specialized class of epigenetic regulators, circulating microRNAs bridge genetic predisposition and environmental response by fine-tuning gene expression at the post-transcriptional level. In HCD, miRNAs regulate pathways involved in BBB integrity, immune activation, iron metabolism, and oxidative stress. For example, miR-21 modulates endothelial survival and inflammatory signaling, while miR-124 promotes anti-inflammatory microglial phenotypes and neuronal protection. Conversely, miR-155 amplifies pro-inflammatory responses and BBB breakdown [37,73]. The dual role of miRNAs as biomarkers and regulators underscores their importance in both disease monitoring and therapeutic development. While several microRNAs, such as miR-21 and miR-124, are consistently associated with neuroprotective and anti-inflammatory effects, others like miR-155 show context-dependent roles, suggesting that their functional impact may vary across disease stages and cellular environments [54].

5. Clinical Translation and Precision Medicine

5.1 From Single Biomarkers to Multimodal Panels

Despite extensive biomarker discovery, translation into clinical practice has been limited by biological heterogeneity and temporal variability. Single biomarkers rarely capture the complexity of hemorrhagic brain injury. Current evidence supports a shift toward multimodal biomarker panels that integrate markers of inflammation, BBB dysfunction, oxidative stress, and neuronal injury [74-76].

Combining circulating biomarkers with neuroimaging parameters, such as hematoma volume, perihematomal edema, and imaging markers of BBB permeability–improves prediction of hematoma expansion, neurological deterioration, and mortality. Biomarker trajectories over time, rather than absolute values, may provide the most clinically relevant information [77,78].

5.2 Biomarkers for Risk Stratification and Prognosis

Early identification of patients at high risk for secondary injury is a major unmet need in hemorrhagic stroke care. Elevated inflammatory and BBB-related biomarkers within the first 24 hours may identify patients prone to rapid edema formation or delayed neurological decline. For instance, increased MMP-9 levels have been associated with higher odds of hematoma expansion and poor functional outcome (reported ORs ranging from ~2 to >20, $p < 0.05$), while elevated neuron-specific enolase concentrations have shown independent predictive value for mortality and unfavorable neurological outcomes in multivariate models. Oxidative stress markers and iron-related proteins may help stratify patients who could benefit from iron-chelation or antioxidant strategies [37,79,80] (Table 2).

Table 2. Diagnostic performance of selected circulating biomarkers in HCD.

Biomarker	Sample Type	Sensitivity (%)	Specificity (%)	Cutoff Value	Clinical Context	Ref.
MMP-9	Plasma	~70-85	~65-80	~140 ng/mL (variable)	Hematoma expansion, poor outcome	[81,82]
NSE	Serum	~65-80	~60-75	~20-25 ng/mL	Mortality, neurological outcome	[83]
S100B	Serum	~70-85	~65-80	~0.1-0.3 µg/L	BBB disruption, functional outcome	[46]
D-dimer	Plasma	~60-80	~55-75	Variable	Thromboinflammatory activity, prognosis	[84]
CRP	Serum	~60-75	~55-70	Variable	Inflammation, mortality risk	[74]

Importantly, genetic and epigenetic markers may inform long-term risk assessment, including hemorrhage recurrence and cognitive decline, particularly in conditions, such as cerebral amyloid angiopathy.

5.3 Toward Precision Therapeutics

Precision medicine in HCD aims to match patients with therapies targeting their dominant injury pathways. Patients with strong inflammatory signatures may benefit from anti-inflammatory or immunomodulatory approaches, whereas those with pronounced oxidative stress profiles may respond better to iron-handling or mitochondrial-targeted interventions. Emerging machine-learning models that integrate clinical variables, imaging data, circulating biomarkers, and genetic information represent a promising avenue for individualized decision-making. Such approaches could guide patient selection for clinical trials and accelerate the development of targeted therapies.

6. Future Directions

Despite substantial advances in understanding the molecular and biomarker landscape of HCD, translation into effective therapies remains limited. Future research must move beyond descriptive biomarker studies toward mechanism-driven, integrative, and patient-centered approaches. Several priority directions are outlined below.

6.1 Longitudinal and Time-Resolved Biomarker Profiling

One of the major limitations in current biomarker research is the reliance on single time-point measurements [85]. Hemorrhagic stroke is a dynamic disease, with molecular processes evolving from acute bleeding to subacute inflammation and chronic neurodegeneration [1,2,4]. Future studies should prioritize longitudinal sampling of blood and cerebrospinal fluid to capture biomarker trajectories rather than static levels.

Time-resolved profiling may improve prediction of hematoma expansion, delayed cerebral edema, and secondary neurological deterioration. Identifying early biomarker patterns that precede clinical worsening could enable preemptive intervention and more accurate prognostication.

6.2 Integration of Multi-Omics Approaches

Single-layer biomarker strategies are unlikely to fully capture the biological complexity of HCD. Future research should integrate multi-omics approaches, including proteomics, transcriptomics (including miRNAs), metabolomics, and epigenomics [39,86-90]. Such integrative analyses can uncover regulatory networks linking inflammation, BBB dysfunction, oxidative stress, and cell death.

Multi-omics datasets, particularly when paired with neuroimaging and clinical phenotypes, may identify novel molecular subtypes of hemorrhagic stroke. These subtypes could explain interindividual variability in outcomes and treatment responses, paving the way for biologically informed classification systems.

6.3 Genetic and Epigenetic Risk Stratification

Genetic susceptibility and epigenetic regulation remain underexplored in hemorrhagic stroke compared with ischemic stroke. Large-scale genome-wide association studies and epigenome-wide association studies focused specifically on ICH and SAH are needed to identify novel risk loci and regulatory mechanisms [91].

Importantly, epigenetic markers, such as DNA methylation patterns and histone modifications–offer the advantage of potential reversibility [92,93]. Future work should explore whether epigenetic signatures can serve not only as prognostic biomarkers but also as therapeutic targets, particularly in modulating inflammation and BBB repair [94-96]. However, emerging evidence suggests that epigenetic responses may vary substantially depending on hemorrhage subtype, anatomical location, and disease stage, highlighting the need for more stratified and longitudinal analyses.

6.4 Biomarker-Guided Therapeutic Targeting

A critical next step is the development of biomarker-guided interventions. Rather than applying uniform treatments to heterogeneous patient populations, future clinical trials should stratify patients based on dominant molecular pathways [97,98]. For example: Patients with strong inflammatory signatures may benefit from targeted anti-inflammatory or immunomodulatory therapies. Those with pronounced oxidative stress and iron overload may respond better to iron-chelation or antioxidant strategies. BBB-focused biomarkers could guide therapies aimed at vascular stabilization.

Such precision-based trial designs may improve therapeutic efficacy and reduce negative or inconclusive trial outcomes that have historically hindered progress in hemorrhagic stroke treatment. Notably, reported effect sizes for biomarkers, such as MMP-9 vary substantially across studies (e.g., ORs ranging from ~2 to >20), reflecting heterogeneity in study design, timing of measurement, and patient populations.

6.5 Artificial intelligence in Epidemiology: Opportunities and Challenges

Artificial intelligence (AI) has emerged as a powerful tool in epidemiological analysis, enabling large-scale integration of behavioral, environmental, and clinical data to identify disease patterns and risk factors. For instance, AI-driven models can improve behavioral intervention strategies by identifying high-risk populations and predicting disease trajectories. However, these benefits are closely intertwined with important limitations, as such models often rely on heterogeneous and incomplete datasets, which may introduce bias and reduce generalizability across different populations [99,100]. Similarly, AI-based analysis of environmental exposures, such as pollution, climate variables, and socioeconomic determinants offers enhanced resolution in understanding disease distribution and triggers. Nevertheless, the accuracy of these insights depends heavily on data quality and representativeness, and disparities in data availability across regions may contribute to the digital divide, limiting applicability in low-resource settings.

AI also holds promise for real-time epidemiological surveillance and public health policy support by enabling rapid data processing and predictive modeling. At the same time, these applications raise critical concerns regarding data privacy, ethical governance, and algorithmic transparency, particularly when sensitive health data are involved. The potential for algorithmic bias further underscores the need for careful validation and regulatory oversight. Taken together, these examples illustrate that the advantages of AI in epidemiology cannot be considered in isolation from their associated challenges. A balanced, context-specific evaluation is essential to ensure that AI-driven approaches are both scientifically robust and ethically responsible [60].

6.6 Translational and Clinical Challenges

For biomarkers to enter routine clinical use, several practical challenges must be addressed, including assay standardization, cost-effectiveness, and rapid turnaround times [101,102]. Future studies should focus on clinically feasible biomarker platforms, such as point-of-care assays or standardized multiplex panels.

International collaboration and harmonization of biomarker protocols will be essential to ensure reproducibility and accelerate regulatory approval.

6.7 Current Evidence Limitations

Despite growing interest in circulating biomarkers and molecular pathways in HCD, several limitations remain [1,4]. Current evidence is characterized by substantial heterogeneity in study design, patient populations, and timing of biomarker sampling, which complicates direct comparison across studies. In addition, many biomarkers lack standardized assay methods and validated clinical thresholds, limiting their translational applicability. The majority of studies are observational and cross-sectional, with relatively few longitudinal investigations assessing biomarker dynamics over time. Furthermore, differences between ICH and SAH are not consistently addressed, contributing to variability in reported findings. Addressing these limitations will be essential for advancing biomarker validation and clinical implementation.

7. Conclusion

HCD remains a major cause of mortality and long-term disability, largely due to complex secondary injury mechanisms that extend beyond the initial vascular rupture. Inflammation, BBB dysfunction, oxidative stress, and thromboinflammatory processes interact dynamically to drive neuronal injury and clinical deterioration. Advances in circulating biomarkers including proteins, microRNAs, and metabolic indicators have provided valuable insight into these molecular pathways and offer promising tools for prognosis and therapeutic stratification. Genetic and epigenetic factors further contribute to disease susceptibility and heterogeneity, highlighting the need for individualized approaches.

Future progress in hemorrhagic stroke care will depend on the successful integration of multi-omics biomarkers, longitudinal profiling, advanced analytics, and precision medicine frameworks. By aligning molecular mechanisms with clinical decision-making, biomarker-guided strategies have the potential to transform HCD from a largely untreatable condition into one managed through targeted, personalized interventions.

Author Contributions

All authors contributed to the conceptualization, literature review, writing, and critical revision of the manuscript. All authors read and approved the final version.

Conflict of Interest

The authors declare that they have no competing interests.

Generative AI Statement

The authors confirm that no generative AI tools were used in the development of the scientific content, data interpretation, or conceptual framework of this manuscript. ChatGPT (OpenAI) was used solely for language editing, including grammatical correction and improvement of readability. The figures included in this manuscript were generated using AI-assisted tools based on concepts, design, and scientific direction provided by the authors. All visual content was carefully reviewed, refined, and validated by the authors to ensure accuracy and alignment with the scientific context. The authors take full responsibility for the originality, accuracy, and integrity of all content presented in this work.

Reference

[1] Hishikawa T, Murai S, Ito YM, Funaki T, Fujimura M, Hamano E, et al. The AMORE score for predicting the 5-year risk of hemorrhagic stroke in asymptomatic moyamoya disease. Cerebrovascular Diseases, 2026, 1-7. DOI: 10.1159/000550273

[2] Kovács KB, Bencs V, Hudák L, Oláh L, Csiba L. Hemorrhagic transformation of ischemic strokes. International Journal of Molecular Sciences, 2023, 24(18), 14067. DOI: 10.3390/ijms241814067

[3] Chavez LM, Huang SS, MacDonald I, Lin JG, Lee YC, Chen YH. Mechanisms of acupuncture therapy in ischemic stroke rehabilitation: A literature review of basic studies. International Journal of Molecular Sciences, 2017, 18(11), 2270. DOI: 10.3390/ijms18112270

[4] Pariona-Vargas F, Mun KT, Lo EH, Starkman S, Sanossian N, Hosseini MB, et al. Circadian variation in stroke onset: Differences between ischemic and hemorrhagic stroke and weekdays versus weekends. Journal of Stroke and Cerebrovascular Diseases, 2023, 32(7), 107106. DOI: 10.1016/j.jstrokecerebrovasdis.2023.107106

[5] Ning B, Zhu X, Wu X, Zhu W, Wang R, Qi C, et al. Efficacy of different traditional Chinese medicine decoctions in the treatment of ischemic stroke: A network meta-analysis. Frontiers in Pharmacology, 2024, 15, 1486458. DOI: 10.3389/fphar.2024.1486458

[6] Aftab N, Masood F, Ahmad S, Rahim SS, Sanami S, Shaker B, et al. An optimized deep learning approach for blood-brain barrier permeability prediction with ODE integration. Informatics in Medicine Unlocked, 2024, 48, 101526. DOI: 10.1016/j.imu.2024.101526
[7] Cao Q, Guo Z, Du S, Ling H, Song C. Circular RNAs in the pathogenesis of atherosclerosis. Life Sciences, 2020, 255, 117837. DOI: 10.1016/j.lfs.2020.117837
[8] Ito M, Uchino H, Fujimura M. Intraoperative cortical indocyanine green extravasation as a predictor of cerebral hyperperfusion following direct revascularization for moyamoya disease: Impact of prolonged observations of indocyanine green videoangiography. Cerebrovascular Diseases, 2026, 55(1), 21-28. DOI: 10.1159/000545333
[9] Pascual-Fernández J, Fernández-Montero A, Córdova-Martínez A, Pastor D, Martínez-Rodríguez A, Roche E. Sarcopenia: Molecular pathways and potential targets for intervention. International Journal of Molecular Sciences, 2020, 21(22), 8844. DOI: 10.3390/ijms21228844
[10] Belbis MD. Skeletal muscle microvascular (dys) function: Mechanisms and therapeutics. Purdue University Graduate School, 2023. DOI: 10.25394/PGS.23721270.v1
[11] Kałużna A, Olczyk P, Komosińska-Vassev K. The role of innate and adaptive immune cells in the pathogenesis and development of the inflammatory response in ulcerative colitis. Journal of Clinical Medicine, 2022, 11(2), 400. DOI: 10.3390/jcm11020400
[12] Gilotra K, Swarna S, Mani R, Basem J, Dashti R. Role of artificial intelligence and machine learning in the diagnosis of cerebrovascular disease. Frontiers in Human Neuroscience, 2023, 17, 1254417. DOI: 10.3389/fnhum.2023.1254417
[13] Chen JX, Cheng CS, Gao HF, Chen ZJ, Lv LL, Xu JY, et al. Overexpression of interferon-inducible protein 16 promotes progression of human pancreatic adenocarcinoma through interleukin-1β-induced tumor-associated macrophage infiltration in the tumor microenvironment. Frontiers in Cell and Developmental Biology, 2021, 9, 640786. DOI: 10.3389/fcell.2021.640786
[14] Presta I, Vismara MFM, Novellino F, Donato A, Zaffino P, Scali E, et al. Innate immunity cells and the neurovascular unit. International Journal of Molecular Sciences, 2018, 19(12), 3856. DOI: 10.3390/ijms19123856
[15] Paranga TG, Mitu I, Pavel-Tanasa M, Rosu MF, Miftode IL, Constantinescu D, et al. Cytokine storm in COVID-19: Exploring IL-6 signaling and cytokine-microbiome interactions as emerging therapeutic approaches. International Journal of Molecular Sciences, 2024, 25(21), 11411. DOI: 10.3390/ijms252111411
[16] Wu JI, Lin YP, Tseng CW, Chen HJ, Wang LH. Crabp2 promotes metastasis of lung cancer cells via HuR and integrin β1/FAK/ERK signaling. Scientific Reports, 2019, 9(1), 845. DOI: 10.1038/s41598-018-37443-4
[17] Abolaji AO, Fasae KD, Iwezor CE, Aschner M, Farombi EO. Curcumin attenuates copper-induced oxidative stress and neurotoxicity in Drosophila melanogaster. Toxicology Reports, 2020, 7, 261-268. DOI: 10.1016/j.toxrep.2020.01.015
[18] Nwanna EE. Protective effect of alkaloid-rich extract of Brimstone tree (Morinda lucida) on eeurotoxicity in the fruit-fly (Drosophila melanogaster) model. African Journal of Biomedical Research, 2021, 24(2), 257-263.
[19] Paula FS, Kabeya LM, Kanashiro A, de Figueiredo AS, Azzolini AE, Uyemura SA, et al. Modulation of human neutrophil oxidative metabolism and degranulation by extract of *Tamarindus indica* L. fruit pulp. Food and Chemical Toxicology, 2009, 47(1), 163-170. DOI: 10.1016/j.fct.2008.10.023
[20] Hahn AW, Gill DM, Pal SK, Agarwal N. The future of immune checkpoint cancer therapy after PD-1 and CTLA-4. Immunotherapy, 2017, 9(8), 681-692. DOI: 10.2217/imt-2017-0024
[21] Hu J, Zhang L, Xia H, Yan Y, Zhu X, Sun F, et al. Tumor microenvironment remodeling after neoadjuvant immunotherapy in non-small cell lung cancer revealed by single-cell RNA sequencing. Genome Medicine, 2023, 15(1), 14. DOI: 10.1186/s13073-023-01164-9
[22] Mishra AK, Kadoishi T, Wang X, Driver E, Chen Z, Wang XJ, et al. Squamous cell carcinomas escape immune surveillance via inducing chronic activation and exhaustion of $CD8^+$ T Cells co-expressing PD-1 and LAG-3 inhibitory receptors. Oncotarget, 2016, 7(49), 81341-81356. DOI: 10.18632/oncotarget.13228
[23] Wang Y, Zhao R, Shen C, Liu W, Yuan J, Li C, et al. Exosomal CircHIPK3 released from hypoxia-induced cardiomyocytes regulates cardiac angiogenesis after myocardial infarction. Oxidative Medicine and Cellular Longevity, 2020, 2020, 8418407. DOI: 10.1155/2020/8418407
[24] Liu Y, Gan Z, Hu F. Effect of hydroxyapatite bioceramics on the growth of osteoblasts and HIF-α/VEGF signal axis in partial hypoxia environment *in vitro*. Technol Health Care, 2022, 30(S1), 363-369. DOI: 10.3233/THC-THC228033
[25] Choueiri TK, Kaelin WG Jr. Targeting the HIF2-VEGF axis in renal cell carcinoma. Nature Medicine, 2020, 26(10), 1519-1530. DOI: 10.1038/s41591-020-1093-z
[26] Yu Y, Yan R, Chen X, Sun T, Yan J. Paeonol suppresses the effect of ox-LDL on mice vascular endothelial cells by regulating miR-338-3p/TET2 axis in atherosclerosis. Molecular and Cellular Biochemistry, 2020, 475(1-2), 127-135. DOI: 10.1007/s11010-020-03865-w
[27] Baru Venkata R, Prasanth DSNBK, Pasala PK, Panda SP, Tatipamula VB, Mulukuri S, et al. Utilizing Andrographis paniculata leaves and roots by effective usage of the bioactive andrographolide and its nanodelivery: Investigation of antikindling and antioxidant activities through *in silico* and *in vivo* studies. Frontiers in Nutrition, 2023, 10, 1185236. DOI: 10.3389/fnut.2023.1185236
[28] Bhardwaj P, Biswas GP, Mahata N, Ghanta S, Bhunia B. Exploration of binding mechanism of triclosan towards cancer markers using molecular docking and molecular dynamics. Chemosphere, 2022, 293, 133550. DOI: 10.1016/j.chemosphere.2022.133550
[29] Shan K, Feng N, Zhu D, Qu H, Fu G, Li J, et al. Free docosahexaenoic acid promotes ferroptotic cell death via lipoxygenase dependent and independent pathways in cancer cells. European Journal of Nutrition, 2022, 61(8), 4059-4075. DOI: 10.1007/s00394-022-02940-w
[30] Shan Z, Liu F. Targeting ferroptosis, pyroptosis and necroptosis for cancer immunotherapy in melanoma: Mechanistic insights and clinical perspectives. Frontiers in Immunology, 2025, 16, 1629620. DOI: 10.3389/fimmu.2025.1629620
[31] Guo Z, Liu Y, Chen D, Sun Y, Li D, Meng Y, et al. Targeting regulated cell death: Apoptosis, necroptosis, pyroptosis, ferroptosis, and cuproptosis in anticancer immunity. Journal of Translational Internal Medicine, 2025, 13(1), 10-32. DOI: 10.1515/jtim-2025-0004

[32] Ali SL, Ali A, Khan A. Identification and assessment of ferroptosis-related genes and their implication as therapeutic agents for pancreatic ductal adenocarcinoma. Annals of Pancreatic Cancer, 2025, 8. DOI: 10.21037/apc-25-2
[33] Jiang C, Yan Y, Long T, Xu J, Chang C, Kang M, et al. Ferroptosis: A potential therapeutic target in cardio-cerebrovascular diseases. Molecular and Cellular Biochemistry, 2025, 480(7), 4379-4399. DOI: 10.1007/s11010-025-05262-7
[34] Karuppagounder SS, Alin L, Chen Y, Brand D, Bourassa MW, Dietrich K, et al. N-acetylcysteine targets 5 lipoxygenase-derived, toxic lipids and can synergize with prostaglandin E2 to inhibit ferroptosis and improve outcomes following hemorrhagic stroke in mice. Annals of Neurology, 2018, 84(6), 854-872. DOI: 10.1002/ana.25356
[35] Razizadeh MH, Zafarani A, Taghavi-Farahabadi M, Khorramdelazad H, Minaeian S, Mahmoudi M. Natural killer cells and their exosomes in viral infections and related therapeutic approaches: where are we? Cell Communication and Signaling, 2023, 21(1), 261. DOI: 10.1186/s12964-023-01266-2
[36] Kinlay S, Libby P, Ganz P. Endothelial function and coronary artery disease. Current Opinion in Lipidology, 2001, 12(4), 383-389. DOI: 10.1097/00041433-200108000-00003
[37] Mir SA, Dar A, Hamid L, Nisar N, Malik JA, Ali T, et al. Flavonoids as promising molecules in the cancer therapy: An insight. Current Research in Pharmacology and Drug Discovery, 2023, 6, 100167. DOI: 10.1016/j.crphar.2023.100167
[38] Akbari B, Baghaei-Yazdi N, Bahmaie M, Mahdavi Abhari F. The role of plant-derived natural antioxidants in reduction of oxidative stress. BioFactors, 2022, 48(3), 611-633. DOI: 10.1002/biof.1831
[39] Zhang W, Zeng Y, Jiao M, Ye C, Li Y, Liu C, et al. Integration of high-throughput omics technologies in medicinal plant research: The new era of natural drug discovery. Frontiers in Plant Science, 2023, 14, 1073848. DOI: 10.3389/fpls.2023.1073848
[40] Tambovtseva RS, Arslan LA, Grigoryeva TA, Abdulkhakov SR, Doludin YV, Stoma IO, et al. Narrative review on bacteria-derived metabolites in the pathogenesis of ulcerative colitis. Clinical Microbiology Reviews, 2025, 38(3), e0021024. DOI: 10.1128/cmr.00210-24
[41] Miao L, Yin RX, Zhang QH, Liao PJ, Wang Y, Nie RJ, et al. A novel circRNA-miRNA-mRNA network identifies circ-YOD1 as a biomarker for coronary artery disease. Scientific Reports, 2019, 9, 18314. DOI: 10.1038/s41598-019-54603-2
[42] Zhu Q, Enkhjargal B, Huang L, Zhang T, Sun C, Xie Z, et al. Aggf1 attenuates neuroinflammation and BBB disruption via PI3K/Akt/NF-κB pathway after subarachnoid hemorrhage in rats. Journal of Neuroinflammation, 2018, 15(1), 178. DOI: 10.1186/s12974-018-1211-8
[43] Zhu W, Fan C, Liu B, Qin J, Fan A, Yang Z, et al. Therapeutic targets for hepatocellular carcinoma identified using proteomics and Mendelian randomization. Journal of Gastroenterology and Hepatology, 2025, 40(1), 282-293. DOI: 10.1111/jgh.16785
[44] Gravina AG, Pellegrino R, Esposito A, Cipullo M, Romeo M, Palladino G, et al. The JAK-STAT pathway as a therapeutic strategy in cancer patients with immune checkpoint inhibitor-induced colitis: A narrative review. Cancers, 2024, 16(3), 611. DOI: 10.3390/cancers16030611
[45] Caponio VCA, Zhurakivska K, Lo Muzio L, Troiano G, Cirillo N. The immune cells in the development of oral squamous cell carcinoma. Cancers, 2023, 15(15), 3779. DOI: 10.3390/cancers15153779
[46] Hu Y, Zeng N, Ge Y, Wang D, Qin X, Zhang W, et al. Identification of the shared gene signatures and biological mechanism in type 2 diabetes and pancreatic cancer. Frontiers in Endocrinology, 2022, 13, 847760. DOI: 10.3389/fendo.2022.847760
[47] Adeloye JB, Aluko PA, Oluwajuyitan TD. *In vitro* α-amylase and α-glucosidase inhibitory activities, antioxidant activity, *in vivo* glycemic response and nutritional quality of dough meals from *Dioscorea alata* and *Vernonia amygdalina*. Journal of Food Measurement and Characterization, 2021, Charact 15, 4083-4097. DOI: 10.1007/s11694-021-00965-z
[48] Shintre SS, Dingli F, Meyerhoefer N, Gorgette O, Thouvenot C, Blumenthal DB, et al. Dynamic phosphoproteomics and proteomics uncover Leishmania donovani-driven ferritin hijacking, contributing to the control of iron homeostasis and iron-related oxidative stress. BioRxiv, 2026, 707907. DOI: 10.64898/2026.02.25.707907
[49] Ndisang JF. Role of heme oxygenase in inflammation, insulin-signalling, diabetes and obesity. Mediators of Inflammation, 2010, 359732. DOI: 10.1155/2010/359732
[50] Hueso M, Cruzado JM, Torras J, Navarro E. ALUminating the path of atherosclerosis progression: Chaos theory suggests a role for alu repeats in the development of atherosclerotic vascular disease. International Journal of Molecular Sciences, 2018, 19(6), 1734. DOI: 10.3390/ijms19061734
[51] Zhou KR, Liu S, Sun WJ, Zheng LL, Zhou H, Yang JH, et al. ChIPBase v2.0: decoding transcriptional regulatory networks of non-coding RNAs and protein-coding genes from ChIP-seq data. Nucleic Acids Research, 2017, 45(D1), D43-D50. DOI: 10.1093/nar/gkw965
[52] Li C, Wang M, Qiu X, Zhou H, Lu S. Noncoding RNAs in medicinal plants and their regulatory roles in bioactive compound production. Current Pharmaceutical Biotechnology, 2021, 22(3), 341-359. DOI: 10.2174/1389201021666200529101942
[53] Huang S, Xiang C, Song Y. Identification of the shared gene signatures and pathways between sarcopenia and type 2 diabetes mellitus. PLoS One, 2022, 17(3), e0265221. DOI: 10.1371/journal.pone.0265221
[54] Wang K, Long B, Liu F, Wang JX, Liu CY, Zhao B, et al. A circular RNA protects the heart from pathological hypertrophy and heart failure by targeting miR-223. European Heart Journal, 2016, 37(33), 2602-2611. DOI: 10.1093/eurheartj/ehv713
[55] Brown CLJ, Scott H, Mulik C, Freund AS, Opyr MP, Metz GAS, et al. Fecal 1H-NMR metabolomics: A comparison of sample preparation methods for NMR and novel *in Silico* baseline correction. Metabolites, 2022, 12(2), 148. DOI: 10.3390/metabo12020148
[56] Liu H, Li L, Krout M, Sheoran S, Zhao Q, Chen J, et al. Protocols for electrophysiological recordings and electron microscopy at *C. elegans* neuromuscular junction. STAR Protocols, 2021, 2(3), 100749. DOI: 10.1016/j.xpro.2021.100749
[57] Tabti K, Ahmad I, Zafar I, Sbai A, Maghat H, Bouachrine M, et al. Profiling the structural determinants of pyrrolidine derivative as gelatinases (MMP-2 and MMP-9) inhibitors using *in silico* approaches. Computational Biology and Chemistry, 2023, 104, 107855. DOI: 10.1016/j.compbiolchem.2023.107855
[58] Ademiluyi AO, Oboh G, Boligon AA, Athayde ML. Effect of fermented soybean condiment supplemented diet on α-amylase and α-glucosidase activities in Streptozotocin-induced diabetic rats. Journal of Functional Foods, 2014, 9, 1-9. DOI: 10.1016/j.jff.2014.04.003

[59] Boor PP, Metselaar HJ, Jonge Sd, Mancham S, van der Laan LJ, Kwekkeboom J. Human plasmacytoid dendritic cells induce $CD8^+$ LAG-3^+ $Foxp3^+$ CTLA-4^+ regulatory T cells that suppress allo-reactive memory T cells. European Journal of Immunology, 2011, 41(6), 1663-1674. DOI: 10.1002/eji.201041229
[60] Song CL, Wang JP, Xue X, Liu N, Zhang XH, Zhao Z, et al. Effect of circular ANRIL on the inflammatory response of vascular endothelial cells in a rat model of coronary atherosclerosis. Cellular Physiology and Biochemistry, 2017, 42(3), 1202-1212. DOI: 10.1159/000478918
[61] Chen X, Luo Y, Zhu Q, Zhang J, Huang H, Kan Y, et al. Small extracellular vesicles from young plasma reverse age-related functional declines by improving mitochondrial energy metabolism. Nature Aging, 2024, 4(6), 814-838. DOI: 10.1038/s43587-024-00612-4
[62] Chen X, Liu J, He B, Li Y, Liu S, Wu B, et al. Vascular endothelial growth factor (VEGF) regulation by hypoxia inducible factor-1 alpha (HIF1A) starts and peaks during endometrial breakdown, not repair, in a mouse menstrual-like model. Human Reproduction, 2015, 30(9), 2160-2170. DOI: 10.1093/humrep/dev156
[63] Gupta A, Verma A, Dhua A, Bhatnagar V. Vascular anomalies: A pediatric surgeon's perspective. Indian Journal of Pediatrics, 2017, 84(8), 612-617. DOI: 10.1007/s12098-017-2355-8
[64] Zhan Y, Brown C, Maynard E, Anshelevich A, Ni W, Ho IC, et al. Ets-1 is a critical regulator of Ang II-mediated vascular inflammation and remodeling. Journal of Clinical Investigation, 2005, 115(9), 2508-2516. DOI: 10.1172/JCI24403
[65] Fortea M, Albert-Bayo M, Abril-Gil M, Ganda Mall JP, Serra-Ruiz X, Henao-Paez A, et al. Present and future therapeutic approaches to barrier dysfunction. Frontiers in Nutrition, 2021, 8, 718093. DOI: 10.3389/fnut.2021.718093
[66] Gunasena M, Alles M, Wijewantha Y, Mulhern W, Bowman E, Gabriel J, et al. Synergy between nk cells and monocytes in potentiating cardiovascular disease risk in severe COVID-19. Arteriosclerosis, Thrombosis, and Vascular Biology, 2024, 44(10), e243-e261. DOI: 10.1161/ATVBAHA.124.321085
[67] Dilbar S, Sher H, Ali H, Ullah R, Ali A, Ullah Z. Antibacterial efficacy of green synthesized silver nanoparticles using *Salvia nubicola* extract against *Ralstonia solanacearum*, the causal agent of vascular wilt of tomato. ACS Omega, 2023, 8(34), 31155-31167. DOI: 10.1021/acsomega.3c03164
[68] Shuja MH, Sajid A, Anwar E, Sajid B, Larik MO. Navigating cardiovascular events in non-cardiac surgery: A comprehensive review of complications and risk assessment strategies. Journal of Cardiothoracic and Vascular Anesthesia, 2025, 39(3), 792-802. DOI: 10.1053/j.jvca.2024.09.149
[69] Cheng W, Gao A, Lin H, Zhang W. Novel roles of METTL1/WDR4 in tumor via m7G methylation. Molecular Therapy Oncology, 2022, 26, 27-34. DOI: 10.1016/j.omto.2022.05.009
[70] Xu J, Wei L, Liu H, Lei Y, Zhu Y, Liang C, et al. CD274 (PD-L1) methylation is an independent predictor for bladder cancer patients' survival. Molecular Therapy Oncology, 2022, 40(3), 228-233. DOI: 10.1080/07357907.2022.2028805
[71] Patel A, Jain P, Ajazuddin. Recent advances in the therapeutics and modes of action of a range of agents used to treat ulcerative colitis and related inflammatory conditions. Inflammopharmacology, 2025, 33(9), 4965-4996. DOI: 10.1007/s10787-025-01906-8
[72] Huggard D, Doherty DG, Molloy EJ. Immune dysregulation in children with down syndrome. Frontiers in Pediatrics, 2020, 8, 73. DOI: 10.3389/fped.2020.00073
[73] Zhidan X, Kedi X, Dan X, Xing S, Rui S. Predictive and carcinogenic roles of necroptosis-related miR-425-5p and miR-16-5p in esophageal squamous cell carcinoma. Annals of Clinical & Laboratory Science, 2024, 54(1), 76-85
[74] Łukaszewicz-Zając M, Zajkowska M, Pączek S, Kulczyńska-Przybik A, Safiejko K, Juchimiuk M, et al. The significance of CXCL1 and CXCR1 as potential biomarkers of colorectal cancer. Biomedicines, 2023, 11(7), 1933. DOI: 10.3390/biomedicines11071933
[75] Mohammed A, Okwor I, Shan L, Onyilagha C, Uzonna JE, Gounni AS. Semaphorin 3E regulates the response of macrophages to lipopolysaccharide-induced systemic inflammation. Journal of Immunology, 2020, 204(1), 128-136. DOI: 10.4049/jimmunol.1801514
[76] Liu S, Li Z, Lan S, Hao H, Baz AA, Yan X, et al. The dual roles of activating transcription factor 3 (ATF3) in inflammation, apoptosis, ferroptosis, and pathogen infection responses. International Journal of Molecular Sciences, 2024, 25(2), 824. DOI: 10.3390/ijms25020824
[77] Dietz S, Avula A, Kumar A, Petersen N, Guasch-Jimenez M, Slowik A, et al. Abstract DP035: White matter hyperintensities are associated with worse outcomes but not greater cerebral edema or hemorrhagic transformation after large vessel occlusion stroke. Stroke, 2026, 57(Suppl_1). DOI: 10.1161/str.57.suppl_1.DP035
[78] Ali A, Alamri A, Mishra VK, Utegenova A, Askarova G, Baiduissenova A, et al. TZ1391: A computationally designed circular mRNA multi-epitope vaccine candidate against Mycobacterium tuberculosis via TLR3 immunomodulation. BMC Immunology, 2026, 27(1), 23. DOI: 10.1186/s12865-025-00795-4
[79] Ononamadu CJ, Alhassan AJ, Ibrahim A, Imam AA, Ihegboro GO, Owolarafe TA, et al. Methanol-extract/fractions of dacryodes edulis leaves ameliorate hyperglycemia and associated oxidative stress in streptozotocin-induced diabetic wistar rats. Journal of Evidence-Based Integrative Medicine, 2019, 24, 2515690X19843832. DOI: 10.1177/2515690X19843832
[80] McLeay Y, Stannard S, Houltham S, Starck C. Dietary thiols in exercise: Oxidative stress defence, exercise performance, and adaptation. Journal of the International Society of Sports Nutrition, 2017, 14, 12. DOI: 10.1186/s12970-017-0168-9
[81] Phromnoi K, Yodkeeree S, Anuchapreeda S, Limtrakul P. Inhibition of MMP-3 activity and invasion of the MDA-MB-231 human invasive breast carcinoma cell line by bioflavonoids. Acta Pharmacologica Sinica, 2009, 30(8), 1169-1176. DOI: 10.1038/aps.2009.107
[82] Li J, Wu Y, Liu H. Expression and role of miR-338-3p in peripheral blood and placenta of patients with pregnancy-induced hypertension. Experimental and Therapeutic Medicine, 2020, 20(1), 418-426. DOI: 10.3892/etm.2020.8719
[83] Signorelli M, Ayoglu B, Johansson C, Lochmüller H, Straub V, Muntoni F, et al. Longitudinal serum biomarker screening identifies malate dehydrogenase 2 as candidate prognostic biomarker for Duchenne muscular dystrophy. Journal of Cachexia, Sarcopenia and Muscle, 2020, 11(2) 505-517. DOI: 10.1002/jcsm.12517
[84] Ryu JK, Yan Z, Montano M, Sozmen EG, Dixit K, Suryawanshi RK, et al. Fibrin drives thromboinflammation and neuropathology in COVID-19. Nature, 2024, 633(8031), 905-913. DOI: 10.1038/s41586-024-07873-4

[85] Davis AP, Wiegers TC, Johnson RJ, Sciaky D, Wiegers J, Mattingly CJ. Comparative toxicogenomics database (CTD): Update 2023. Nucleic Acids Research, 2023, 51(D1), D1257-D1262. DOI: 10.1093/nar/gkac833
[86] Pavel I, Irina L, Tatiana G, Denis P, Philipp K, Sergei K, et al. Comparison of the Illumina NextSeq 2000 and GeneMind Genolab M sequencing platforms for spatial transcriptomics. BMC Genomics, 2023, 24(1), 102. DOI: 10.1186/s12864-023-09192-w
[87] Xue Q, Fan H, Yao F, Cao XX, Liu MM, Sun J, et al. Transcriptomics and targeted metabolomics profilings for elucidation of pigmentation in *Lonicera japonica* flowers at different developmental stages. Industrial Crops and Products, 2020, 145, 111981. DOI: 10.1016/j.indcrop.2019.111981
[88] Sher H, Sharif H, Zaheer T, Khan SA, Ali A, Javed H, et al. Employing computational tools to design a multi-epitope vaccine targeting human immunodeficiency virus-1 (HIV-1). BMC Genomics, 2023, 24(1), 276. DOI: 10.1186/s12864-023-09330-4
[89] Bhattacharjee A, Hosen MR, Lamisa AB, Ahammad I, Chowdhury ZM, Jamal TB, et al. An integrated comparative genomics, subtractive proteomics and immunoinformatics framework for the rational design of a Pan-Salmonella multi-epitope vaccine. PLoS One, 2024, 19(7), e0292413. DOI: 10.1371/journal.pone.0292413
[90] Khan MT, Mahmud A, Hasan M, Azim KF, Begum MK, Rolin MH, et al. Proteome exploration of legionella pneumophila to identify novel therapeutics: A hierarchical subtractive genomics and reverse vaccinology approach. Microbiology Spectrum, 2022, 10(4), e0037322. DOI: 10.1128/spectrum.00373-22
[91] Schubach M, Maass T, Nazaretyan L, Röner S, Kircher M. CADD v1.7: Using protein language models, regulatory CNNs and other nucleotide-level scores to improve genome-wide variant predictions. Nucleic Acids Research, 2024, 52(D1), D1143-D1154. DOI: 10.1093/nar/gkad989
[92] Yang T, Liu X, Kumar SK, Jin F, Dai Y. Decoding DNA methylation in epigenetics of multiple myeloma. Blood Reviews, 2022, 51, 100872. DOI: 10.1016/j.blre.2021.100872
[93] Duan L, Hu J, Xiong X, Liu Y, Wang J. The role of DNA methylation in coronary artery disease. Gene, 2018, 646, 91-97. DOI: 10.1016/j.gene.2017.12.033
[94] Dong C, Huang S, Sun L, Yao J, Yan J, Yin X. DLGAP4 acts as an effective prognostic predictor for hepatocellular carcinoma and is closely related to tumour progression. Scientific Reports, 2022, 12(1), 19775. DOI: 10.1038/s41598-022-23837-y
[95] Abdipourbozorgbaghi M, Vancura A, Radpour R, Haefliger S. Circulating miRNA panels as a novel non-invasive diagnostic, prognostic, and potential predictive biomarkers in non-small cell lung cancer (NSCLC). British Journal of Cancer, 2024, 131(8), 1350-1362. DOI: 10.1038/s41416-024-02831-3
[96] Huang P, Zhang B, Zhao J, Li MD. Integrating the epigenome and transcriptome of hepatocellular carcinoma to identify systematic enhancer aberrations and establish an aberrant enhancer-related prognostic signature. Frontiers in Cell and Developmental Biology, 2022, 10, 827657. DOI: 10.3389/fcell.2022.827657
[97] Ashok Kumar P, Graziano SL, Danziger N, Pavlick D, Severson EA, Ramkissoon SH, et al. Genomic landscape of non-small-cell lung cancer with methylthioadenosine phosphorylase (MTAP) deficiency. Cancer Medicine, 2023, 12(2), 1157-1166. DOI: 10.1002/cam4.4971
[98] Ren Q, Li Q, Shao C, Zhang P, Hu Z, Li J, et al. Establishing a prognostic model based on immune-related genes and identification of BIRC5 as a potential biomarker for lung adenocarcinoma patients. BMC Cancer, 2023, 23(1), 897. DOI: 10.1186/s12885-023-11249-8
[99] Deng J, Yang Z, Ojima I, Samaras D, Wang F. Artificial intelligence in drug discovery: Applications and techniques. Briefings in Bioinformatics, 2022, 23(1), bbab430. DOI: 10.1093/bib/bbab430
[100] Martorell-Marugán J, Chierici M, Bandres-Ciga S, Jurman G, Carmona-Sáez P. Machine learning applications in the study of Parkinson's disease: A systematic review. Current Bioinformatics, 2023, 18(7), 576-586. DOI: 10.2174/1574893618666230406085947
[101] Javed R, Abbas T, Khan AH, Daud A, Bukhari A, Alharbey R. Deep learning for lungs cancer detection: A review. Artificial Intelligence Review, 2024, 57, 197. DOI: 10.1007/s10462-024-10807-1
[102] Ezugwu JA, Okoro UC, Ezeokonkwo MA, Kurma SH, Mithun R, David IU, et al. Design, synthesis, molecular docking, molecular dynamics and *in vivo* antimalarial activity of new dipeptide-sulfonamides. ChemistrySelect, 2022, 7(5), e202103908. DOI:10.1002/slct.202103908