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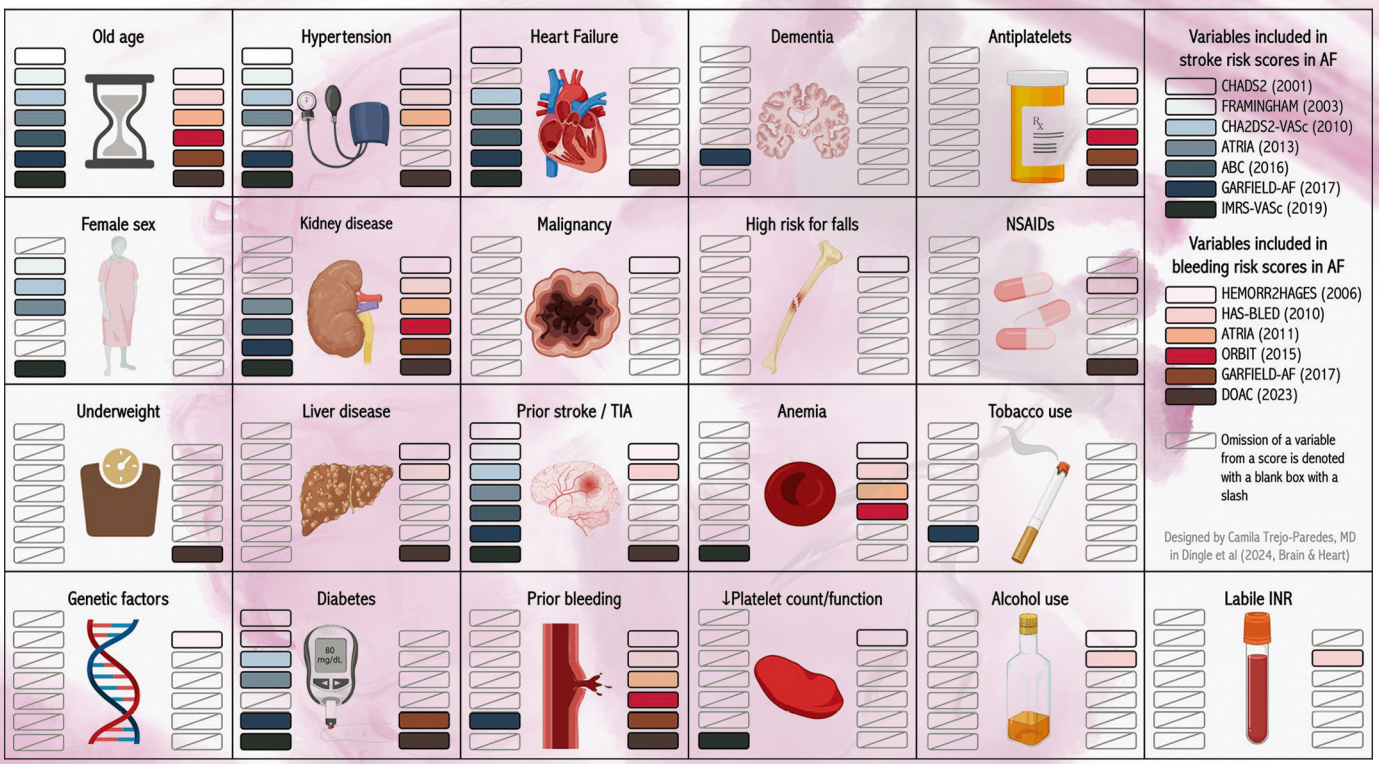

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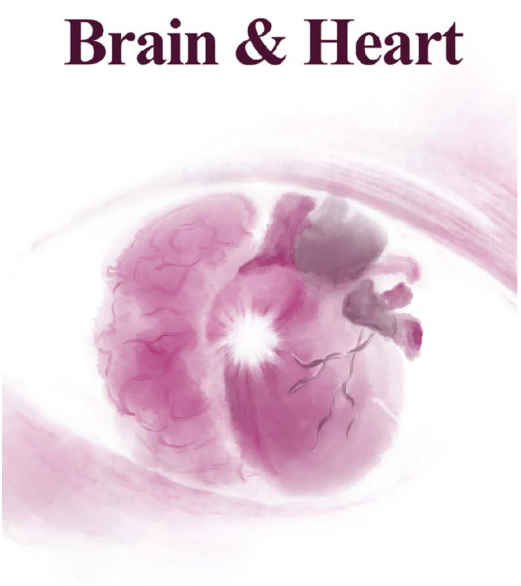

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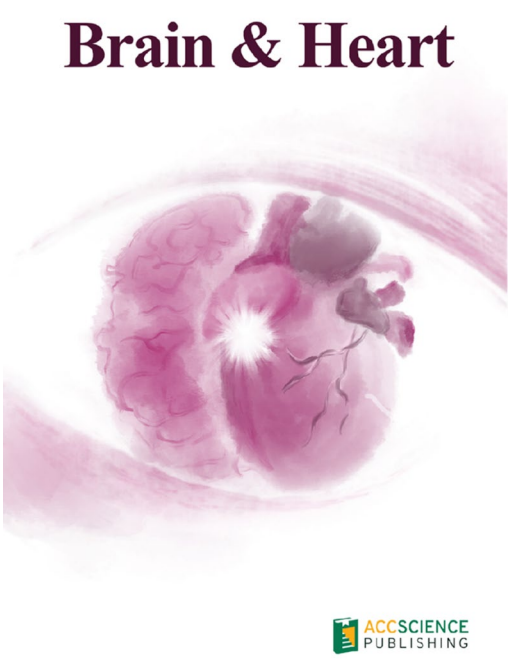

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REVIEW ARTICLE

Cerebral ischemia biomarkers: Their roles in early diagnosis and prognosis with potential clinical applications

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Abstract

Cerebral ischemia, caused by a disruption in blood supply to the brain, remains a significant cause of morbidity and mortality worldwide. Identifying reliable biomarkers for the early diagnosis and prognosis of cerebral ischemia is crucial for timely intervention and improved patient outcomes. This review aims to provide a comprehensive overview of the current knowledge on cerebral ischemia biomarkers, focusing on their potential role in early detection and prediction of clinical outcomes. Specifically, we discuss the current advances in the field of cerebral ischemia biomarkers, which serve as essential tools for early diagnosis and monitoring of ischemic stroke. We examine several promising biomarkers, including omics data, genetic (*FOXF2* and *ATP5H*), physiological and neuroinflammatory biomarkers, neuroimaging markers, blood-based biomarkers (proteins, microRNAs, and metabolites), and newer modalities such as exosomes, microvesicles, and cell-free deoxyribonucleic acid (cfDNA). In addition, we highlight the challenges and future directions in translating these biomarkers into clinical practice. Standardization and reproducibility, methodological limitations, and cost and accessibility are critical challenges in the translation of biomarkers into clinical practice. Addressing these challenges requires multi-stakeholder collaborations and coordinated efforts to establish standardized protocols, improve analytical methods, and develop cost-effective biomarker assays. The use of point-of-care testing devices or miniaturized lab-on-a-chip technologies can reduce costs and improve accessibility, particularly in low-resource settings. Furthermore, collaborations between academia, industry, and regulatory agencies can facilitate the translation of biomarkers by addressing regulatory and reimbursement hurdles that affect the affordability and availability of these tests.

Keywords: Cerebral ischemia; Biomarkers; Neuroimaging; Blood-based markers; Early diagnosis; Prognosis; Monitoring

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1. Introduction

Cerebral ischemia is a complex, heterogeneous condition characterized by insufficient blood supply to the brain. This lack of blood flow, primarily caused by reduced cerebral blood flow or occlusion of cerebral arteries, results in an inadequate supply of oxygen and nutrients to brain tissue, triggering a cascade of cellular events that ultimately lead to brain damage.[1] The pathophysiology of cerebral ischemia involves a complex interplay

of mechanisms that lead to neuronal injury, cell death, and, ultimately, neurological deficits.[1] Early intervention to restore blood flow and prevent further ischemic damage is critical for improving outcomes in patients with cerebral ischemia.

Despite advances in acute stroke management, the timely diagnosis and prognosis of cerebral ischemia depend largely on clinical assessment, radiological imaging, and neurological examinations, which may have limited sensitivity and specificity.[1] Therefore, the search for reliable biomarkers that facilitate early detection, risk stratification, and therapeutic monitoring remains a priority.

This review provides a comprehensive overview of the current state of knowledge on cerebral ischemia biomarkers, focusing on their potential role in early detection and prediction of clinical outcomes. It addresses various potential biomarkers for the diagnosis of cerebral ischemia, the methods of detection and monitoring, and the translational challenges of these biomarkers in this complex and clinically important field. In addition, the review discusses several promising biomarkers at different structural levels, such as neuroimaging biomarkers, omics data biomarkers, protein biomarkers, genetic biomarkers, physiological biomarkers, neuroinflammatory biomarkers, and blood-based biomarkers (Figure 1).

2. Neuroimaging biomarkers in cerebral ischemia

Neuroimaging plays a key role in detecting, characterizing, and monitoring the progression of ischemic brain injury.

2.1. Computed tomography (CT) and magnetic resonance imaging (MRI)

CT and MRI are two commonly used neuroimaging techniques that provide detailed images of the ischemic brain. CT uses X-ray technology to create cross-sectional images of the brain, whereas MRI uses powerful magnets and radio waves to generate high-resolution images.[2,3]

CT scans are often used in emergency settings to quickly assess patients with head trauma or suspected stroke. CT is widely available and useful for early detection of ischemic changes. Non-contrast CT is commonly employed to detect hyperacute ischemic lesions by visualizing neuronal hypodensity or loss of differentiation between gray and white matter.[2] CT angiography and CT perfusion provide additional information on vascular occlusion and tissue viability, respectively.[4]

MRI, on the other hand, provides more detailed images of the brain compared to CT scans. It can detect subtle changes in brain anatomy and is particularly useful in diagnosing diseases such as multiple sclerosis, brain tumors, and neurodegenerative diseases such as Alzheimer's disease (AD). MRI is also used to examine the blood vessels in and around the brain, aiding in the diagnosis and management of conditions such as stroke and arteriovenous malformations.[3]

2.1.1. Diffusion-weighted imaging (DWI)

DWI is an important neuroimaging technique that measures the movement of water molecules in the brain, providing information about the integrity of the white matter and aiding in the diagnosis of acute stroke. DWI can detect regions of restricted diffusion that indicates ischemia or tissue damage.[5]

As an MRI technique, DWI provides crucial information about ischemic lesions. The restricted diffusion observed in DWI images helps detect early ischemic damage, facilitating therapeutic decisions and predicting treatment outcomes.[6] DWI detects ischemic damage within minutes and remains the most sensitive non-contrast MRI technique. Restricted water diffusion identifies affected tissue (hyperintense signal) due to cytotoxic edema and enables early detection and differential diagnosis of acute stroke.[3]

2.1.2. Perfusion imaging and cerebral blood flow measurement

Perfusion imaging and cerebral blood flow measurement techniques provide information about the blood supply to the brain. These techniques are used to evaluate patients with suspected ischemic stroke. They can help to determine the extent of tissue damage and identify areas at risk of further damage. Perfusion imaging can also assist in treatment decisions, such as administering thrombolytic therapy for acute stroke.[6] Perfusion-weighted imaging (PWI) assesses cerebral perfusion by detecting changes in regional cerebral blood flow. The discrepancy between the affected region on DWI and the hypoperfused region on PWI can be used to identify potential candidates for reperfusion therapies.[4] PWI helps assess the ischemic penumbra, which indicates potentially salvageable tissue. Cerebral blood flow, cerebral blood volume, and mean transit time can be quantified to identify areas of reduced perfusion.[6] In combination with DWI, PWI helps characterize the ischemic core and penumbra.

2.2. Magnetic resonance spectroscopy (MRS)

MRS provides insights into the biochemical changes associated with ischemia. For example, decreased N-acetylaspartate and increased lactate levels correlate with ischemic injury and neuronal dysfunction.[7] MRS can assess response to treatment and long-term outcomes.

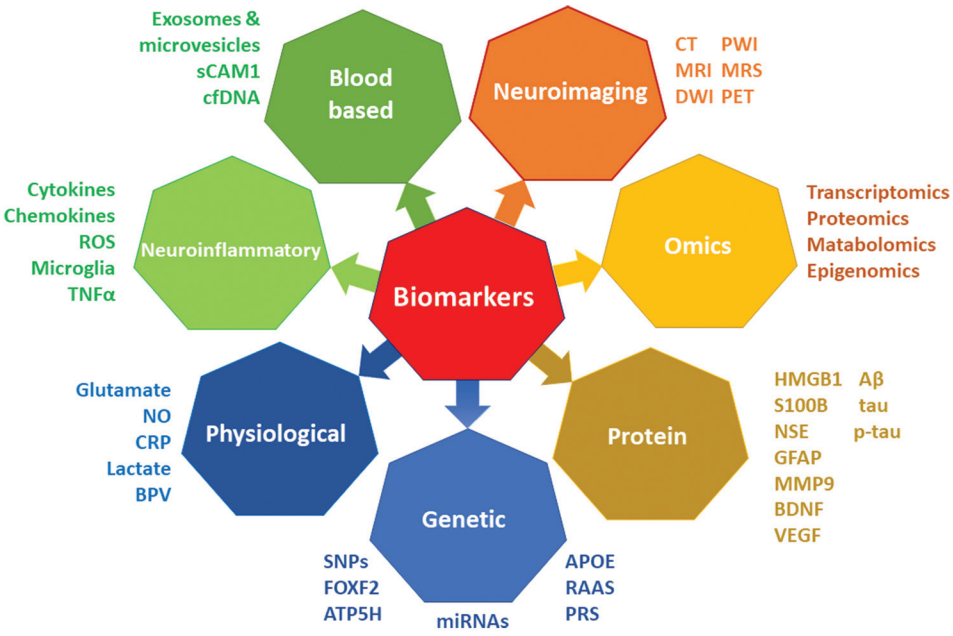

Figure 1. Cerebral ischemia biomarkers presented at different structural levels
Abbreviations: APOE: Apolipoprotein E; ATP5H: ATP synthase subunit; βA: beta-amyloid; BDNF: Brain-derived neurotrophic factor; BPV: Blood pressure variability; cfDNA: Cell-free deoxyribonucleic acid; CRP: C-reactive protein; CT: Computed Tomography; DWI: Diffusion-weighted imaging; FOXF2: Forkhead box F2; HMGB1: High mobility group box 1 protein; GFAP: Glial fibrillary acidic protein; MMP-9: Matrix metalloproteinases 9; MRI: Magnetic resonance imaging; MRS: Magnetic resonance spectroscopy; NO: Nitric oxide; NSE: Neuron-specific enolase; PET: Positron emission tomography; PRS: Polygenic risk score; PWI: Perfusion-weighted imaging; RAAS: Renin-angiotensin-aldosterone system; ROS: Reactive oxygen species; S100B: S100 calcium-binding protein B; sCAMs: Soluble adhesion molecules; SNPs: Single-nucleotide polymorphisms; TNF: Tumor necrosis factor; VEGF: Vascular endothelial growth factor.

2.3. Positron emission tomography (PET)

PET imaging is a functional neuroimaging technique that measures the metabolism of different substances in the brain. It uses radioactive tracers to detect changes in glucose metabolism, oxygen consumption, or neurotransmitter activity.[3] Glucose is the primary energy source for the brain, and an adequate glucose supply is essential for normal brain function. During cerebral ischemia, the reduced blood flow and oxygen supply lead to a decrease in glucose uptake and utilization by brain cells, resulting in a state of energy depletion known as cerebral metabolic distress. Monitoring glucose levels in brain tissue or blood may provide valuable information about the severity and progression of cerebral ischemia. Studies have shown that changes in glucose metabolism measured using PET or blood glucose levels serve as biomarkers of cerebral ischemia and can help assess the effectiveness of interventions to restore normal brain function.[3] PET scans are particularly useful in the diagnosis and management of conditions such as cerebral ischemia.

PET also allows the assessment of the ischemic penumbra and the detection of secondary injury cascades.[7] It can assess response to treatment and provide prognostic information.

Neuroimaging biomarkers provide valuable information about the structure, function, and metabolism of cerebral ischemia. These techniques aid in the diagnosis, monitoring, and treatment of cerebral ischemia and improve patient outcomes and quality of life. Continued advances in neuroimaging technology are expected to further enhance our understanding of cerebral ischemia and improve patient care in the future.

Although neuroimaging biomarkers have made significant contributions to the management of cerebral ischemia, challenges remain. These challenges include the limited availability of advanced imaging techniques, discrepancies in standardization between institutions, and the need for further validation of newer biomarkers.

3. Application of omics data as biomarkers in cerebral ischemia

Omics data, encompassing genomics, transcriptomics, proteomics, metabolomics, and epigenomics, have emerged as valuable tools for understanding the

pathophysiology of cerebral ischemia and identifying potential biomarkers for early detection and prognosis. Genomics has revolutionized our understanding of the genetic component of cerebral ischemia and has enabled the identification of several genetic variants associated with an increased risk of stroke. One notable example is the locus 9p21, which is associated with large vessel ischemic stroke and other cardiovascular diseases.[8] In addition, the identification of single nucleotide polymorphisms (SNPs) in candidate genes involved in endothelial dysfunction, platelet aggregation, and lipid metabolism has contributed to the discovery of new therapeutic targets.[9]

3.1. Transcriptomics biomarkers in cerebral ischemia

Transcriptomics enables the study of gene expression patterns in cerebral ischemia and provides insights into the molecular mechanisms involved in ischemic injury. For instance, microarray analyses have revealed differentially expressed genes involved in inflammation, neuronal apoptosis, and oxidative stress following cerebral ischemia. Researchers have identified potential biomarkers such as pituitary adenylate cyclase-activating polypeptide and S100 calcium-binding protein B (S100B) as early indicators of ischemic brain injury.[10]

A potential biomarker for cerebral ischemia is hypoxia-inducible factor 1 (HIF1). HIF1 is a transcription factor that plays a crucial role in the cellular response to low oxygen levels—hypoxia. During cerebral ischemia, the brain is exposed to hypoxia, leading to the activation of HIF1. Studies have shown that serum HIF-1α is closely correlated with hemorrhagic severity and poor outcomes and may serve as a potential prognostic biomarker for this condition.[11] In addition, HIF1 is involved in the regulation of genes related to cell survival, angiogenesis, and metabolism,[11] all of which are important processes in the pathophysiology of cerebral ischemia.

In addition, transcriptomic profiling has facilitated the discovery of genes associated with post-stroke recovery, offering potential targets for neurorestorative therapies.[8]

3.2. Proteomic biomarkers in cerebral ischemia

Proteomic biomarkers are molecules measurable in various body fluids or tissues that have shown promise in elucidating the biological processes involved in cerebral ischemia. A frequently used approach for discovering proteomic biomarkers is mass spectrometry. This technique identifies and quantifies proteins in a sample based on their mass-to-charge ratio. By comparing protein profiles between healthy individuals and those with stroke, researchers can identify potential biomarkers for further validation and clinical use.

A comprehensive proteomic analysis of plasma samples from ischemic stroke patients and healthy controls identified nine significantly dysregulated proteins in the ischemic stroke group, including vasoactive intestinal peptide, complement C3, and haptoglobin.[12] These proteins are implicated in biological processes related to inflammation, oxidation, and coagulation, highlighting their potential as blood-based biomarkers of cerebral ischemia.

Proteomics aims to identify and quantify protein expression patterns, providing valuable insights into the functional consequences of changes in gene expression. Proteomic studies have identified numerous differentially expressed proteins associated with cerebral ischemia, including matrix metalloproteinases (MMPs), heat shock proteins, and neuroinflammatory markers.[13] For example, elevated levels of MMP-9 are associated with blood-brain barrier (BBB) dysfunction and an increased risk of hemorrhagic transformation in ischemic stroke patients.[14] Proteomic analysis has also revealed the potential of plasma biomarkers such as copeptin and miR-134 as predictors of stroke severity and patient outcomes.[15]

Proteomic biomarkers have shown great potential for improving the detection, diagnosis, and monitoring of cerebral ischemia. Several proteomic studies have identified dysregulated proteins associated with inflammation, oxidative stress, neuronal cell death, and other biological processes involved in cerebral ischemia.[16] These biomarkers provide valuable insights into the pathophysiology of cerebral ischemia, aid early diagnosis, and may guide targeted therapeutic approaches. However, further research is needed to validate these proteomic biomarkers and translate them into routine clinical practice for treating patients with cerebral ischemia.

3.3. Metabolomic biomarkers in cerebral ischemia

Conventional diagnostic imaging techniques such as CT or MRI provide valuable information about the extent and location of ischemic lesions. However, they lack the ability to assess the underlying metabolic changes that occur during the ischemic event. Metabolomic profiling offers a promising approach to identifying and quantifying different metabolites that may serve as biomarkers for ischemic stroke.

Metabolomics is a comprehensive analysis of small molecule metabolites (e.g., amino acids, lipids, and sugars) present in biological samples. By analyzing changes in the metabolome, metabolomic studies aim to unravel the complex metabolic pathways altered during cerebral ischemia. This approach has the potential to identify new biomarkers that can aid in the early diagnosis, risk

stratification, treatment monitoring, and prognosis of patients with ischemic stroke.

Metabolomics analyzes the dynamic changes of small molecules involved in cellular processes. Metabolomic profiling in cerebral ischemia has revealed altered concentrations of various metabolites, including amino acids, lipids, and energy-related compounds. Studies have identified metabolic signatures associated with ischemic stroke and its subtypes, suggesting their potential use as diagnostic markers.[17,18] For example, decreased glycerophosphocholine levels and increased 5-aminolevulinic acid levels have been suggested as markers of ischemic brain injury.[19] In addition, metabolomic studies have shed light on the effects of neuroprotective agents and provided insights into therapeutic targets in cerebral ischemia.[20]

Several metabolomic biomarkers associated with cerebral ischemia have been identified.[21] Plasma samples from patients with ischemic stroke were analyzed by liquid chromatography-mass spectrometry, identifying 10 potential metabolic biomarkers. The results showed that decreased levels of α-ketoglutarate, glutamate, and phosphocholine and increased levels of phenylalanine, tyrosine, and diacetylspermine were significantly associated with cerebral ischemia. It has been confirmed that there are changes in metabolites related to energy metabolism (e.g., lactate and alanine), neurotransmission (e.g., glutamate and γ-aminobutyric acid), and lipid metabolism (e.g., glycerolipids and choline metabolites) in patients with cerebral ischemia.[21]

Oxidative stress plays a crucial role in the pathophysiology of cerebral ischemia. The metabolic profile of urine from patients with ischemic stroke has been studied, revealing alterations in antioxidant metabolites, including glutathione and related metabolites.[22] These changes were attributed to a disturbance in the regulation of oxidative stress in the ischemic brain. Furthermore, plasma levels of sphingomyelins and phosphatidylcholines were found to be significantly reduced in patients with ischemic stroke, suggesting disturbances in lipid metabolism.[23]

Metabolomic studies have also demonstrated the potential of cerebrospinal fluid (CSF) as a valuable source for identifying biomarkers of ischemic stroke. In one study, CSF metabolites were analyzed using nuclear MRS, and several metabolites, including lactate, succinate, acetate, and glutamine, were identified to be significantly altered in patients with ischemic stroke compared to control subjects.[24] These metabolites are involved in energy metabolism, mitochondrial dysfunction, and neuroinflammation, highlighting their potential as biomarkers for ischemic stroke.

Metabolomic profiling has shown great promise in identifying biomarkers that can aid in the diagnosis and prognosis of cerebral ischemia. The identified metabolites shed light on the underlying metabolic dysregulations that occur during ischemic stroke, highlighting different metabolic pathways involved, such as energy metabolism, oxidative stress, neurotransmission, and lipid metabolism. However, further validation and standardization of these metabolomic biomarkers are necessary to ensure their clinical utility.

In addition to diagnosis and prognosis, metabolomic profiling holds potential for individualized treatment strategies. By characterizing the metabolic status of patients with ischemic stroke, clinicians may be able to tailor interventions that target specific pathways or molecules affected by cerebral ischemia. This personalized approach could potentially improve patient outcomes and reduce long-term disability.

Despite the growing body of evidence supporting the role of metabolomic biomarkers in cerebral ischemia, several challenges need to be addressed. Standardization of protocols for sample collection, storage, and analysis is crucial to ensure reproducibility and comparability between different studies. Furthermore, large-scale multicenter studies involving diverse populations are required to establish robust reference ranges and diagnostic thresholds.

3.4. Epigenomics biomarkers in cerebral ischemia

Epigenomics investigates changes in gene expression caused by modifications to DNA and histones. Epigenetic changes in cerebral ischemia have been associated with both acute and chronic outcomes. DNA methylation patterns have been identified as potential biomarkers for predicting stroke risk, recurrence, and prognosis.[25] In addition, histone modifications, such as acetylation and methylation, have been linked to neuroplasticity and recovery after stroke.[26] It is noteworthy that advances in epigenomics, such as next-generation sequencing and chromatin immunoprecipitation, have paved the way for the identification of novel epigenetic biomarkers in cerebral ischemia.[27]

The integration of omic data as biomarkers in cerebral ischemia has the potential to revolutionize not only early diagnosis and prognosis but also the development of personalized therapeutic approaches. Genomics, transcriptomics, proteomics, metabolomics, and epigenomics together provide comprehensive insights into the complex molecular pathways underlying cerebral ischemia. Future research should focus on the validation and translation of omic biomarkers into clinical practice

to enable tailored interventions and improve outcomes for patients with cerebral ischemia.

4. Protein biomarkers in cerebral ischemia

Protein biomarkers are specific proteins measurable in various body fluids or tissues that can indicate the presence or severity of a disease. Several protein biomarkers have been identified for cerebral ischemia, and their levels may provide insights into the extent of brain damage and predict disease outcomes. Cerebral ischemia triggers a complex series of molecular events, including neuroinflammation, oxidative stress, and excitotoxicity. Numerous proteins contribute to these events and serve as potential biomarkers for cerebral ischemia. For instance, the high mobility group box 1 protein, which is released by damaged neurons and endothelial cells, has been identified as a mediator of inflammation and a potential diagnostic marker for cerebral ischemia.[12] Similarly, as mentioned above, S100B, a calcium-binding protein released by astrocytes and damaged neurons, has emerged as a potential biomarker for detecting ischemic brain injury and predicting prognosis. During cerebral ischemia, astrocytes are activated and release S100B into the bloodstream. Elevated levels of S100B have been found in the CSF and blood of patients with cerebral ischemia, correlating with the severity of the injury.[28,29] S100B has been shown to be a reliable biomarker for predicting neurological outcomes and determining the effectiveness of treatment interventions.

Another promising protein biomarker for the diagnosis and prognosis of cerebral ischemia is neuron-specific enolase (NSE), a glycolytic enzyme predominantly found in neurons. When cerebral ischemia occurs, NSE is released into the bloodstream due to neuronal damage. Elevated levels of NSE have been detected in CSF and blood samples from patients with ischemic stroke, indicating its potential as a biomarker for brain injury. NSE levels have been found to correlate with the extent of neurological deficit and clinical outcome.[30-32]

Other protein biomarkers have also been investigated for cerebral ischemia, including glial fibrillary acidic protein (GFAP), which is predominantly expressed in astrocytes and is elevated in CSF and blood samples from patients with ischemic stroke. GFAP levels have been associated with the presence and severity of brain damage.[29,33] Another previously mentioned protein biomarker, MMP-9, is an enzyme involved in the degradation of the extracellular matrix. Elevated levels of MMP-9 have been found in the CSF and blood of patients with cerebral ischemia, and this biomarker has been associated with disruption of the BBB and an increased risk of hemorrhagic transformation.[34]

The identification of protein biomarkers for cerebral ischemia is important not only for diagnosis and prognosis but also for the development of new therapeutic interventions. By understanding the molecular pathways involved in ischemic brain injury, potential targets for therapeutic interventions can be identified. Consequently, protein biomarkers can also guide therapeutic interventions in cerebral ischemia. For example, brain-derived neurotrophic factor, a neuroprotective protein, has been shown to be a potential therapeutic target in cerebral ischemia.[35] In addition, vascular endothelial growth factor, which is involved in angiogenesis and neuroprotection, has been investigated as a potential therapeutic target for cerebral ischemia.[34]

In summary, protein biomarkers play an important role in the detection, diagnosis, and management of cerebral ischemia. Further research is needed to validate these biomarkers and explore their potential therapeutic implications. Although protein biomarkers are promising in the field of cerebral ischemia, several challenges remain, including the standardization of measurement methods, validation in different populations, and integration into clinical practice guidelines. In addition, further research is necessary to identify new protein biomarkers and determine their role in the different stages of cerebral ischemia.

4.1. β-amyloid, tau, and phospho-tau as biomarkers in cerebral ischemia

An important aspect of the pathophysiology of stroke is the disruption of protein homeostasis and the subsequent accumulation of misfolded proteins. In recent years, several studies have investigated the potential diagnostic and prognostic value of β-amyloid (Aβ), tau, and phospho-tau biomarkers in cerebral ischemia.[36]

Aβ is a known pathological hallmark of AD and is implicated in neurodegenerative processes. However, emerging evidence suggests that Aβ may also have a role in cerebral ischemia. A study examining the plasma of patients with acute ischemic stroke found significantly higher Aβ levels compared to healthy controls.[37] These results support the notion that Aβ may play a role in the acute phase of cerebral ischemia. Furthermore, Aβ deposition has been observed in post-stroke brains, possibly contributing to long-term cognitive impairment.[38] These studies highlight the importance of Aβ as a potential biomarker and therapeutic target in cerebral ischemia.

The tau protein has been extensively studied in the context of neurodegenerative diseases, particularly AD.[39] Tau undergoes phosphorylation, leading to the formation of phospho-tau, which is implicated in the formation of neurofibrillary tangles. Studies investigating tau and phospho-tau in cerebral ischemia are limited; however,

some intriguing findings have been reported.[40] Tau protein levels have been shown to be increased in the CSF of patients after severe traumatic brain injury, which is also associated with vascular insults. This observation suggests that tau may serve as a potential biomarker for vascular brain injury, including cerebral ischemia.

Further evidence for the role of tau in cerebral ischemia is provided by elevated tau levels in post-stroke patients, with higher levels being associated with poorer cognitive performance.[41] In addition, increased levels of tau phosphorylation have been reported in ischemic brain tissue, indicating the activation of tau pathology in this condition.[42]

Emerging evidence suggests that Aβ, tau, and phospho-tau may serve as valuable biomarkers in cerebral ischemia. However, further research is essential to fully understand their role in disease progression, diagnosis, and prognosis. The interplay between these protein abnormalities and other pathological features of cerebral ischemia remains an active area of research. Future studies should focus on elucidating the molecular mechanisms linking Aβ, tau, and phospho-tau to cerebral ischemia, which will lead to the development of novel therapeutic interventions for stroke patients.

5. Genetic biomarkers in cerebral ischemia

Genetic biomarkers have emerged as promising tools for predicting, diagnosing, and monitoring cerebral ischemia.

5.1. Single-nucleotide polymorphisms as biomarkers in cerebral ischemia

Several studies have implicated SNPs in genes related to vascular function, inflammation, and coagulation in the pathogenesis of cerebral ischemia. For example, the rs1801133 SNP in the methylenetetrahydrofolate reductase (*MTHFR*) gene has been associated with an increased risk of cerebral ischemia.[8] Similarly, the rs1800795 SNP in the interleukin-6 (*IL6*) gene has been linked to both ischemic stroke susceptibility and outcome.[43] Certain genetic variants, such as factor V Leiden, *MTHFR* C677T, and angiotensin-converting enzyme (ACE) insertion/deletion polymorphisms, have also been associated with increased susceptibility to cerebral ischemia.[44]

5.2. *FOXF2* and *ATP5H* as biomarkers in cerebral ischemia

In recent years, the transcription factor gene *FOXF2* and the ATP synthase subunit gene *ATP5H* have emerged as promising biomarkers for cerebral ischemia, providing valuable insights into the pathophysiological processes and potential therapeutic targets. Further research to validate their clinical utility and explore their mechanisms of action is essential for their successful integration into routine clinical practice. *FOXF2* and *ATP5H* hold promise for improving the diagnosis, prognosis, and management of patients with cerebral ischemia and may contribute to better treatment outcomes in the future.

FOXF2 (forkhead box F2) is a transcription factor that plays a crucial role in neuronal development and vascular homeostasis. Emerging evidence suggests that it is involved in multiple aspects of cerebral ischemia, including angiogenesis, neuroprotection, and inflammation regulation. *FOXF2* expression is upregulated in cerebral ischemia and has been associated with improved clinical outcomes in several studies. For instance, a recent study found that increased *FOXF2* expression in animal models of cerebral ischemia correlated significantly with reduced infarct size and improved neurological function.[45] Furthermore, increased expression of *FOXF2* was associated with enhanced angiogenesis and reduced brain edema. These results suggest that *FOXF2* may serve as a potential biomarker for predicting the severity and prognosis of cerebral ischemia.

ATP5H is a subunit of ATP synthase, a key enzyme responsible for ATP production in the mitochondria. Due to its involvement in cellular energy metabolism and cerebral homeostasis, its encoding gene has recently emerged as a potential biomarker for cerebral ischemia. Several studies have demonstrated altered *ATP5H* expression in ischemic brain tissue. For example, *ATP5H* has been found to be downregulated in cerebral ischemia models, indicating impaired mitochondrial function.[10] This dysregulation of *ATP5H* could contribute to the energy depletion observed in ischemic brain tissue. The potential diagnostic utility of *ATP5H* as a biomarker in cerebral ischemia requires further investigation.

The diagnostic and prognostic significance of *FOXF2* and *ATP5H* in cerebral ischemia is gaining increasing attention. The identification of these biomarkers enables early detection and risk stratification in patients at high risk of stroke. In addition, monitoring the expression of these biomarkers during treatment can provide valuable insights into the response to treatment and help to adjust therapeutic strategies accordingly. Several studies have highlighted the potential of *FOXF2* and *ATP5H* as therapeutic targets in cerebral ischemia. For instance, pharmacological interventions targeting *FOXF2* have shown neuroprotective effects in animal models.[46] Similarly, strategies aimed at restoring *ATP5H* expression or function may have therapeutic potential to alleviate cerebral ischemic injury.

Although *FOXF2* and *ATP5H* show promising potential as biomarkers of cerebral ischemia, several

challenges remain to be addressed. Validation studies in larger patient cohorts are needed to confirm the diagnostic accuracy and prognostic value of these biomarkers. In addition, standardization of detection methods and the establishment of reference ranges are essential for widespread clinical application. Future research should focus on elucidating the underlying mechanisms of FOXF2 and ATP5H in cerebral ischemia to develop targeted therapeutic strategies.

5.3. MicroRNAs as biomarkers in cerebral ischemia

MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression post-transcriptionally. Since miRNAs are stable in blood and readily measurable, they have proven to be attractive biomarkers for disease detection and monitoring. Dysregulation of miRNAs has been observed in cerebral ischemia and is thought to contribute to the pathological processes in stroke. For instance, miR-124 and miR-21, known for their roles in regulating apoptosis and oxidative stress, have been shown to modulate neuronal survival, neuroinflammation, and BBB integrity in experimental models of ischemic stroke.[47] Moreover, circulating miRNAs such as miR-126, miR-134, and miR-223 have been investigated as potential diagnostic and prognostic biomarkers for cerebral ischemia.[48,49]

Another miRNA, miR-146a, is associated with the regulation of inflammation and immune responses. Elevated levels of miR-146a have been observed in both animal models and stroke patients, suggesting its potential as a diagnostic and prognostic marker for cerebral ischemia.[50] Other miRNAs, such as miR-9, miR-124, miR-210, and miR-424, have been investigated for their potential as biomarkers. These miRNAs have been found to be dysregulated in animal models or in patients with cerebral ischemia, providing insights into the complex molecular mechanisms underlying ischemic injury.[51]

The identification of miRNA biomarkers promises to improve the diagnosis and management of cerebral ischemia. MiRNAs can be detected in various body fluids, including blood, plasma, serum, and CSF, making them attractive candidates for non-invasive diagnostics. Several studies have demonstrated the diagnostic and prognostic value of miRNA biomarkers in different subtypes of ischemic stroke. For instance, miR-150 and miR-21 have shown the potential to differentiate cardioembolic stroke from other types of ischemic stroke.[52]

In addition, miRNAs have been explored as potential therapeutic targets for cerebral ischemia. By modulating the expression of specific miRNAs, researchers aim to attenuate ischemic injury and promote repair mechanisms. Methods such as miRNA mimics or inhibitors have shown promise in pre-clinical studies, and ongoing clinical trials are investigating their potential therapeutic applications.[53]

MiRNAs have great potential as biomarkers for cerebral ischemia due to their stability, tissue specificity, and involvement in important cellular signaling pathways. Numerous studies have identified specific miRNAs that exhibit dysregulated expression during cerebral ischemia, suggesting their potential as diagnostic and prognostic markers. In addition, miRNAs represent a new avenue for therapeutic interventions and provide hope for improved outcomes in patients with cerebral ischemia. Future research should focus on validating these miRNA biomarkers in larger cohorts and exploring their potential as therapeutic targets to accelerate clinical translation.

5.4. Apolipoprotein E (*APOE*) gene as a biomarker in cerebral ischemia

The *APOE* gene is one of the most extensively studied genes in cerebral ischemia. The *APOE*-ε4 allele has been consistently associated with an increased risk of developing cerebral ischemia, particularly in younger individuals.[53] In addition, the *APOE*-ε4 allele has been associated with poorer outcomes and increased mortality in stroke patients.[54] Therapies targeting the APOE signaling pathway are being explored as potential treatment strategies for cerebral ischemia.

5.5. Genes in the renin-angiotensin-aldosterone system (RAAS) as biomarkers in cerebral ischemia

Genes involved in the RAAS play a crucial role in the regulation of blood pressure and fluid balance. Genetic variations in the *ACE* and angiotensinogen (*AGT*) genes have been associated with an increased risk of cerebral ischemia.[55] These variations affect the function and production of key molecules in the RAAS cascade, resulting in altered vascular tone and increased susceptibility to ischemic events.

5.6. Summary of genetic biomarkers in cerebral ischemia

In summary, genetic biomarkers provide unique opportunities for stroke risk stratification, personalized treatment, and prognosis prediction. Combining genetic information with traditional risk factors can improve the accuracy of stroke prediction models.[56] Genetic biomarkers may also help in selecting appropriate treatment strategies and identifying individuals who would benefit from preventive measures such as lifestyle modifications or pharmacological interventions.

Advances in genomic research have facilitated the development of polygenic risk scores (PRS) for cerebral

ischemia. The PRS utilizes multiple genetic variants associated with stroke to create a cumulative genetic risk score. Patients with a higher PRS are more likely to develop cerebral ischemia and may be eligible for more aggressive preventive interventions.[50]

Genetic biomarkers associated with cerebral ischemia have the potential to revolutionize stroke management by enabling personalized risk assessment, prevention, and treatment strategies. However, further research is required to validate these findings in larger and more diverse populations and facilitate their translation into routine clinical practice.

6. Physiological biomarkers in cerebral ischemia

Cerebral ischemia leads to various physiological changes. The identification of physiological biomarkers for cerebral ischemia can aid in the diagnosis, prognosis, and monitoring of the condition. Nevertheless, further research is needed to validate and refine the use of these biomarkers in clinical practice.

6.1. Glutamate as a biomarker in cerebral ischemia

Glutamate is an excitatory neurotransmitter in the brain that plays a crucial role in normal brain function. However, during cerebral ischemia, excessive release of glutamate can lead to excitotoxicity, causing neuronal damage. Several studies have investigated glutamate as a potential biomarker for cerebral ischemia. For example, glutamate levels were shown to be significantly higher in patients with acute ischemic stroke compared to control subjects.[57] In addition, higher glutamate levels were associated with larger infarct sizes and worse neurological outcomes.

6.2. Nitric oxide (NO) as a biomarker in cerebral ischemia

NO is a signaling molecule involved in various physiological processes, including the regulation of blood vessels. During cerebral ischemia, the production of NO is disturbed, contributing to the pathogenesis of ischemic brain damage.[58] Studies have shown that NO concentrations in CSF were significantly higher in patients with acute ischemic stroke compared to controls.[59] A positive correlation was also found between NO levels and the severity of the stroke.

6.3. C-reactive protein (CRP) as a biomarker in cerebral ischemia

CRP is an acute-phase protein produced by the liver in response to inflammation. There is increasing evidence that CRP can serve as a biomarker for cerebral ischemia. For instance, elevated CRP levels were found to be associated with poor functional outcomes and increased mortality in patients with acute ischemic stroke.[60] Similarly, higher CRP levels have been reported to be associated with an increased risk of recurrent ischemic stroke.[61]

6.4. Lactate as a biomarker in cerebral ischemia

Lactate is a metabolite produced during anaerobic metabolism, indicating a state of cellular hypoxia. During cerebral ischemia, lactate levels may increase as a result of impaired energy production. Studies have shown that lactate levels were significantly higher in patients with acute ischemic stroke compared to control subjects.[62] In addition, higher lactate levels were positively correlated with infarct volume and were associated with poorer neurological outcomes.

6.5. Abnormal blood pressure variability (BPV) as a biomarker in cerebral ischemia

Abnormal BPV has been identified as a potential marker for the severity and prognosis of cerebral ischemia. Elevated BPV is associated with an increased risk of stroke, while decreased variability indicates poor cerebral autoregulation.[63]

7. Neuroinflammatory biomarkers in cerebral ischemia

Cerebral ischemia leads to severe neuroinflammation, causing neuronal damage and functional impairment. The identification and understanding of neuroinflammatory biomarkers associated with cerebral ischemia are crucial for both diagnosis and therapeutic interventions.

Neuroinflammation is a complex process involving the activation of immune cells, such as microglia and astrocytes, in response to cerebral ischemia.[64] These activated cells release various pro-inflammatory mediators, including cytokines, chemokines, and reactive oxygen species (ROS), which contribute to the deleterious effects of cerebral ischemia in the brain.[65]

The use of neuroinflammatory biomarkers in clinical practice could enable early intervention and personalized treatment strategies, leading to improved outcomes for patients with cerebral ischemia.

7.1. Cytokines as biomarkers in cerebral ischemia

Cytokines play a crucial role in the initiation and propagation of neuroinflammation in cerebral ischemia. Tumor necrosis factor-alpha (TNF-α), IL-1 beta, and IL-6 are among the most important pro-inflammatory cytokines that play a role in cerebral ischemia.[66] Studies have demonstrated increased levels of these cytokines

in animal models of cerebral ischemia and patients with ischemic stroke.[66] Cytokines contribute to BBB disruption, neuronal death, and immune cell recruitment by activating downstream inflammatory signaling pathways.

7.2. Chemokines as biomarkers in cerebral ischemia

Chemokines are small chemotactic proteins that regulate the transport and activation of leukocytes in the brain. In cerebral ischemia, chemokines such as monocyte chemoattractant protein-1 and macrophage inflammatory protein-1 alpha play a crucial role in mediating the infiltration of immune cells into the ischemic region. Elevated levels of these chemokines have been detected in experimental models and stroke patients, suggesting that they may serve as biomarkers of cerebral ischemia severity and prognosis.[66]

7.3. ROS as biomarkers in cerebral ischemia

ROS production increases during cerebral ischemia, leading to oxidative stress and neuronal damage. Several studies have found increased levels of ROS and lipid peroxidation markers, such as malondialdehyde, in animal models and stroke patients.[58,67,68] These markers reflect the extent of oxidative damage caused by neuroinflammation and could be used as biomarkers to assess the severity of cerebral ischemia and monitor therapeutic interventions.

7.4. Microglial activation markers as biomarkers in cerebral ischemia

Microglia, the resident immune cells of the central nervous system, plays a crucial role in neuroinflammation during cerebral ischemia.[69] Activation of microglia is associated with the release of pro-inflammatory cytokines and chemokines and the phagocytosis of damaged neurons. Several microglia activation markers, including ionized calcium-binding adapter molecule 1, cluster of differentiation 11b, and triggering receptor expressed on myeloid cells 2, have been investigated as potential biomarkers of cerebral ischemia.[70,71] These markers provide information on the degree of microglial activation and help assess the progression of neuroinflammation in ischemic brain tissue.

7.5. Glial TNF signaling as a biomarker in cerebral ischemia

New evidence suggests that the glial TNF signaling pathway is involved in the pathophysiology of cerebral ischemia. Adenosine monophosphate-activated protein kinase (AMPK) regulates TNF-α production in glial cells after cerebral ischemia, leading to tissue damage. Targeting AMPK signaling pathways has shown neuroprotective effects in attenuating ischemia injury in animal models.[72] Understanding glial TNF signaling and its role as a potential therapeutic target may open new avenues for the treatment of cerebral ischemia.

8. Blood-based biomarkers in cerebral ischemia

Blood-based biomarkers refer to specific molecules or substances in the blood that can indicate the presence of a disease or physiological condition, such as ischemic stroke. These biomarkers can provide valuable information about an individual's health status, help in the early detection of stroke, and monitor progression and assess response to treatment. Blood-based biomarkers can be divided into several categories, including the proteomic and metabolomic biomarkers already mentioned, as well as exosomes and microvesicles, soluble adhesion molecules (sCAMs), and cfDNA.

8.1. Exosomes and microvesicles as biomarkers in cerebral ischemia

Exosomes and microvesicles, which are small membrane-bound extracellular vesicles, have attracted considerable attention due to their involvement in intercellular communication and signaling in various pathological conditions. These vesicles play an important role in intercellular communication, carrying specific cargoes such as proteins, lipids, RNAs, and miRNAs that can regulate target cell behavior and influence disease progression. The analysis of exosomes and microvesicles in blood samples has shown promise for the identification of disease-specific biomarkers.[73]

Exosomes and microvesicles are enriched with specific molecules, including miRNAs and proteins, which can be isolated and analyzed for biomarker discovery. These vesicles can provide information about the tissue of origin and the specific pathophysiological processes occurring in a particular disease. Analyzing exosomes and microvesicles in blood samples can provide valuable insights into disease mechanisms and potential therapeutic targets.[73] Exosomes and microvesicles, released by various cells into biofluids such as blood, CSF, and urine, have the potential to serve as minimally invasive biomarkers and are important players in the pathophysiology of cerebral ischemia.

During cerebral ischemia, exosomes and microvesicles play a crucial role in cellular communication. They act as carriers of signaling molecules and transfer bioactive molecules to recipient cells, influencing their gene expression and cellular functions. For instance, exosomes released by endothelial cells can modulate the permeability of the BBB and alter the microenvironment of the ischemic brain.[73]

Exosomes and microvesicles are involved in the initiation and propagation of neuroinflammation following cerebral ischemia. They mediate the intercellular transfer of pro-inflammatory cytokines, chemokines, and immune-related miRNAs, leading to the activation of neuroinflammatory signaling pathways.[15] Studies have shown that the cargo of exosomes derived from activated microglia or astrocytes can influence the polarization of microglia toward pro-inflammatory phenotypes or exacerbate neuroinflammation.[74]

Exosomes and microvesicles derived from different cell types, including neural stem cells, mesenchymal stem cells, and astrocytes, have shown neuroprotective properties in experimental models of cerebral ischemia.[75] They promote neurogenesis, angiogenesis, and synaptic remodeling, thereby improving neuronal survival and functional recovery. These vesicles carry neurotrophic factors, anti-apoptotic proteins, and miRNAs that modulate the neuroprotection and regeneration cascades.[15]

Recent advances have highlighted the potential of using exosomes and microvesicles as therapeutic tools in cerebral ischemia. These vesicles can serve as natural drug delivery systems due to their inherent biocompatibility and ability to cross the BBB. Various strategies, including genetic modification and pre-treatment of the parent cells, have been explored to enhance their therapeutic potential.[15] Exosomes loaded with specific cargo molecules, such as growth factors or anti-inflammatory agents, have shown promising results in pre-clinical studies.[74,75]

In summary, blood-based biomarkers, such as proteomic biomarkers, metabolomic biomarkers, exosomes, and microvesicles, have revolutionized disease diagnosis, prognosis, and treatment monitoring. These biomarkers provide non-invasive and easily accessible tools for disease detection and monitoring, enabling early intervention and better patient outcomes. However, further research and validation are needed to fully realize the potential of blood-based biomarkers in clinical practice.

8.2. sCAMs as biomarkers in cerebral ischemia

Recently, sCAMs have emerged as potential biomarkers of cerebral ischemia due to their involvement in the inflammatory response and disruption of the BBB.

Adhesion molecules play a crucial role in the recruitment of leukocytes, the interaction between leukocytes and endothelial cells, and the subsequent inflammatory cascade. Soluble forms of these adhesion molecules can be released from the cell surface by proteolytic cleavage or induced release. Elevated levels of sCAMs have been observed in various inflammatory and ischemic conditions, making them promising biomarkers for cerebral ischemia.[76]

Several studies have indicated the potential of sCAMs as diagnostic biomarkers for cerebral ischemia. For instance, vascular cell adhesion molecule-1 (VCAM-1) has been shown to be elevated in ischemic stroke patients compared to healthy controls, with elevated levels observed within 24 h of the onset of symptoms.[77] Intercellular adhesion molecule-1 (ICAM-1) has also been shown to be a promising diagnostic biomarker, with significantly higher levels in ischemic stroke patients compared to controls.[78] These studies support the potential of sCAMs, such as VCAM-1 and ICAM-1, as complementary diagnostic biomarkers for cerebral ischemia.

Prognostic biomarkers facilitate the prediction of the severity, progression, and outcome of cerebral ischemia. Several studies have investigated the correlation between sCAM levels and the clinical outcome of ischemic stroke patients. For instance, elevated levels of soluble P-selectin were associated with a higher risk of recurrent stroke.[79] Similarly, increased levels of soluble E-selectin were found to be associated with a poorer prognosis and an increased risk of neurological deterioration in patients with acute ischemic stroke.[80] These results suggest that sCAMs, such as soluble P-selectin and soluble E-selectin, may serve as prognostic biomarkers for cerebral ischemia.

Disruption of the BBB is a critical event in cerebral ischemia, leading to increased permeability and infiltration of inflammatory cells into the brain. Adhesion molecules play an important role in regulating the integrity of the BBB. Studies have shown that sCAMs, such as soluble ICAM-1, soluble VCAM-1, and soluble E-selectin, are associated with BBB disruption in cerebral ischemia. For instance, a significant increase in the concentrations of soluble ICAM-1 and soluble VCAM-1 was demonstrated in patients with ischemic stroke and impaired BBB compared to patients without BBB.[77] These results suggest that sCAMs may serve as biomarkers for BBB disruption in cerebral ischemia.

The use of sCAMs as biomarkers of cerebral ischemia holds great promise for early diagnosis, prognostic assessment, and monitoring of therapeutic interventions. Further research is needed to validate and optimize the utility of sCAMs as biomarkers, taking into account factors such as the timing of sample collection, sample storage conditions, and the influence of comorbidities.

8.3. cfDNA as a biomarker in cerebral ischemia

In recent years, interest in the potential use of cfDNA as a biomarker in cerebral ischemia has increased, as it offers several advantages over traditional diagnostic methods. CfDNA refers to circulating DNA fragments released into the bloodstream following cell death or

cellular stress. These fragments originate from a variety of sources, including neurons, glial cells, and blood vessels. An ischemic insult triggers cell death pathways that lead to the release of cfDNA into the bloodstream. Numerous studies have explored the potential of cfDNA as a biomarker for cerebral ischemia, as it is easily accessible and quantifiable.

The use of cfDNA as a biomarker offers the potential for early detection and prompt management of cerebral ischemia. For example, it has been shown that elevated cfDNA levels in stroke patients could be detected within the 1st h of symptom onset, allowing timely intervention and reducing the risk of long-term damage.[81] CfDNA released from neutrophils into plasma during stroke is a key player in the post-stroke inflammatory response and has recently been shown to trigger recurrent vascular events through an inflammasome-dependent mechanism leading to rupture of atherosclerotic plaques.[82,83] These examples represent initial approaches for a new, targeted approach to secondary stroke prevention.

Ischemic stroke can occur in various subtypes, including large artery atherosclerosis, cardioembolism, small vessel occlusion, and stroke of other etiologies. Accurate identification of stroke subtypes is crucial for the selection of appropriate treatment. Specific cfDNA fragments have been associated with different stroke subtypes, emphasizing the potential of cfDNA as a tool to differentiate stroke subtypes.[84]

Determining the severity of cerebral ischemia is crucial for optimizing treatment strategies. It has been shown that increased cfDNA levels are associated with larger ischemic lesion volumes and higher stroke severity, indicating their potential as a biomarker for assessing the extent and severity of cerebral ischemia.[85]

A prognostic assessment is essential for estimating the long-term outcomes of stroke patients. Several studies have shown a correlation between cfDNA levels and functional outcomes after cerebral ischemia. For instance, higher cfDNA levels have been associated with poor functional outcomes, highlighting the potential use of cfDNA as a prognostic marker in cerebral ischemia.[86]

Despite the promising potential of cfDNA as a biomarker in cerebral ischemia, several challenges need to be addressed before widespread clinical application. These challenges include the standardization of cfDNA extraction and quantification methods, the establishment of reference ranges for cfDNA levels in healthy individuals and stroke patients, and the assessment of the influence of confounding factors such as age, comorbidities, and medication use on cfDNA levels.

Future research should focus on large-scale prospective studies to validate the diagnostic and prognostic value of cfDNA in different patient populations and to explore the specific cfDNA fragments associated with the pathogenesis of cerebral ischemia. Furthermore, investigating the dynamic changes in cfDNA levels during the course of cerebral ischemia could provide valuable insights into disease progression and response to treatment.

9. Challenges in biomarker translation

Biomarkers play a vital role in the diagnosis, prognosis, and treatment of cerebral ischemia. They provide objective and measurable indicators of biological processes, thus enabling early detection, monitoring, and personalized treatment approaches. However, the translation of biomarkers from the laboratory to clinical practice is associated with various challenges. This review discusses three major challenges in biomarker translation: standardization and reproducibility, methodological limitations, and cost and accessibility (Figure 2).

9.1. Standardization and reproducibility

Standardization and reproducibility are major challenges in the translation of biomarkers. Biomarker discovery and validation studies often use different platforms, sample types, and statistical methods across different research laboratories. This lack of standardization makes it difficult to compare and validate biomarker results between different studies. In addition, the lack of reproducibility of biomarker studies has raised concerns about the reliability and validity of these biomarkers. Many biomarker candidates fail to replicate their original results in independent validation studies, leading to false positive or false negative results. This inconsistency

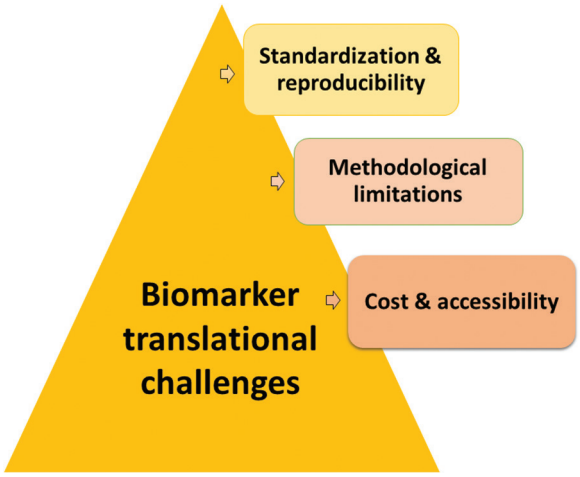

Figure 2. Major challenges in biomarker translation

hampers the progress of biomarker research and limits its clinical utility.

To overcome these challenges, standardized protocols and guidelines for biomarker discovery, validation, and implementation are needed. Standardization efforts should also extend to sample handling, processing, and storage to minimize experimental variation. In addition, rigorous validation studies with large and diverse cohorts should be conducted to ensure the reproducibility and generalisability of biomarker results. Joint efforts between research institutions and industry partners can promote consensus on standardization and improve the translation of biomarkers into clinical practice.

9.2. Methodological limitations

Methodological limitations represent another major challenge in the translation of biomarkers. Biomarker studies often rely on sophisticated technologies and analytical techniques, such as genomics, proteomics, and metabolomics. However, these methods have inherent limitations that can affect the accuracy and reliability of biomarker measurements. For example, DNA sequencing technologies can produce false positive or false negative results due to technical errors or sample contamination. Similarly, mass spectrometry-based proteomics can be affected by variations in sample preparation and instrument calibration.

In addition, the diversity of biomarker types, such as genetic, protein, and imaging biomarkers, requires different methods for their measurement and interpretation. This heterogeneity poses a challenge in standardizing analytical methods and establishing universal thresholds or cutoff values for clinical decision-making. Biomarker validation studies should address these methodological limitations by systematically evaluating the analytical performance of the measurement tests and considering multiple validation metrics, such as sensitivity, specificity, and predictive value.

9.3. Cost and accessibility

Cost and accessibility are major challenges in the implementation of biomarkers in clinical practice. Many biomarkers rely on expensive and complex technologies, making their routine use in the clinic financially burdensome. The high cost of biomarker tests hinders their widespread use, especially in resource-limited settings. In addition, the availability and accessibility of biomarker assays vary across healthcare systems, limiting the global dissemination and adoption of these diagnostic tools.

To overcome these challenges, efforts should be made to develop cost-effective biomarker assays that can be readily used in clinical laboratories. The use of point-of-care testing devices or miniaturized lab-on-a-chip technologies can reduce costs and improve accessibility, especially in low-resource settings. Furthermore, collaboration between academia, industry, and regulatory agencies can facilitate the implementation of biomarkers by addressing regulatory and reimbursement hurdles that affect the affordability and availability of these tests.

In summary, standardization and reproducibility, methodological limitations, cost, and accessibility are key challenges in the translation of biomarkers into clinical practice. Overcoming these challenges requires multi-stakeholder collaboration and coordinated efforts to establish standardized protocols, improve analytical methods, and develop cost-effective biomarker tests. Addressing these hurdles will not only increase the clinical utility of biomarkers but also pave the way for precision medicine and personalized medicine.

10. Future directions and conclusion

The search for reliable biomarkers for cerebral ischemia has made promising progress in recent years. Imaging techniques such as CT, MRI, DWI, and PET have provided valuable insights into the pathophysiology and localization of ischemic damage. Blood-based biomarkers, including proteins, miRNAs, and metabolites, offer the potential for non-invasive and cost-effective detection and prognostication. However, before these biomarkers can be successfully integrated into routine clinical practice, further research is needed to establish standardized protocols, overcome methodological limitations, and address clinical challenges. Future studies should focus on large-scale validation trials to determine the diagnostic and prognostic accuracy of these biomarkers and evaluate their potential for decision-making in the treatment of cerebral ischemia.

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REVIEW ARTICLE

Digital tools and scores for estimating the risk of stroke and bleeding in atrial fibrillation

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Abstract

Atrial fibrillation (AF) is a significant risk factor for cardioembolic stroke; however, therapeutic anticoagulation for stroke prevention in patients with AF also increases the risk of bleeding. Over the past two decades, extensive efforts have focused on refining patient stratification into risk categories to guide the selection of anticoagulant therapy. Various scores have been developed to estimate stroke risk in patients with AF, including $CHADS_2$, Framingham, CHA_2DS_2-VASc, ATRIA, ABC, GARFIELD-AF, and IMRS-VASc, with CHA_2DS_2-VASc being the most widely utilized. Similarly, scores such as $HEMORR_2HAGES$, HAS-BLED, ORBIT, ATRIA, GARFIELD-AF, and the newly introduced DOAC are utilized to estimate bleeding risk, with HAS-BLED being predominant. In recent years, numerous digital tools have been developed to assist physicians and patients in visualizing the risk of stroke and bleeding in an interactive manner, to strengthen the use of evidence-based medicine, and to facilitate shared decision-making. These tools include the American College of Cardiology's AnticoagEvaluator app, Mayo Clinic Anticoagulation Choice Decision Aid, Stroke Prevention in Atrial Fibrillation Risk Tool (SPARCtool), GARFIELD-AF Risk Calculator, and CardioSmart Atrial Fibrillation and Bleeding Risk Calculator. This review explores the evolution of these risk scores, evaluating their merits and drawbacks while highlighting common variables across multiple risk scores. In addition, it describes the landscape of novel digital tools designed for decision-making, offering insights from the perspective of physician users.

Keywords: Atrial fibrillation; Anticoagulation; Bleeding; Stroke; Digital tools; Risk score

1. Introduction

Atrial fibrillation (AF) is the most common arrhythmia in adults, with an estimated global prevalence of 50 million in 2020.[1] In the United States (US) alone, the prevalence of AF is projected to increase to 12.1 million in 2030 from 5.2 million in 2010, and the incidence of AF is projected to increase to 2.6 million in 2030 from 1.2 million in 2010.[2,3] AF not only contributes significantly to mortality but also to morbidity related to

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stroke and bleeding. It is responsible for 10% of strokes and is associated with a two-fold increased risk of death.[4] In most patients with AF, the rate of thromboembolic events is five- to eight-fold higher than bleeding rates.[5] However, patients on anticoagulation therapy face an increased risk of major bleeding, ranging from 1.4% to 3.4% while using warfarin.[6,7] A central issue in the management of AF is effectively preventing cardioembolic strokes while carefully balancing the bleeding risks associated with therapy.

The selection of stroke risk reduction therapy is guided by the patient's risk of stroke, bleeding risks associated with the therapy, and individual preferences. Given the dynamic nature of these factors, it is crucial to periodically reassess all decisions related to stroke prevention therapy and to make use of validated scores for risk estimation. To support ongoing discussions and provide guidance to both patients and providers, various digital tools have been developed to augment the role of these risk scores. In this paper, we review the historical development of scores used to estimate stroke and bleeding risks in AF, as well as the current landscape of digital tools available for this purpose.

2. Scores for estimating stroke risk in AF

Over the years, at least 19 risk scores and 76 updates to risk scores have been published to estimate stroke risk in AF.[8] Major scores for estimating stroke risk in AF include $CHADS_2$ (2001), Framingham (2003), CHA_2DS_2-VASc (2010), ATRIA (2013), ABC (2016), GARFIELD-AF (2017), and IMRS-VASc (2019) (Table 1). By the late 1980s, the association between AF and stroke became apparent, even in patients without mitral valve disease.[9] The Framingham Heart Study documented a dramatic increase in AF incidence with each successive decade of age.[10] In addition to age, independent risk factors for AF included diabetes, hypertension, valve disease, and congestive heart failure (HF).[11] The Stroke Prevention in AF (SPAF) I, II, and III trials compared the efficacy of warfarin, aspirin, and placebo in stroke prevention for patients with non-valvular AF.[12] Collectively, SPAF I, II, and III demonstrated greater stroke reduction in patients prescribed with warfarin (with a target international normalized ratio [INR] of 2.0 – 3.0) compared to those on combination low-dose warfarin/aspirin or aspirin monotherapy, and this established warfarin as the standard therapy for stroke prevention in AF.[13-15] The SPAF trials identified age, female sex, systolic blood pressure (BP)>160 mmHg, and prior stroke or transient ischemic attack (TIA) as risk factors for stroke in AF.[12] At the same time, the AF investigators (AFI) pooled data from five trials and identified age, hypertension, prior stroke, or TIA, and diabetes mellitus as additional risk factors for stroke in AF.[16]

The identification of stroke risk factors in patients with AF spurred the development of scoring systems for risk estimation. $CHADS_2$, introduced in 2001, was the first widely adopted scoring system for this purpose. The $CHADS_2$ score was constructed by amalgamating the independent risk factors identified from SPAF and AFI, and its validation utilized the National Registry of AF (NRAF), a dataset assembled from Medicare claims of patients hospitalized for non-rheumatic AF who had not received anticoagulation therapy.[17] Congestive HF, hypertension, age ≥75 years, and diabetes mellitus were each assigned 1 point in the score, whereas history of stroke or TIA was each assigned 2 points. In contrast to subsequent scores, hypertension was defined as "any history of hypertension" rather than using a numeric BP threshold, and congestive HF (CHF) was defined as "recent heart failure exacerbation." Scores were initially categorized as low (0 – 1), moderate (2 – 3), and high (≥4) risk levels.[17] Subsequently, moderate risk was denoted as 1 point and high risk as ≥2 points.[18]. During this time, treatment with warfarin, the only pharmacologic option available for anticoagulation, was generally recommended for patients with a $CHADS_2$ score of ≥2, irrespective of gender.[18]

Since the development of $CHADS_2$, efforts have been underway to refine scores for stroke risk assessment in AF, particularly because a substantial number of patients with AF were placed into the moderate risk category, according to $CHADS_2$. An alternate scoring system based on Framingham Heart Study data was introduced in 2003.[19] Similar to the $CHADS_2$, advancing age, increasing systolic blood pressure, female sex, diabetes, and history of stroke or TIA were identified as stroke risk factors in the Framingham score. However, the Framingham score assigns more points based on age and severity of risk factors. While the Framingham score improved the identification of patients with AF at low risk for stroke and who may not have derived significant benefit from anticoagulation, it did not estimate risk reduction with anticoagulation therapy. In 2004, a study involving patients with non-valvular AF who were taking aspirin revealed that $CHADS_2$ had the greatest discrimination in identifying high-risk patients compared to the AFI, SPAF, and Framingham risk scores. However, these scoring systems showed minimal differences in their ability to discriminate among low-risk patients.[20]

In 2008, a validation study of the $CHADS_2$ score revealed that although it was a good predictor of stroke risk in patients with AF, incorporating additional factors such as sex, extending age categories, and reweighing existing risk factors could result in improved accuracy.[21] Addressing this, the Birmingham 2009 score, more popularly termed the CHA_2DS_2-VASc score, was introduced in 2010.[22] The

Table 1. Factors used in various scoring systems for assessing stroke risk in patients with atrial fibrillation (AF)

Factors	$CHADS_2$ (2001)	Framingham (2003)	CHA_2DS_2-VASc (2010)	ATRIA Stroke (2013)	ABC (2016)	GARFIELD-AF (2017)	IMRS-VASc (2019)
Number of variables	5	5	7	8	4	10	10 (male) and 12 (female).
Old age	≥75 years old.	Increasing risk points for age groups >60.	65 – 74 years: 1 point; ≥75 years old: 2 points.	Eight categories of age+stroke history: 65 – 74; 75 – 84; and≥85. Increasing points for patients with older age and prior stroke.	Increasing points ≥60 years old.	✔	As per CHA2DS2-VASc.
Diagnosed hypertension	Any history of hypertension (no BP cutoff defined).	Increasing points for every 10 mmHg above 120 mmHg systolic BP.	✔	✔		Diastolic BP.	✔
Renal disease				Two variables: (i) eGFR <45 by MDRD equation or ESRD; (ii) proteinuria: urine dipstick protein≥1+ (≥30 mg/dL) without urinary tract infection.		Moderate-to-severe CKD stage III – IV.	Serum creatinine.
Bleeding history						✔	
Anemia							Hematocrit, hemoglobin, and mean corpuscular hemoglobin concentration.
Prior stroke or TIA	Stroke or TIA symptoms.	✔	History of vascular disease, including stroke, MI, PAD, or aortic plaque.	✔	✔	✔	As per CHA2DS2-VASc.
Platelets							Platelet count and mean platelet volume.
Female sex		✔	✔	✔			✔
Diabetes mellitus	✔	✔	✔	✔		✔	✔
Heart failure	Recent CHF exacerbation. No LVEF criterion.		Recent CHF exacerbation. No LVEF criterion.	✔	Increasing points for NTproBNP >50.	History of heart failure or LVEF <40%.	✔
Additional factors					Increasing points for high-sensitivity troponin.	Current tobacco use, dementia, and history of CAD and PAD.	Other CBC: white blood cell count and red cell distribution width. Other BMP: sodium, potassium, bicarbonate, calcium, and glucose. IMRS uses sex-specific values.

Abbreviations: BMP: Basic metabolic panel; BP: Blood pressure; CAD: Coronary artery disease; CBC: Complete blood count; CKD: Chronic kidney disease; eGFR: Estimated glomerular filtration rate; ESRD: End-stage renal disease; LVEF: Left ventricular ejection fraction; MDRD: Modification of diet in renal disease; MI: Myocardial infarction; NTproBNP: N-terminal B-type natriuretic peptide; PAD: Peripheral artery disease; TIA: Transient ischemic attack; ✔: Variable used in scoring.

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CHA_2DS_2-VASc score added three additional factors: female sex, age 65 – 74 years, and history of vascular disease (myocardial infarction, peripheral artery disease, or aortic plaque). One point was assigned for the presence of each additional factor. Patients with ages ≥75 years were given two points. While $CHADS_2$ had classified 61.9% of patients into the moderate risk stratum, CHA_2DS_2-VASc classified only 15.1% of patients into the moderate risk stratum.[22] The score was validated using data from the Swedish Atrial Fibrillation Cohort Study, a national cohort study in Denmark, and an administrative claims database in the US, and demonstrated CHA_2DS_2-VASc to be superior to CHADS2 in predicting "truly low risk (composite thromboembolism event rate of 0.3% per year)." [23-25] CHA_2DS_2-VASc was then promptly adopted by the European Society of Cardiology in their 2010 guidelines and the American College of Cardiology (ACC)/American Heart Association (AHA)/American College of Chest Physicians (ACCP)/Heart Rhythm Society (HRS) in 2014.[26,27] Although other scoring methods have since been introduced CHA_2DS_2-VASc remains the most widely used score for stroke risk estimation in patients with AF.

The definition of "C" (congestive HF) in the CHA_2DS_2-VASc has sparked ongoing debate.[28] Both $CHADS_2$ and CHA_2DS_2-VASc scores were derived at a time before the classification of HF into left ventricular ejection fraction (LVEF)-based groups — reduced (<40%; HFrEF), mid-range (40 – 49%; HFmrEF), and preserved (>50%; HFpEF) — was incorporated in clinical guidelines.[29] The original study, which proposed the $CHADS_2$ score, defined "C" as a recent exacerbation of HF without specifying an LVEF criterion.[17] In contrast, the original study, which proposed the CHA_2DS_2-VASc score, defined "C" as the presence of signs and symptoms of either right (e.g., dependent edema, elevated central venous pressure, and hepatomegaly) or left ventricular failure (e.g., pulmonary venous congestion, exertional dyspnea, rales, cough, fatigue, orthopnea, paroxysmal nocturnal dyspnea, cardiac enlargement, and gallop rhythm) or both, confirmed by non-invasive or invasive measurements demonstrating objective evidence of cardiac dysfunction.[22] There was no LVEF criterion. Although not explicitly reported, patients with HFpEF are assumed to be included because they met study criteria by signs and symptoms of HF without requiring left ventricular systolic failure.[28] The recent AF guidelines provide limited clarification on this definition.[30] Early research suggested a higher stroke risk associated with lower LVEF in patients with AF. However, a meta-analysis of 33,773 participants from seven studies found no significant differences in stroke incidence between patients with AF with HFrEF and those with HFpEF.[31] Further guidance is needed, particularly as the prevalence of AF is now higher in patients with HFpEF compared to patients with HFrEF.[32]

In 2013, data from the Anticoagulation and Risk Factors in Atrial Fibrillation (ATRIA) score was used to create another scoring system.[33] The ATRIA score was notable for assigning a different set of point values for age in primary prevention and secondary patients, giving 6, 5, 3, and 0 points for ≥85, 75 – 84, 65 – 74, and <65 year olds with no prior stroke, and 9, 7, 7, and 8 points for ≥85, 75 – 84, 65 – 74, and <65 year olds with a prior stroke.[33] Moreover, the ATRIA score incorporated markers of renal function associated with increased thromboembolic states, including proteinuria and eGFR <45, or end-stage renal disease. The score was developed in the ATRIA cohort of patients with non-valvular AF and validated using the ATRIA-CVRN cohort, both in the California Kaiser Permanente system.[33,34]

Meanwhile, there has been growing interest in incorporating serum biomarkers to refine risk assessment for stroke in AF.[35] In 2016, the ABC (age, biomarkers, and clinical history) stroke risk score was introduced, incorporating cardiac biomarkers such as N-terminal fragment B-type natriuretic peptide (NT-proBNP) and high-sensitivity cardiac troponin (hs-cTn).[36] An increasing number of points were assigned based on the levels of NTproBNP and hs-cTn, measured on a continuous scale. The score was developed using data from patients with AF in the Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation (ARISTOTLE) trial and validated in patients with AF in the Stabilization of Atherosclerotic Plaque by Initiation of Darapladib Therapy (STABILITY) trial. It demonstrated an improved c-statistic for predicting stroke and systemic embolic events compared to CHA_2DS_2-VASc.[36,37] The drawback of ABC was the requirement for laboratory measurement of biomarkers, in contrast to preceding scores, which could be calculated based on clinical variables readily available in the chart.

Recognizing that prior scores were primarily developed and validated in the era when warfarin was the only anticoagulant available to prevent stroke in AF, there was an increasing need in the late 2010s to develop a score in a patient population receiving either warfarin or a direct-acting anticoagulant (DOAC). In 2017, the GARFIELD-AF score was introduced and was the first to allow simultaneous estimation of the risk of ischemic stroke, mortality, and internal bleeding.[38] In addition to traditional risk factors (age, sex, and history of stroke) for ischemic stroke, the GARFIELD-AF score also considers factors such as diastolic blood pressure and dementia. Another innovation of GARFIELD-AF was its provision of differences in event

rate should the patient receive treatment with DOACs, treatment with warfarin, or no treatment. The score was developed using prospective data from the international GARFIELD-AF registry and validated using data from the Outcome Registry for Better Informed Treatment of AF (ORBIT-AF) trial.[38] GARFIELD-AF showed better predictive value compared to CHA_2DS_2-VASc for stroke or systemic embolism.[38] A major limitation of GARFIELD-AF is that the weights for each variable are not readily available, so they cannot easily be calculated from memory, unlike more commonly used scores such as CHA_2DS_2-VASc.

As the aforementioned scores improved risk stratification in moderate-risk patients, there was a need to improve the prediction of stroke in low-risk patients. In 2019, the ABCD score was introduced as a means of refining the risk stratification of low-risk patients who had a non-gender CHA_2DS_2-VASc (i.e., CHA_2DS_2-VA) score of 0 or 1.[39] The ABCD score includes age (≥60 years), NT-proBNP ≥300 pg/mL, creatinine clearance <50 mL/min, and left atrial dimension (≥45 mm).[39] The score was developed in Korea by a retrospective review of patients with non-valvular AF who experienced cardioembolic stroke, with a control group of patients with AF who did not experience cardioembolic stroke, matched with a nearest-neighbor approach.[40] The ABCD score for estimating the risk of stroke in patients with AF should not be confused with the ABCD2 score commonly used to estimate the risk of stroke in patients with TIA.[41]

In addition to ABCD, two other scores were published in 2019: the Anticoagulation-specific Stroke (ACTS) score and the Intermountain Mortality Risk Score (IMRS). It was noted that for patients with a CHA_2DS_2-VASc score of ≥2, anticoagulation was recommended, but provided little guidance on choosing between warfarin and DOACs. Therefore, ACTS was designed to refine the estimation of stroke risk by the type of anticoagulant used, focusing on those at high risk for stroke.[42] It was developed using health claims data from MarketScan and validated with data from Optum Clinformatics.[42] Variables in ACTS incorporate prescribed pharmacotherapy, including anticoagulants, antiplatelet medications, beta-blockers, anti-diabetics, antihyperlipidemic, and antiarrhythmic (type II and type III) medications. However, ACTS did not show improved discrimination compared to simpler models such as CHA_2DS_2-VASc.[42]

The IMRS score was developed by investigators at Intermountain Medical Center by combining their previously established IMRS, which assesses mortality and morbidity using laboratory markers across various diseases, with the CHA_2DS_2-VASc score.[43-45] The IMRS uses components from the complete blood count (hematocrit, platelet count, white blood cell count, mean platelet volume, red cell distribution width, mean corpuscular hemoglobin concentration, and mean platelet volume) and the basic metabolic panel (sodium, potassium, bicarbonate, calcium, glucose, and creatinine), The resultant IMRS score estimates risk using different models for males and females. It demonstrated that among patients within the stratum of a traditional CHA_2DS_2-VASc score of 2, there was a four-fold separation between those with a high IMRS score and those with a low IMRS score. To date, the IMRS score has not been validated in patients with AF outside the Intermountain Health-care dataset.

While the addition of increasing numbers of laboratory-based biomarkers may improve the predictive accuracy of the score, as indicated by superior c-statistics, this may not necessarily translate into a net clinical benefit in real-world practice due to the corresponding offset in simplicity and practicality for clinical decision-making.[46] In a study examining the addition of ≥2 biomarkers compared to one biomarker to the CHA_2DS_2-VASc and HAS-BLED scores in real-world patients with AF on warfarin, the predictive ability of CHA_2DS_2-VASC for ischemic stroke was not significantly increased, whereas the predictive ability of HAS-BLED for major bleeding was only slightly increased.[47] All things considered, the CHA_2DS_2-VASc score remains the most validated among the scores for estimating stroke risk in AF, with 82 validation studies.[8] This is followed by the CHADS2 score, with 46 validation studies; the ATRIA score, with 11 validation studies; the AFI score, with seven validation studies; the GARFIELD-AF score, with four validation studies; the SPAF score, with five validation studies; the Framingham score, with six validation studies; and the ABC score, with five validation studies.[8]

3. Estimation of stroke risk in patients with AF using non-discrete predictors

Findings derived from electrocardiographic and echocardiographic data, including non-discrete features, have been observed to predict outcomes in patients with AF.[48] In fact, left atrial abnormality, as defined by abnormal P wave morphology, and paroxysmal supraventricular tachycardia have both been associated with the development of ischemic stroke, consistent with the paradigm that the presence of the left atrial substrate itself predicts stroke independent of the presence of AF.[49,50] In a study examining different types of P-wave abnormalities — prolonged P-wave duration, abnormal P-wave axis, advanced interatrial block, and abnormal P-wave terminal force in lead V1 — an abnormal P-wave axis was identified as the only P-wave index associated with increased ischemic stroke risk independent of the

variables in CHA_2DS_2-VASc, resulting in a meaningful improvement in stroke prediction.[51]

Information derived from echocardiography also provides insights into the development of stroke.[52] Recently, left atrial (LA) size has emerged as a useful marker to characterize stroke risk. The Northern Manhattan Stroke Study concluded that moderate-to-severe LA enlargement was associated with recurrent strokes in patients with and without AF.[53] The creators of the ABCD score cited this finding when adding echocardiographic LA dilation as a risk factor.[39] In a more recent study, LA deformation characterized by LA strain using a three-beat method had a higher predictive value for ischemic stroke compared to traditional CHA_2DS_2-VASc scoring, although the improved performance was attenuated when the global longitudinal strain was added to the model.[54]

Artificial intelligence (AI) tools incorporating these non-discrete measures are being developed to predict the risk of AF in patients.[55] Although validated AI tools specifically for predicting thromboembolic stroke in patients with AF are not yet in clinical use, it is likely that in the coming years, AI-augmented risk prediction may enhance existing validated risk scores and further inform clinical decision-making, especially for patients classified as "intermediate risk" by conventional scoring methods.

4. Scores for estimating bleeding risk in AF

Stroke prevention therapy in patients with AF must balance the risks of ischemic stroke against the increased risks of bleeding with treatment. Various scoring systems to assess bleeding risk have been introduced. However, employing these scores can be challenging given that several comorbid conditions and risk factors simultaneously elevate both stroke and bleeding risk, including advanced age, hypertension, renal impairment, and a history of stroke. The overlap in variables across scores for estimating both stroke and bleeding risk is illustrated in Figure 1. Fortunately, many risk factors for bleeding are reversible or controllable, such as alcohol use, elevated blood pressure, and the use of NSAIDs. Assessment of risk factors specific to bleeding may better inform interventions to reduce bleeding risk and recommendations on anticoagulation. Major scores for estimating bleeding risk in AF include $HEMORR_2HAGES$ (2006), HAS-BLED (2010), ATRIA (2011), ORBIT (2015), GARFIELD-AF (2017), and DOAC (2023) (Table 2). Most scores are designed to estimate the risk of major bleeding at critical sites, including intracranial bleeding, retroperitoneal bleeding, intraspinal bleeding, and pericardial bleeding. Major bleeding can also be defined by the specific treatments required, such as the transfusion of two or more units of packed red blood cells.[56] Among these scores, HAS-BLED is the most widely validated and commonly used score in clinical practice. However, each scoring system has its merits and drawbacks, which will be explored in the following parts.

The $HEMORR_2HAGES$ score was introduced in 2006 to help identify patients with AF who may benefit from anticoagulation but require closer monitoring while on anticoagulation therapy.[57] This score was created based on the bleeding risk factors identified in the National Registry of AF, the same registry from which the $CHADS_2$ score was derived.[17] $HEMORR_2HAGES$ assigns 2 points for a history of bleeds and 1 point for hepatic or renal disease, ethanol abuse, malignancy, age >75 years, reduced platelet count or function, uncontrolled hypertension, anemia, genetic factors, excessive fall risk, and stroke. A score of 0 or 1 is interpreted as a low risk, 2 or 3 as an intermediate risk, and ≥4 as a high risk.[57]

Noting that $HEMORR_2HAGES$ was developed on a historical cohort a decade earlier, and had a high degree of overlap with risk factors for estimating stroke, Pisters *et al.*[58] introduced the HAS-BLED score in 2010. HAS-BLED was developed and validated in the Euro Heart Survey on the AF cohort. In addition to several risk factors included in $HEMORR_2HAGES$, such as uncontrolled hypertension, abnormal renal and liver function, stroke history, and history of prior bleeding, HAS-BLED also includes a labile international normalized ratio, simultaneous use of drugs and alcohol, and qualifies older age as >65 years. Each factor was assigned 1 point except for abnormal renal and liver function and drug/alcohol use, which have a maximum of 2 points should both individual factors be present. Scores allow the classification of patients with AF into three risk strata, in which a score of 0 indicates low risk, 1 – 2 moderate risk, and ≥3 high risk for bleeding.[58,59]

In 2011, the ATRIA score for assessing the risk of hemorrhage associated with warfarin use in patients with AF was introduced.[60] The ATRIA score for risk of bleeding was derived from split-sample testing of a cohort of patients with non-valvular AF in the Kaiser Permanente system of Northern California, which was also used to derive the 2013 ATRIA score for assessing the risk of stroke in AF.[33] ATRIA included the following factors selected by bootstrapping: severe renal disease (3 points), anemia (3 points), age ≥75 years (2 points), prior bleeding (2 points), and hypertension (1 point). Notably, patients aged 65 – 74 did not receive a point, hypertension was defined as any history of hypertension rather than a specific numeric BP threshold, and concomitant aspirin use was not included as a risk factor for bleeding. In the ATRIA cohort, this score demonstrated good discrimination performance and net reclassification improvement compared to prior

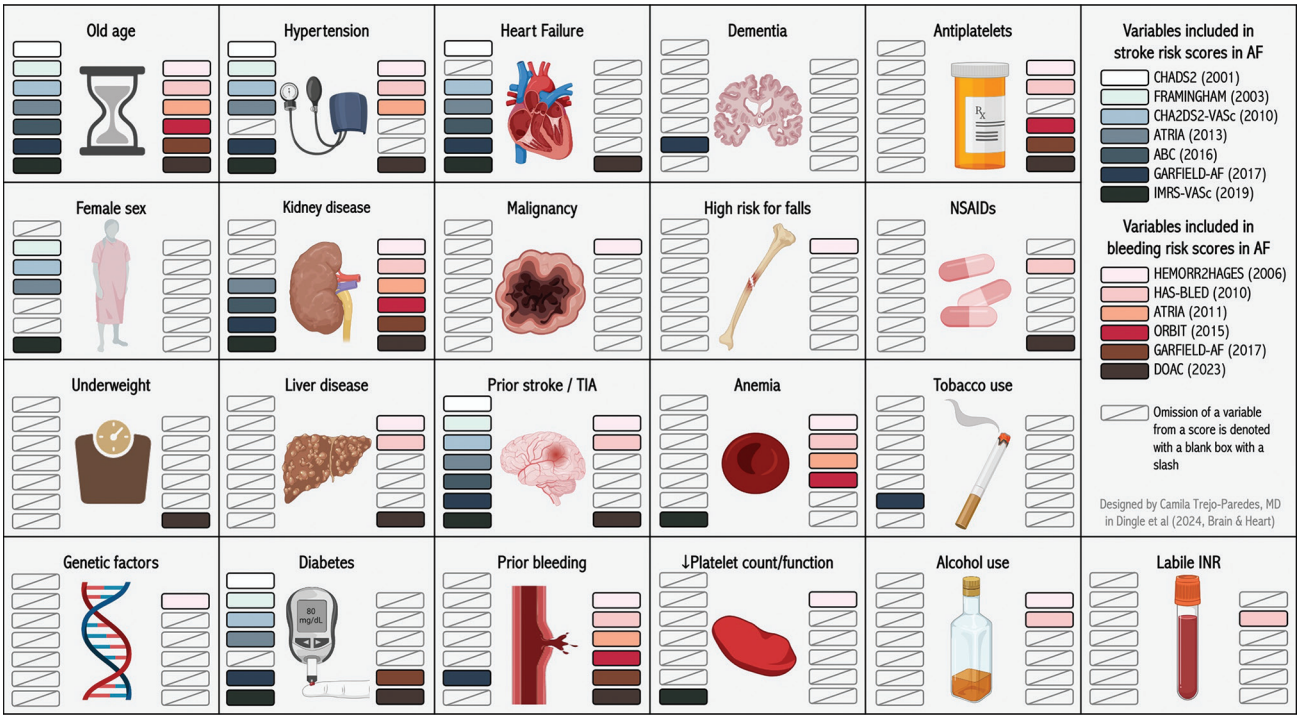

Figure 1. Common variables used in scores for estimating the risk of stroke and bleeding in atrial fibrillation (AF). Shades of blue indicate that the variable is included in stroke risk scores, such as CHADS2 (2001), Framingham (2003), CHA2DS2-VASc (2010), ATRIA (2013), ABC (2016), GARFIELD-AF (2017), or IMRS-VASc (2019). Shades of red indicate that the variable is included in bleeding risk scores, such as HEMORR$_2$HAGES (2006), HAS-BLED (2010), ATRIA (2011), ORBIT (2015), GARFIELD-AF (2017), or DOAC (2023). The omission of a variable from a score is denoted as a blank box with a slash. Note: Please refer to Tables 1 and 2 for a full list of variables for each score, including the specific definitions of each variable. Source: Icons were created using BioRender.
Abbreviations: INR: International normalized ratio; TIA: Transient ischemic attack.

scores, including HEMORR$_2$HAGES.[60] However, in several validation cohorts, ATRIA demonstrated a poorer ability to predict intracranial bleeding compared with HAS-BLED.[61,62]

Noting the limitations of scores such as HAS-BLED and HEMORR$_2$HAGES, which were formulated from a limited number of incidents and where HEMORR$_2$HAGES demonstrated variable reliability across external validation groups, O'Brien *et al.*[63] proposed the ORBIT score in 2015, intending to improve bleeding risk prediction using only readily available clinical variables. The ORBIT score was derived from the prospective Outcomes Registry for Better Informed Treatment of AF, which enrolled patients with AF from 176 sites across the US. The five strongest predictors of major bleeding events were identified using a backward selection approach and formed the basis of a risk score with the same acronym as the prospective cohort. The acronym ORBIT stands for older (age ≥75 years); reduced hemoglobin, hematocrit, or history of anemia; bleeding history; insufficient kidney function; and treatment with an antiplatelet agent. Each of these factors was assigned 1 point except reduced hemoglobin, hematocrit, history of anemia, and bleeding history, which are assigned 2 points each. The ORBIT score classified patients as having low (0 – 2), medium (3), and high risk for bleeding (≥4). ORBIT was validated using an external clinical trial population from the ROCKET-AF trial, which randomized patients with non-valvular AF to rivaroxaban or warfarin.[63,64] ORBIT demonstrated similar discrimination but improved calibration compared to HAS-BLED and ATRIA scores.

Various validation studies have compared the accuracy of these bleeding risk scores. HAS-BLED and HEMORR$_2$HAGES were validated in a "real-world" cohort of patients with AF from Denmark, where they demonstrated similar performance in predicting major bleeding events, although HAS-BLED was preferred due to its greater simplicity.[25] Additional studies further supported HAS-BLED's superior performance. HAS-BLED was also found to have better predictive ability than ATRIA in a cohort of AF outpatients from an anticoagulation clinic.[62] When all four scores were evaluated using data from the AMADEUS trial, HAS-BLED was the best predictor of clinically relevant bleeding, defined as major bleeding or any non-major clinically relevant bleeding.[61,65] Consequently, HAS-BLED has become the predominant score used today to assess bleeding risk in patients with AF.

Table 2. Factors used in various scoring systems for assessing bleeding risk in patients with atrial fibrillation (AF)

Factors	HEMORR$_2$HAGES (2006)	HAS-BLED (2010)	ATRIA (2011)	ORBIT (2015)	GARFIELD-AF (2017)	DOAC (2023)
Number of variables	11	9	5	5	8	10
Old age	>75 years old	>65 years old	≥75 years old	≥75 years old	✔	≥65 years old
Diagnosed hypertension	Uncontrolled	Uncontrolled, >160 mmHg systolic blood pressure	✔			History
Renal disease	ESRD or creatinine >2.5 mg/dL	Dialysis, transplant, Cr >2.26 mg/dL or >200 μmol/L	eGFR <30 mL/min or dialysis-dependent	eGFR <60 mg/dL/1.73 m^2	CKD III-IV	eGFR≤60 mL/min
Bleeding history	✔	✔ (2)	✔ (3)	✔	✔	History or major or minor bleeding event
Anemia	✔	✔ (Included within the factor bleeding history)	Hb <13 g/dL in Men; Hb <12 g/dL in Women	Hb <13 mg/dL or Hct <40% in Men; Hb <12 mg/dL or Hct <36% in Women		
Hepatic disease	End-stage liver disease or cirrhosis	Cirrhosis or bilirubin >2 × normal with AST/ALT/AP >3 × normal				AST, ALT, ALP≥3 × ULN, ALP≥2 × ULN, or Cirrhosis
Alcohol excess	✔	≥8 drinks/week				
Prior stroke or TIA	✔	Particularly lacunar				Includes TIA and/or systemic embolism
Malignancy	✔					
Reduced platelet counts or function	Thrombocytopenia and blood dyscrasias (Hemophilia)					
Excessive fall risk	High risk of falling, dementia, Parkinson's or psychiatric disease					
Genetic factors	CYP 2C9 single-nucleotide polymorphisms					
Labile INR		Unstable/high INRs, time in therapeutic range <60%				
Treatment with antiplatelets	Aspirin use	✔ (Included in the category medications predisposing to bleeding)		✔	✔	Current
Diabetes mellitus					✔	✔
Underweight						BMI <18.5 kg/m^2
NSAID use		✔ (Included in the category medications predisposing to bleeding)				Current

(Cont'd...)

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Table 2. ***(Continued)***

Factors	HEMORR2HAGES (2006)	HAS-BLED (2010)	ATRIA (2011)	ORBIT (2015)	GARFIELD-AF (2017)	DOAC (2023)
Heart failure						History. No LVEF criterion
Additional factors					Carotid occlusive disease, history of CAD or PAD, pulse	
Additional definitions	Variables defined based on ICD-9-CM Codes from baseline hospitalization	Major bleeding refers to any bleeding requiring hospitalization and/or causing a decrease in hemoglobin level of >2 g/L and/or requiring blood transfusion that was not a hemorrhagic stroke	Defined as any prior outpatient or inpatient ICD-9 diagnosis code of hemorrhage, including by specific organ system (e.g., prior intracranial or gastrointestinal bleeding), in the aggregate (e.g., all-cause prior bleeding), and by timing (within 90 days or >90 days)			

Abbreviations: ALP: Alkaline phosphatase; ALT: Alanine transaminase; AST: Aspartate transaminase; BMI: Body mass index; CAD: Coronary artery disease; CKD: Chronic kidney disease; eGFR: Estimated glomerular filtration rate; ESRD: End-stage renal disease; Hb: Hemoglobin; INR: International normalized ratio; LVEF: Left ventricular ejection fraction; NSAID: Non-steroidal anti-inflammatory; PAD: Peripheral artery disease; TIA: Transient ischemic attack; ULN: Upper limit of normal; ✔: Variable used in scoring.

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HAS-BLED features a warfarin-specific variable: labile INR. When "time in therapeutic INR range," a variable inversely related to labile INR, was added to the ATRIA and ORBIT scores, their predictive performance improved.[65] This evidence highlights the importance of considering labile INR and/or time in the therapeutic INR range when assessing bleeding risk in patients with AF receiving warfarin. However, DOACs, including rivaroxaban, apixaban, edoxaban, and dabigatran, are increasingly prevalent. Since the data used to create the aforementioned scoring systems for estimating bleeding risk is mostly derived from cohorts of patients with AF on warfarin, these may not reflect patients' true risk of bleeding while on DOACs.

To address this issue, a novel DOAC score was introduced in 2023. Developed from individuals in both the Randomized Evaluation of Long-Term Anticoagulation Therapy (RE-LY trial) and the GARFIELD-AF registry, the DOAC score includes 10 different risk factors that were each assigned a specific number of points. One point each is given for underweight, stroke/TIA/embolism history, hypertension, diabetes, and non-steroidal anti-inflammatory use. Two points are awarded for liver disease, and 3 points if the individual has a history of bleeding. For the risk factors of age, creatinine clearance/glomerular filtration rate, and antiplatelet use, different point values are assigned based on specific categories within each factor. For example, within age, 2 points were given for 65 – 69 years, 3 points for 70 – 74 years, 4 points for 75 – 79 years, and 5 points for ≥80 years. One point is awarded if the individual has a creatinine clearance/glomerular filtration rate of 30 – 60 mL/min and 2 points if ≤30 mL/min. For antiplatelet use, aspirin use is assigned 2 points, while dual-antiplatelet therapy is assigned 3 points. The score classifies patients into risk categories of very low (0 – 3 points), low (4 – 5), moderate (6 – 7), high (8 – 9), and very high (≥10 points) risk. The DOAC score was externally validated in the COMBINE-AF and RAMQ cohorts, where it also had stronger predictive performance than HAS-BLED for major bleeding.[66] Several factors have been hypothesized to explain the improved predictive performance of the DOAC score. First, it assigns a different number of points to each variable rather than an equal point to each, reflecting the different magnitudes of bleeding risk for each variable. Second, it assigns a higher cumulative risk for those taking multiple antiplatelet/anticoagulant medications, whereas previous scores treated those with any combination of NSAIDs, aspirin, and dual antiplatelet therapy as having similar risks. Third, it accounts for differences in the propensity for bleeding at different levels of kidney function. Fourth, it was developed for a contemporary population, reflecting current standards of care and DOAC use.

5. Recent guideline updates

Current guidelines from the European Society of Cardiology/European Association for Cardio-Thoracic Surgery/European Heart Rhythm Association (ESC/EACTS/EHRA) in 2020 and the Asia Pacific Heart Rhythm Society (2021) recommend the CHA_2DS_2-VASc score for estimating stroke risk and the HAS-BLED score for assessing bleeding risk in the setting of AF.[67,68] The 2023 AHA/ACC/ACCP/HRS guidelines for the diagnosis and management of AF, which represent the newest society guidelines for managing AF to date, note that the risk of stroke can be characterized as low (<1%/year), intermediate (1 – 2%/year), or high (>2%/year) and that the use of a validated clinical risk score such as CHA2DS2-VASc, ATRIA, or GARFIELD-AF can do the calculation of this risk.[30] They do not endorse a specific risk score. The recommendation to pursue anticoagulation therapy is made based on the annual percent risk of thromboembolism rather than the point summation of a specific risk score. Anticoagulation therapy for patients with ≥2% annual thromboembolic risk (Class I recommendation) is recommended and considered reasonable for patients with 1 – 2% annual thromboembolic risk (Class IIa recommendation).[30] Patients with AF who fall into the intermediate annual risk category of thromboembolic events (<2%) may still benefit from consideration of additional factors that could potentially affect their risk of stroke. These factors include a higher AF burden, obesity, hypertrophic cardiomyopathy, poorly controlled hypertension, impaired kidney function (GFR <45 mL/h), proteinuria (>150 mg/24 h), and left atrial enlargement (volume ≥73 mL or diameter (≥4.7 cm). Direct oral anticoagulants (DOACs) such as apixaban, dabigatran, edoxaban, and rivaroxaban are first-line medications and are preferred over warfarin to reduce the risk of mortality, stroke, systemic embolism, and intracranial hemorrhage (Class I recommendation), except in patients with mitral stenosis or mechanical heart valves. The guidelines also emphasize the need to examine drug interactions between DOACs and other medications, namely inhibitors and inducers of CYP3A4 and p-glycoprotein.

Concerning the bleeding risk scores, the 2023 AHA/ACC/ACCP/HRS guidelines recommend using them as part of the comprehensive clinical assessment of patients with AF and as guidance to determine modifiable risk factors to reduce a patient's bleeding risk. Specifically, the guidelines note that unless there is an absolute contraindication to anticoagulation, the bleeding risk scores have limitations in clinical guidance. Bleeding risk scores must not be interpreted in isolation, as they do not balance the risk of bleeding against the risk of stroke or provide an assessment of the net clinical benefit of using anticoagulation.[30] In the

rationale for this recommendation, the guidelines note that population-based studies have shown that the benefits of anticoagulation in AF generally outweigh the risks of bleeding, even in patients at high bleeding risk.[69]

For patients who have prohibitive contraindications to anticoagulation, it is worth noting that in the 2023 AHA/ACC/ACCP/HRS guidelines, percutaneous left atrial appendage occlusion (pLAAO) implantation of a Watchman device is given a Class IIa recommendation in patients with AF with a moderate to high risk of stroke (CHA_2DS_2-VASc score ≥2) and a contraindication to long-term oral anticoagulation due to a non-reversible cause.[30] In patients with AF with a moderate to high risk of stroke and a high risk of major bleeding on oral anticoagulation but without an absolute contraindication to oral anticoagulation, pLAAO is considered a reasonable alternative to oral anticoagulation, based on patient preference, with a Class IIb recommendation.[30]

Concerning digital tools, the 2023 AHA/ACC/ACCP/HRS guidelines provide a Class IIb recommendation for using evidence-based decision aids for guidance in stroke reduction therapy treatment decisions to improve decision quality and both patient engagement and satisfaction.[30] The guidelines acknowledge that online ATRIA, GARFIELD-AF, and CHA2DS2-VASc calculators are available. However, there has not been a systematic evaluation of the weaknesses and strengths of each digital tool in the literature to date.

6. Digital tools for estimating the risk of stroke or bleeding

6.1. The need for digital tools

The role of digital tools in estimating stroke or bleeding risk in AF has become increasingly important for several reasons. First, in an era of increasing patient care volumes and message volumes, physicians need the capability to quickly estimate patient-specific risks using validated risk scores and estimate the impact of each treatment option on mitigating or augmenting these risks. Findings from large cohort studies are only useful if they can be deployed at the point of care. Second, the development of models that use uneven weights for each variable rather than simple point-based systems necessitates using online calculators, to sum up weights, as seen with GARFIELD-AF. Manual calculation of these scores is not easy. While scores with greater complexity may increase predictive value, the complexity may serve as a barrier for providers and patients, who may struggle to garner insight in the absence of a computerized interface that contextualizes the output. Third, the variables that comprise each score are often defined in medical language, and the outputs associated with each score are often defined in statistical language. The development of digital tools that translate medical and statistical language into comparative visual aids allows both patients and physicians to improve shared decision-making engagement. Fourth, legal mandates worldwide to ensure transparency of health data, such as the 21st Century Cures Act in the US, provide patients with access to all results and documentation in the electronic health record. While patients may not necessarily understand all the data required to calculate a certain risk score, access to this data opens avenues for such interactions to take place. Patient decision aids, described in the last section of this review, can assist patients in understanding their risks, and support the value placed on the fundamental rights of patient autonomy and access to personal information in the modern health-care system.

6.2. Landscape of available tools

We conducted a search for tools to assist in the decision-making process for estimating bleeding or stroke risk among patients with AF across the Apple App Store, Google Play Store, and web platforms. We found five major digital tools for estimating bleeding or stroke risk in AF that are publicly available and free of charge: the Mayo Clinic Anticoagulation Choice Decision Aid, the Stroke Prevention in Atrial Fibrillation Risk Tool (SPARCtool), the GARFIELD-AF Risk Calculator, the ACC AnticoagEvaluator Application, and the CardioSmart Atrial Fibrillation and Bleeding Risk Calculator (Table 3). Four out of the five tools allow users to assess both stroke and bleeding risk among patients with AF, while one specifically focuses on stroke risk estimation. While all five tools are available online, the ACC AnticoagEvaluator app and the GARFIELD-AF risk calculator are also available as mobile apps. Among these tools, the ACC offers two: the ACC AnticoagEvaluator app, designed for use by physicians, and the CardioSmart Atrial Fibrillation and Bleeding Risk Calculator, intended for patient use.

Of note, several stroke risk scores exist on general medical calculator websites such as MDCalc and QxMD, including $CHADS_2$, CHA_2DS_2-VASC, ATRIA, and GARFIELD-AF.[70-73] Bleeding risk scores that exist on general medical calculator websites include $HEMORR_2HAGES$, HAS-BLED, ATRIA, ORBIT, and GARFIELD-AF.[73-77] General medical calculator websites hosting risk scores across different organ systems are beyond the scope of this present review. The GARFIELD-AF risk calculator, which is hosted on the domain of the GARFIELD-AF registry through which the score used by the digital tool was developed, qualified for inclusion in this review due to its incorporation of additional visual and comparative features for patient-shared decision-making.

Table 3. Digital tools for estimating stroke and/or bleeding risk in patients with atrial fibrillation (AF), including the comparison of their web applications, mobile applications, and associated evidence

Digital tool name	Type of risk	Description of digital tool purpose	Main host of tool and URL	Intended user	Last update date present	Presence of a dedicated mobile application	Validation of the digital tool been done?	Based on what score?	Source equations/ evidence on page?
Mayo Clinic Anticoagulation Choice Decision Aid	Stroke risk	Generates a decision-making report for patients to be informed on the best anticoagulation option for them by exploring their risk of a stroke with an underlying history of AF under no anticoagulation, being on warfarin, or being on a DOAC	Mayo Clinic https://anticoagulationdecisionaid.mayoclinic.org/index.php/site/anticoagulation	Both	No	No	Effect on decision-making and encounter length examined[78,79]	CHA_2Ds_2-VASc	No
Stroke Prevention in Atrial Fibrillation Risk Tool (SPARCtool)	Both stroke and bleeding risk	Estimates the stroke risk and presents the benefits and risks of antithrombotic therapy among chronic non-valvular AF patients.	SPARCtool https://www.sparctool.com/	Provider	Yes: February 2024	No	Effect on decision-making examined[80]	$CHADS_2$ & CHA_2DS_2-VASc	Yes
GARFIELD-AF Risk Calculator	Both stroke and bleeding risk	Provides the risk of mortality, ischemic stroke or systemic embolism, and major bleeding, including hemorrhagic stroke, for up to 24 months	GARFIELD Registry https://af.garfieldregistry.org/garfield-af-risk-calculator	Provider	No	Yes, on both iOS and Android. By QxMD and MD Calc.	Validation of score (not digital tool specifically) published[81]	GARFIELD-AF	Yes
ACC Anticoag Evaluator Application	Both stroke and bleeding risk	Calculates a patient's stroke risk and renal function while reviewing factors that contribute to bleeding risk in patients with AF	ACC https://tools.acc.org/anticoag/#!/content/calculator/	Provider	Yes: 2019	Yes, on both iOS and Android. By ACC (available as "AnticoagEvaluator")	No	CHA_2DS_2-VASc (& Cockcraft-Gault)	No
CardioSmart Atrial Fibrillation and Bleeding Risk Calculator	Both stroke and bleeding risk	Estimates of the stroke and major bleeding risk in AF patients.	ACC https://www.cardiosmart.org/stroke-and-bleeding-risk-calculator	Patient	No	No	No	Not Listed	N/A

Abbreviations: ACC: American College of Cardiology; DOAC: Direct-acting oral anticoagulant.

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Based on the complexity of input variables and outputs, three out of the five tools are physician-facing; the CardioSmart Atrial Fibrillation and Bleeding Risk Calculator are patient-facing; and the Mayo Clinic Anticoagulation Choice Decision Aid is both patient- and physician-facing. Two of the five tools provide information about when the tool was last updated, either with new information or any indication of further maintenance being done. Four out of the five tools include instructions on what the digital tool and calculator are for on a user input page (Table A1).

With regard to the outcomes predicted, all digital tools, except for the CardioSmart Atrial Fibrillation and Bleeding Risk Calculator, provided distinct risk estimates for the scenarios of no intervention compared with any possible pharmacological intervention but did not distinguish between specific drugs (Table 4). The ACC AnticoagEvaluator provides distinct risk estimates for the use of no therapy, aspirin, aspirin + clopidogrel, apixaban, dabigatran, edoxaban, rivaroxaban, and warfarin. The greatest diversity of medications and associated risks was presented by the SPARCtool, which provides distinct risk estimates for the use of no therapy, aspirin, aspirin + clopidogrel, warfarin, dabigatran 110 mg, dabigatran 150 mg, rivaroxaban, apixaban, edoxaban 30 mg, and edoxaban 60 mg. Meanwhile, the CardioSmart Atrial Fibrillation and Bleeding Risk Calculator, which is patient-facing, presents the risk of stroke in a categorization of low, moderate, high, and very high, with no numerical presentation of these variables.

With regard to governance information for each of these digital tools, all five tools include a medical disclaimer, an accessible privacy policy on the digital tool interface, and terms and conditions (Table A2). Only the GARFIELD-AF Risk Calculator has a "frequently asked questions" section related to understanding the tool and its findings. Both the GARFIELD-AF Risk Calculator and the Mayo Clinic Anticoagulation Choice Decision Aid also include a feedback section where users can suggest improvements to the web application or its exportable materials.

6.3. Distinguishing between risk scores and digital tools of risk scores

All the calculators evaluated in this review source their recommendations from validated predictive risk scores but additionally provide the ability to combine findings, present findings, and export findings for provider and patient decision-making. Almost all digital tools that were evaluated for this review, except for the GARFIELD-AF calculator and the CardioSmart Atrial Fibrillation and Bleeding Risk calculator, explicitly state that they are based on the CHA_2DS_2-VASc score. While many predictive risk scores have their own calculators accessible through both web and mobile applications, digital tools differ in design by including interactive elements that extend calculations with details not present in the original output of the score. Digital tools are not designed to improve the predictability of a risk score but to increase their understandability and accessibility. This includes contextualization through organizing the output in statistical measures to improve the meaning of results, including graphical representations of outputs in both web format and exportable format for communication, improving interactivity with inputs and outputs by providing point of access explanation of the variables involved in the score and explanation of the results, and aggregation of evidence and influencing information on the predictive risk scores over which these tools function. SPARCtool features dynamic bar graphs to visualize risks, while the Mayo Clinic Anticoagulation Choice Decision Aid uses risk pyramids to picture this. As explored in the subsequent sections, digital tools also aid in reducing the complexity of output to a form that is far more easily understood by patients, best illustrated by the Mayo Clinic Anticoagulation Choice Decision Aid and the CardioSmart Atrial Fibrillation and Bleeding Risk Calculator. In summary, a digital tool is not only an output for the predictive risk score but a means to enrich findings beyond solely the risk predicted to broaden understanding and ease accessibility for patients and providers.

7. Features of currently available digital tools

The digital tools presented in this review are notable for several features that improve ease of use and understanding. These features should be considered for incorporation in the future development of similar applications.

7.1. Ability to export results for patient education

The Mayo Clinic Anticoagulation Choice Decision Aid offers users the capability to export a specific document that summarizes the results of their assessment. This document explains the implications of the output regarding the patients' risk of developing stroke, and it outlines how this risk is mitigated or augmented by the decision to pursue no anticoagulation, or anticoagulation using warfarin, or a direct oral anticoagulant. Similarly, the ACC AnticoagEvaluator application provides an option to email results in a concise text statement along with a graphical representation of stroke and bleeding risk estimation. This accessibility enables patients to be well-informed and facilitates the easy sharing of information with family members involved in their care.

Table 4. Comparison of features for understanding the output of each digital tool for estimating stroke and/or bleeding risk in patients with atrial fibrillation (AF)

Digital tool name	Statistical measure (s) of results	Do outcomes have discrete categories?	What are outcomes stratified between?	Result output as text?	Result output as graphic?	Able to copy statements for EHR?	Able to export/email results?	Education tools provided?
Mayo Clinic Anticoagulation Choice Decision Aid	Ratio	Stroke risk (partitioned between #/100 of no stroke event, #/100 for non-disabling stroke event, and #/100 for fatal or disabling stroke)	(i) No anticoagulation (ii) Warfarin (iii) DOAC	Yes	Yes	Yes	Yes (Export)	Yes
Stroke Prevention in Atrial Fibrillation Risk Tool (SPARCtool)	Annual risk percentage, relative risk reduction, absolute risk reduction, and net clinical benefit for each agent	Yes (partitioned between no risk of stroke, risk of event with no neurological deficit, and risk of stroke or neurological deficit)	(i) No therapy (ii) Aspirin (iii) Aspirin+clopidogrel (iv) Warfarin (v) Dabigatran 110 (vi) Dabigatran 150 (vii) Rivaroxaban (viii) Apixaban (ix) Edoxaban 30 (x) Edoxaban 60	Yes	Yes	Yes	No	No
GARFIELD-AF Risk Calculator	Percentage (risk-adjusted based on slide for days after diagnosis to calculate the risk of the event)	Yes (partitioned between mortality, ischemic stroke or systemic embolism, and major bleeding including hemorrhagic stroke)	(i) No oral anticoagulation treatment (ii) Vitamin K antagonist (iii) Direct oral anticoagulant	Yes	Yes	No	No	No
ACC AnticoagEvaluator Application	Stroke: • Annual risk of stoke+thromboembolism by selected agent • Relative risk reduction • Absolute risk reduction • Chance of benefit per year Bleeding risk: • Annual risk of major bleed • Annual chance of being harmed by selected agent • Patients annual risk of major bleed • Annual chance of being harmed by selected agent	Yes (partitioned between stroke risk/benefit and bleed risk)	(i) No therapy (ii) Aspirin (iii) Aspirin+Clopidogrel (iv) Apixaban (v) Dabigatran (vi) Edoxaban (vii) Rivaroxaban (viii) Warfarin	Yes	No	No	Yes (email)	No
CardioSmart Atrial Fibrillation and Bleeding Risk Calculator	None	Not described	Presentation of stroke risk: (i) Low (ii) Moderate (iii) High (iv) Very high	Yes	No	No	No	Yes

Abbreviations: ACC: American College of Cardiology; ASA: Aspirin; DOAC: Direct-acting oral anticoagulant; EHR: Electronic health record.

7.2. Ability to export results for physician documentation

Most of the tools also generate a dynamic text output that can be used for electronic health record documentation. These statements generally involve an explanation of what the tool has calculated and what the associated risk is (expressed as a percentage). They furthermore provide distinction on recommendations for what is required based on the digital tool output. With patients having access to their records, such statements are vital to understanding provider decision-making and influencing patient self-advocacy.

7.3. Augmentation/mitigation of risk based on anticoagulant/antiplatelet used

An advantage of these digital tools is that they allow for the consolidation of risk data derived from different validation studies beyond the original point-based score, incorporating multiple types of risk estimation into a single platform. While stroke and/or bleeding risk are essential outputs of these digital tools, it is especially valuable for physicians and patients to be able to see the impact of anticoagulation or antiplatelet therapy on the mitigation or augmentation of bleeding or stroke risk. The SPARCtool and the ACC AnticoagEvaluator Application present risks based on each specific pharmacological agent being considered. The SPARCtool additionally provides a graphical representation of the comparative risk of various agents.

7.4. Statistical contextualization of risk

While most of the digital tools evaluated present the exact proportion of risk, the ACC AnticoagEvaluator Application, the SPARCtool, and the GARFIELD-AF risk calculator include statistical effect estimates of their outcomes. These features commonly include the relative risk (RR), the relative risk reduction (RRR), the absolute risk reduction, and the net clinical benefit. The RR is aimed at measuring the strength of the association between the risk of developing bleeding or stroke on anticoagulation compared to being without anticoagulation. Any RR >1 suggests a higher risk within the anticoagulation group compared to the no anticoagulation group, while the inverse, meaning a ratio < 1, indicates a lower risk in the anticoagulation group compared to the no-anticoagulation group. For both the risk of stroke and the risk of bleeding, an RR <1 is desired. RRR is a measure of how much an intervention reduces the risk of an outcome compared to a baseline or control condition, expressed as a percentage. In the context of the output from the digital tools discussed in this study, the relative risk reduction is the ratio between the risk of developing a stroke or bleeding complication among patients with AF with anticoagulation subtracted by the same defined risk without anticoagulation and the risk of developing a stroke or a bleeding complication in patients solely with AF. Absolute risk reduction (ARR) is an important measure of the effect of a particular intervention. The actual reduction is formed by taking the difference between the risk of stroke and bleeding complications in patients with AF without any anticoagulation and the risk with anticoagulation in this population, represented as an actual value.[82] The net clinical benefit (NCB) is a quantification of the relationship between benefit and harm. It is the difference between the benefit received and the risk of harm, both based on a specifically defined outcome. The derivation of the NCB is typically based on the specific outcomes studied. Among the various digital tools analyzed in this review, the measures of benefit and risk differ depending on the tool's focus. In general, the benefit is defined as the reduction in the risk of bleeding or stroke in patients with AF, whereas harm is defined as the risk of a specific complication associated with the particular agent used. For instance, the ACC AnticoagEvaluator app reports on the annual probability of experiencing harm from pharmacological agents that modify either stroke or bleeding risk in AF.[83]

Each of these statistical measures produces a comparative conclusion on the benefit of an intervention. While the predictive risk scores described in Tables 1 and 2 estimate bleeding or stroke risk, digital tools incorporate data from other studies that estimate the modification of this risk from different interventions. These statistical representations are important aids in communicating risk to both patients and other providers and should be critical features in the further development of digital.

8. Validation studies for digital tools

To determine if these digital tools were clinically validated, we performed searches on Pubmed and Google Scholar and directly contacted the primary creators of each tool to inquire about any published studies validating their clinical performance. Out of the five authors contacted, three returned requests for literature: The Mayo Clinic Anticoagulation Decision Aid, the GARFIELD-AF Risk Calculator, and the SPARCtool. However, we did not receive a response or find any validating studies for the ACC AntiCoagEvaluator and the CardioSmart Atrial Fibrillation and Bleeding Risk Calculator.

8.1. Studies associated with the Mayo Clinic Anticoagulation Decision Aid

The Shared Decision Making for Atrial Fibrillation (SDM4AFIB) consortium published an initial whitepaper

in 2019 regarding the creation of the Mayo Clinic Anticoagulation Decision Aid,[84] noting that results presented as a large graphic with minimal data points and numbers allowed physicians to elaborate on them during clinic visits and thereby engage in shared decision-making. It was positively received by patients and improved their understanding of the indication for anticoagulation, without increasing the duration of the clinical encounter.[79] To determine if adherence to anticoagulation was improved with the implementation of the Mayo Clinic Anticoagulation Decision Aid, the SDM4AFIB investigators designed a randomized controlled trial; the only randomized trial among all the featured digital tools.[85] Patient encounters were randomized to either shared decision-making tool use (SDM; 463 encounters) or no tool (usual care [UC]; 459 encounters). The primary outcomes were primary adherence (adherence to prescribed anticoagulant at 10 months) and secondary adherence (determined as times filling prescriptions or INR within therapeutic range if patients were prescribed warfarin). No difference in primary or secondary adherence to anticoagulation between both groups was found; 78% in the SDM group vs. 81% in the UC group filled their first prescription.[78] The investigators also provided a resource page with their research and approaches for further validating shared decision-making tools.[86]

8.2. Studies associated with the GARFIELD-AF Risk Calculator

The GARFIELD-AF Risk Calculator is a digital tool that allows interaction with the GARFIELD-AF predictive risk score specifically. The GARFIELD-AF score itself was previously validated in a large study against HAS-BLED and CHA_2DS_2-VASc, as described above.[87] To date, the utility of the GARFIELD-AF digital tool in enhancing the use of the GARFIELD-AF score has not been studied.

8.3. Studies associated with the SPARCtool

The creators of the SPARCtool published a white paper describing the development of the tool in 2003.[88] In 2019, a small observational pilot study enrolled 37 participants.[80] The digital tool was effective in reducing decisional conflict, eliciting patients' values, increasing patient knowledge, and successfully showing individualized therapy options for patients to use.

8.4. Need for further validation studies

The scarcity of literature on digital tool validation should not detract from its potential value in improving patient understanding and shared decision-making. Nonetheless, further efforts to evaluate the general applicability of these digital tools, particularly those lacking supporting studies, are warranted. Outcomes that could be examined in future studies include patients' adherence to anticoagulation, patients' understanding of their clinical profile and evidence-based recommendations, patients' perception of the congruence of the output according to their personal values and preferences, and provider and patients' perception of the role of the digital tool in enhancing the clinical encounter.

9. Online resources for patient education

In addition to the digital tools for decision-making described in Tables 3 and 4, there are several additional online resources that deserve mention. These patient-centered online resources are educational portals with written, illustrated, and video materials to enhance patient understanding of both the risks and benefits of anticoagulation in AF. They serve as decision aids for patients by summarizing information and engaging users to make an informed decision about a diagnosis of AF and the risks and benefits associated with available interventions. In contrast to the digital tools for decision-making previously described, these resources do not dynamically generate different outputs based on a calculated risk score.

First is the Stanford Afib Guide.[89] This tool is designed to enhance patient comprehension of AF through an interactive, gamified, web-based interface that contains both textual and video resources. It is aimed at patients who seek a deeper understanding of AF by mixing video material with easy-to-understand graphics that serve as questions to test understanding.

Second is the module on AF: Should I Take an Anticoagulant to Prevent Stroke? by the Ottawa Health Research Institute in collaboration with Healthwise.[90] This tool serves as a decision aid that presents information on aspects of AF diagnosis and care to help patients understand their treatment options and the associated risks and benefits. It features a dashboard guiding patients through six major steps organized into different tabs. The first two steps introduce information about AF and available medical treatments. Steps three and four provide space for patients to reflect on their thoughts regarding an AF diagnosis, concerns about stroke risk, and confidence in adhering to treatment options. Subsequently, both steps four and five allow patients to assess their readiness to start treatment and their understanding of the presented material, respectively. The output of these interactions is presented as a summary in step six, which the patient can keep for personal records or share with a physician.

Third is the Atrial Fibrillation: Stroke Prevention Decision Aid by the Colorado Program for Patient-Centered Decisions.[91] This aid presents information about the risks and benefits of interventions to prevent stroke

in AF. Assuming a calculated CHA_2DS_2-VASC score, it offers four categories of materials according to the patient's CHA_2DS_2-VASC score of =0, =1, =2, or >3. Each section has its own literature and video, with a subsequent decision aid in Spanish or English. This tool focuses its patient education on the treatment aspect of AF, whereas the two aforementioned patient decision aids also provide patient education materials to develop an understanding of AF as a whole. This tool also requires patients to be aware of what their calculated CHA_2DS_2-VASC is before determining which set of information would be most useful for them.

Taken together, these online resources are an important component in the physician's toolkit for shared decision-making, as emphasized in the most recently published guidelines for the management of AF.[30]

10. Conclusion

The method for estimating stroke and bleeding risks in patients with AF has significantly advanced over the past two decades. Risk estimation scores have progressively evolved by incorporating biomarkers and non-discrete variables, such as electrocardiographic P-wave morphologies, to refine predictive accuracy. In recent years, the emergence of digital tools has enhanced physicians' and patients' ability to engage in evidence-based shared decision-making. Digital tools provide numeric and visual context to the output of risk scores, allow for the incorporation of risk mitigation or augmentation estimates from specific anticoagulation and antiplatelet agents, and improve the ease and accessibility of exporting and sharing decision-making material. These features have the potential to facilitate patients' understanding of their health issues, such that patients can become better informed and empowered to advocate for their care.

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Conflict of interest

The authors declare that they have no competing interests.

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Availability of data

Further educational material can be found at www.AtrialHarmony.org

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Appendix

Table A1. Comparison of interactive features of each digital tool for estimating stroke and/or bleeding risk in patients with atrial fibrillation (AF)

Digital tool name	Digital tool description present on tool page	Legend/variable key (descriptor for each variable)	Tools instructions present?	Continuous variables with sliders present	Discrete value entry present (if applicable for variables)	Able to exchange units (metrics vs. imperial)?
Mayo Clinic Anticoagulation Choice Decision Aid	Yes	Yes	Yes	N/A	Yes	N/A
Stroke Prevention in Atrial Fibrillation Risk Tool (SPARCtool)	Yes	Yes	Yes	N/A	Yes	No
GARFIELD-AF Risk Calculator	Yes	Yes	Yes	Yes	Yes	No
ACC AnticoagEvaluator Application	Yes	Yes	No	N/A	Yes	Yes
CardioSmart Atrial Fibrillation and Bleeding Risk Calculator	Yes	Yes	Yes	N/A	Yes	N/A

Table A2. Governance information for each digital tool for estimating stroke and/or bleeding risk in patients with atrial fibrillation (AF)

Digital tool name	Medical disclaimer?	Privacy policy accessible?	Terms and conditions accessible?	Frequently asked questions section	Feedback for tool improvement present?
Mayo Clinic Anticoagulation Choice Decision Aid	Yes	Yes	Yes	No	Yes
Stroke Prevention in Atrial Fibrillation Risk Tool (SPARCtool)	Yes	Yes	Yes	No	No
GARFIELD-AF Risk Calculator	Yes	Yes	Yes	Yes	Yes
ACC AnticoagEvaluator Application	Yes	Yes	Yes	No	No
CardioSmart Atrial Fibrillation and Bleeding Risk Calculator	Yes	Yes	Yes	Yes	No

ACCSCIENCE PUBLISHING

Brain & Heart

PERSPECTIVE ARTICLE

Pictorial rendition of author's observations on balloon valvuloplasty/angioplasty procedures: Aortic stenosis

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Abstract

Balloon aortic valvuloplasty (BAV) effectively decreases peak pressure gradients through the aortic valve both at the time of the procedure and during follow-up. This paper presents the author's observations on the utility of BAV in treating congenital aortic valve stenosis (AVS). Previous work by the author noted intermediate-term restenosis and late-onset aortic insufficiency (AI). Factors contributing to restenosis include age under 3 years and post-balloon residual aortic valve gradients exceeding 30 mmHg. Repeat balloon valvuloplasty has been found to address restenosis effectively. However, the onset of AI at late follow-up is a significant disadvantage of BAV. Despite this, BAV is currently considered a beneficial treatment option for managing AVS in children, adolescents, and young adults. In contrast, seniors with calcific AVS do not experience relief from obstruction with BAV and are candidates for aortic valve replacement through transcatheter methodology, a procedure not reviewed in this paper. BAV provides relief of obstruction across the aortic valve and functions as an alternative to surgical intervention. Consequently, BAV is considered a preferred choice in addressing aortic stenosis in the pediatric patient.

Keywords: Aortic stenosis; Balloon aortic valvuloplasty; Restenosis; Aortic insufficiency

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1. Introduction

Following a brief description of the historical aspects of transcatheter interventional procedures, a pictorial rendition of the author's published studies on balloon dilatation of pulmonary valve obstruction has been presented in a prior publication.[1] In this paper, a pictorial rendition of the author's studies on balloon aortic valvuloplasty (BAV) for aortic valve stenosis (AVS) is described.

2. Congenital AVS

The technique developed by Dotter, Grüntzig, and their associates[2-6] was employed by Lababidi *et al.*[7,8] to treat AVS. Following this development, BAV became the initial therapy of choice for treating congenital AVS.[9-11] The indications for BAV are analogous to those employed for surgical intervention, namely aortic valve peak pressure gradients higher than 70 mmHg or a gradient higher than 50 mmHg, along with symptoms and/or ST-T wave abnormalities.[9-12] In this section, the technique and results of BAV to treat valvar AVS are reviewed.

2.1. BAV technique

Most commonly, a retrograde arterial approach using one balloon is utilized for BAV (Figures 1 and 2); the balloon-annulus (BA) ratio is set between 0.8 and 1.0. If the aortic valve annulus is too large for one balloon, a dual balloon method (Figures 3 and 4) may be used. If a two-balloon method is utilized, the combined balloon diameter may be estimated by equation I[13,14]:

$$\text{Combined balloon diameter} = 0.82\ (D_1 + D_2) \qquad \text{(I)}$$

Since there is a potential for femoral artery occlusion in newborns and infants, different routes of approach, such as umbilical artery[15] and umbilical vein[16] (Figures 5 and 6), have been utilized for BAV. Other approaches, such as subscapular, axillary, and carotid artery, have also been advocated by some interventionalists; however, these routes of access are not popular.

3. Immediate results

Lababidi[8] was the earliest investigator to publish immediate outcomes of BAV; he reported the results of 23 children with AVS. The peak aortic valve pressure gradient was reduced from 113 ± 48 to 32 ± 15 mmHg ($p < 0.001$) after BAV. Mild aortic regurgitation developed

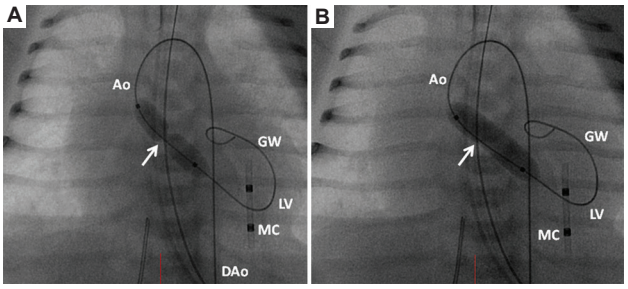

Figure 1. Diagrams illustrating the location of the balloon valvuloplasty catheter through the aortic valve in a neonate in anteroposterior projections. Waisting of the balloon is seen in frame A (arrow), which is completely abolished (arrow in B) on the additional filling of the balloon. The ascending aorta (Ao), descending aorta (DAo), guide wire (GW), left ventricle (LV), and marker catheter (MC) are shown. Reproduced from Agu and Rao.[12]

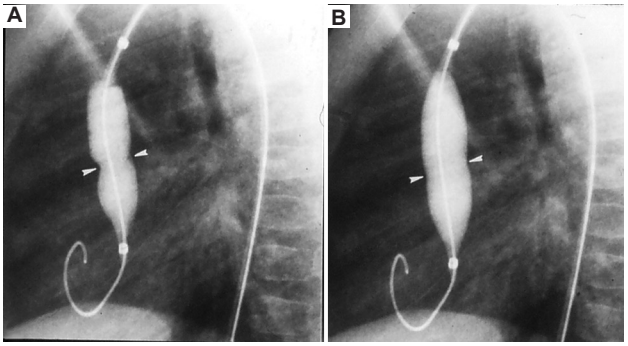

Figure 2. Diagrams illustrating the location of the balloon valvuloplasty catheter through the aortic valve in a child in lateral projections. Waisting of the balloon is seen in the frame a (arrow-heads), which is completely abolished (arrow-heads in b) on additional inflation of the balloon.

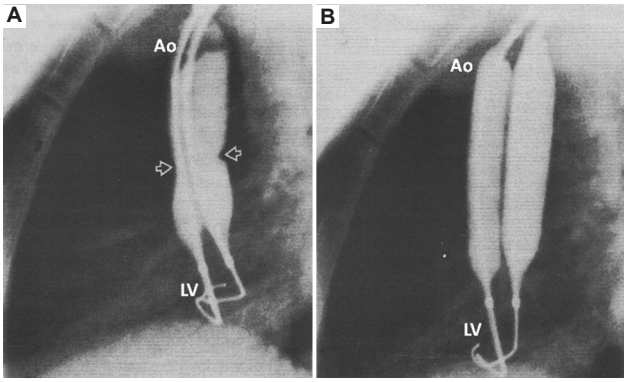

Figure 3. Diagrams illustrating the position of two balloon valvuloplasty catheters through the aortic valve in a patient in lateral projections. Waisting of the balloons is seen in frame A (arrows), which is totally eliminated after additional balloon inflation (B). The aorta (Ao) and left ventricle (LV) are labeled. Replicated from Rao.[10]

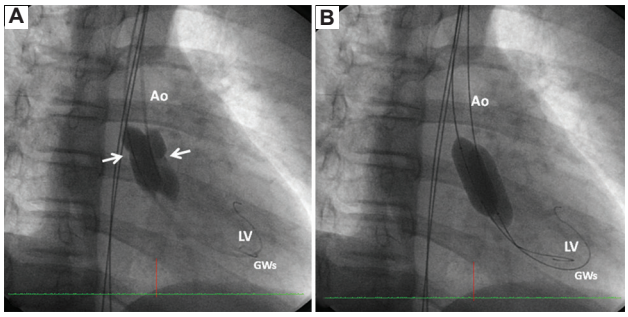

Figure 4. Diagrams illustrating the position of two balloon valvuloplasty catheters through the aortic valve in a child in right anterior oblique projections. Waisting of the balloons is seen in frame A (arrows), which is totally eliminated after additional balloon inflation (B). Aorta (Ao), guide wires (GWs), and left ventricle (LV) are labeled. Reproduced from Agu and Rao.[12]

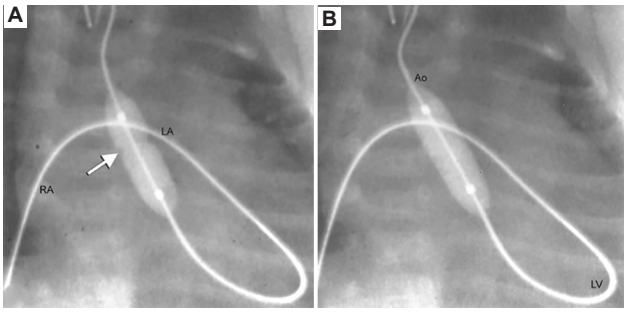

Figure 5. Diagrams illustrating the location of the balloon valvuloplasty catheter through the aortic valve in a neonate in anteroposterior projections. Waisting of the balloon is seen in frame A (arrow) which is completely abolished (arrow in B) on the additional balloon inflation. The ascending aorta (Ao), left atrium (LA), left ventricle (LV), and right atrium (RA) are shown. Reproduced from Rao.[17]

in 10 of these children, and two patients required surgical intervention. We evaluated the immediate outcomes of BAV in the late 1980s.[18] Subsequently, immediate outcomes for a higher number of patients were investigated.[19] Results of BAV for calcific AVS in the elderly are not included in this paper. Reduction in peak aortic valve pressure gradients (Figures 7-9) and left ventricular (LV) systolic and end-diastolic pressures was demonstrated following BAV; the cardiac index did not change.[18-20] A 60% drop in the aortic valve pressure gradient was observed (Figure 10). The extent of aortic insufficiency (AI) did not worsen (Figure 11), and none of the patients had grade 3+ AI. In fact, AI improved in some children, which was thought to be due to better coaptation of the aortic valve leaflets after BAV. Echocardiographic studies showed no change in the end-diastolic LV dimension, the LV posterior wall measurement in diastole, and the contractile function of the LV following BAV (Figure 12). Except for neonates, almost all children were discharged the day after the procedure. The immediate outcomes of BAV documented by other cardiologists[21-35] during the 5-year period (1983 – 1988) following the initial description of BAV are similar to the author's observations.[18,19] More recent studies of BAV reported between 2019 and 2023[36-62] also show outcomes similar to those described above.

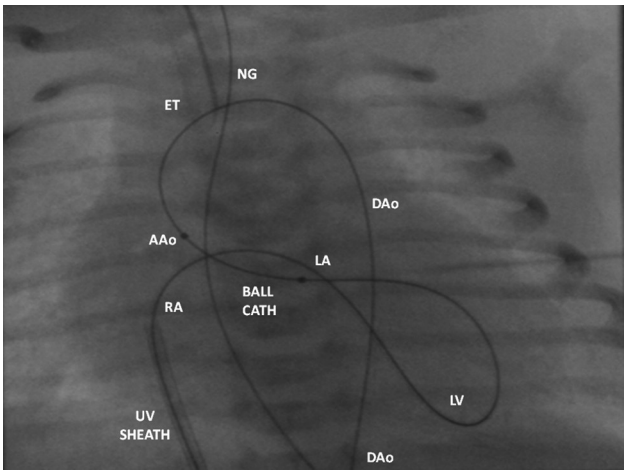

Figure 6. Diagram illustrating the course of the balloon valvuloplasty catheter (BALL CATH) across the aortic valve in a neonate in anteroposterior projections. The ascending aorta (AAo), descending aorta (DAo), endotracheal tube (ET), left atrium (LA), left ventricle (LV), a nasogastric tube (NG), right atrium (RA), and umbilical venous (UV) are shown. Reproduced from Agu and Rao.[12]

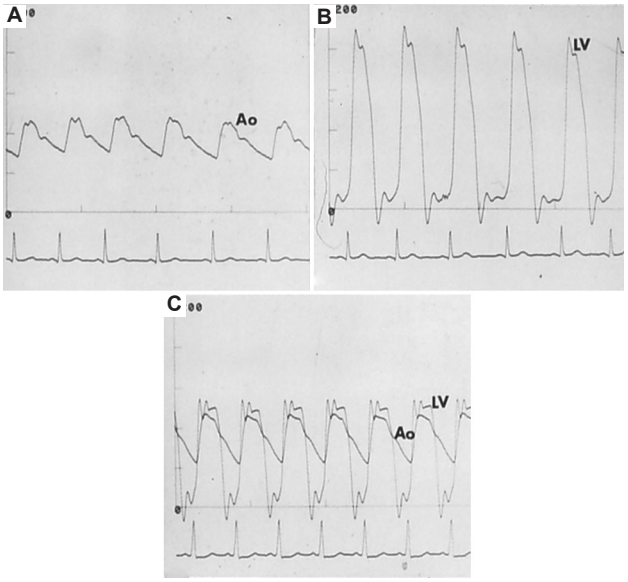

Figure 7. Pressure recordings from the aorta (Ao) (A) and left ventricle (LV) (B) before balloon aortic valvuloplasty (BAV) indicated a significant aortic valve gradient. Following BAV (C), the aortic valve pressure gradient is remarkably reduced. Reproduced from Rao.[10]

4. Intermediate-term results

Intermediate-term results, defined as 6 months to 2 years after BAV, have been evaluated by several authors. Short-term data reported by Lababidi *et al.*,[8] Walls *et al.*,[21] and others, as summarized elsewhere,[10] demonstrate sustained relief of aortic valve obstruction with residual aortic valve gradients in the high 30s to low 40s mmHg.[6,10,21] In the author's study subjects,[10,18,19] the peak-to-peak aortic valve systolic pressure gradients remained lower than pre-BAV aortic valve gradients (Figures 9 and 13). The decline in the pressure gradients was evident through both cardiac catheterization (Figure 9) and Doppler studies (Figure 13).[10,18,19] The end-diastolic diameter of the LV, posterior wall thickness of the LV in diastole, and the LV shortening fraction did not significantly alter ($p > 0.1$) at short-term follow-up. The magnitude of AI also remained unchanged in the short term.[17] Nonetheless, when assessing the outcomes of each child, recurrence of AVS (aortic valve peak gradient >50 mmHg) was observed in 23% of patients (Figure 14). During the author's early experience with BAV, four patients had surgical valvotomy, and subsequently, two children had a second BAV; these interventions were undertaken 9 months (median) following the first BAV. Short-term results documented by other researchers[36,38-40,42,48,51,52,53,55,57] were similar to the author's previous work.

Causes of restenosis were investigated[18] by examining differences between the good and poor results groups (Figure 15). The factors associated with poor outcomes

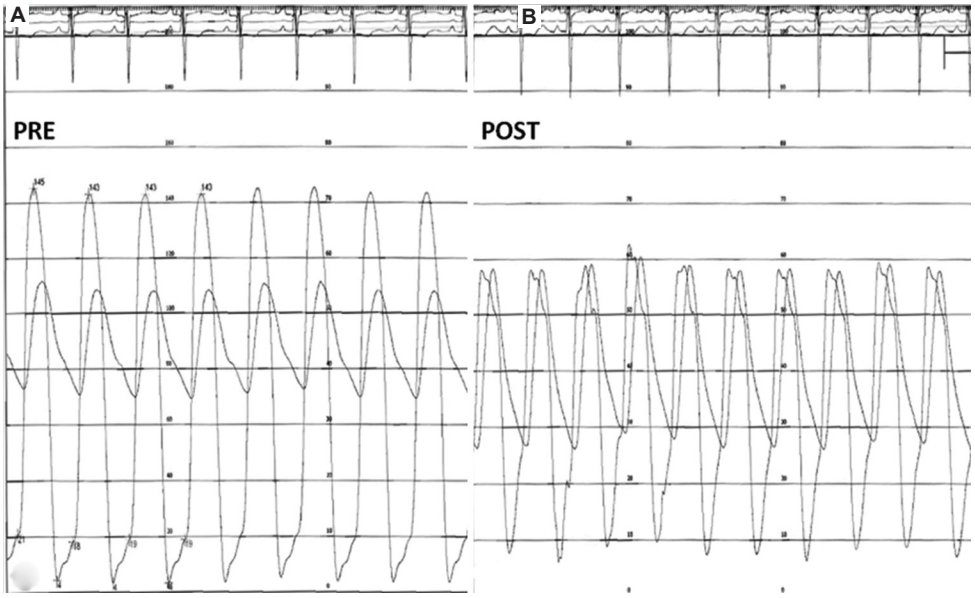

Figure 8. Pressure tracings from the left ventricle and aorta were recorded simultaneously (A) before balloon aortic valvuloplasty (BAV), indicating a significant aortic valve pressure gradient. Following BAV (B), the aortic valve pressure gradient was abolished. The fall in diastolic pressure in the aorta following BAV is probably due to the development of aortic insufficiency. Reproduced from Agu and Rao.[12]

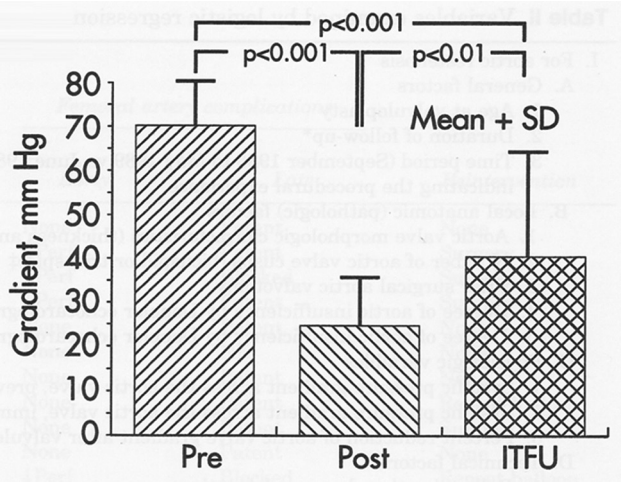

Figure 9. Graph depicting outcomes of balloon aortic valvuloplasty (BAV). Significant ($p < 0.001$) decline in aortic valve peak gradients after BAV is shown: Pre (before) vs. Post (after) BAV. Repeat cardiac catheterization at a mean of 16 months after BAV in 15 patients revealed an increase ($p < 0.01$) at intermediate-term follow-up (ITFU). However, the pressure gradients are lower ($p < 0.001$) than those before BAV. Reproduced from Galal *et al.*[19]

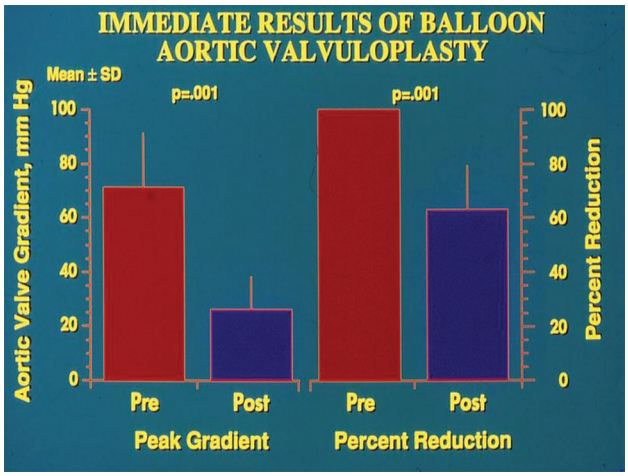

Figure 10. Graph depicting outcomes of balloon aortic valvuloplasty (BAV). Both the aortic valve peak gradients (left panel) and percentage drop (right panel) decrease ($p = 0.001$) after BAV. Mean ± standard deviation (*SD*) is indicated. Notes: Pre: before BAV; Post: after BAV. Reproduced from Rao.[11]

were found to be age under 3 years and post-BAV residual aortic gradients exceeding 30 mmHg.[18,19] Notably, the higher the number of risk factors, the greater the probability of obstruction (Figure 16).[18,19] Other investigators[63] studied this issue but could not identify any key factors. The re-stenosed valve can be successfully balloon dilated by repeat BAV (Figure 17).[64]

5. Late follow-up outcomes

Late follow-up (more than a median or mean of 5 years) outcomes post-BAV have been evaluated by several cardiologists. Hawkins *et al.*,[65] Kuhn *et al.*,[66] and Demkow *et al.*[67] observed the need for surgical intervention in 33% to 45% of patients at long-term follow-up, either to address recurrent AVS or newly developed AI. Reports by other investigators were summarized elsewhere.[75] In our

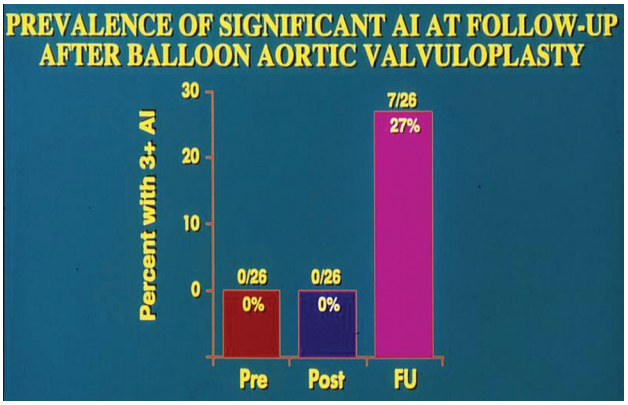

Figure 11. Diagram showing the occurrence of grade 3 aortic insufficiency (AI) before (Pre), the day after (Post) balloon aortic valvuloplasty (BAV), and at long-term follow-up (FU). No rise in AI was observed immediately after BAV. However, AI increased at long-term FU. Reproduced from Rao.[11]

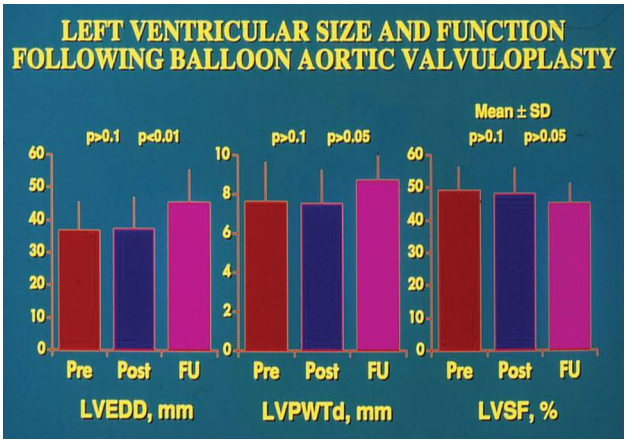

Figure 12. Diagram depicting left ventricular end-diastolic dimension (LVEDD) (left section), left ventricular posterior wall thickness in diastole (PWTd) (middle section), and left ventricular shortening fraction (LVSF) (right section) before (Pre), on the day following (Post), and at long-term follow-up (FU). The LVEDD (mm), LVPWTd (mm), and LVEF (%) did not change ($p > 0.1$) the day after balloon aortic valvuloplasty. However, the LVEDD increased ($p < 0.01$) at FU, but the LVPWTd and LVEF remained unaltered ($p > 0.05$). Mean ± standard deviation (*SD*) is indicated. Reproduced from Rao.[20]

study subjects.[19,20] there was a further reduction in peak-to-peak aortic valve gradients (Figure 13). However, the level of AI increased in the long term (Figure 18). The end-diastolic diameter of the LV increased, most probably due to the AI (Figure 12). However, there was no evidence of LV hypertrophy nor a reduction in LV systolic function (Figure 12). Reinterventions related to restenosis and AI were included in calculating re-intervention-free rates, which were 76 and 60% at 5- and 10-year follow-ups, respectively (Figure 19). The late findings we observed are generally similar to those found by other investigators[65-74] and were summarized in a prior publication.[75] We have

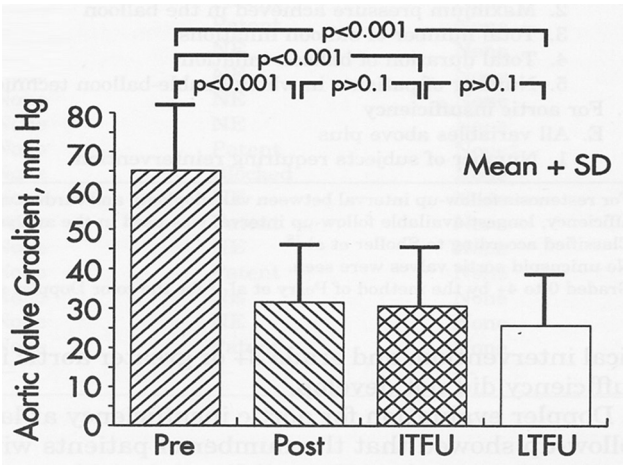

Figure 13. Graph depicting outcomes of balloon aortic valvuloplasty (BAV) as evaluated by Doppler evaluation. A significant ($p < 0.001$) decline in aortic valve peak instantaneous gradients after BAV was observed (Pre vs. Post). Repeat Doppler studies at intermediate-term (ITFU) and long-term (LTFU) evaluation revealed no change ($p > 0.1$). However, these Doppler gradients are significantly lower ($p < 0.001$) than pre-BAV values
Notes: Pre: before BAV; Post: after BAV. Replicated from Galal *et al.*[19]

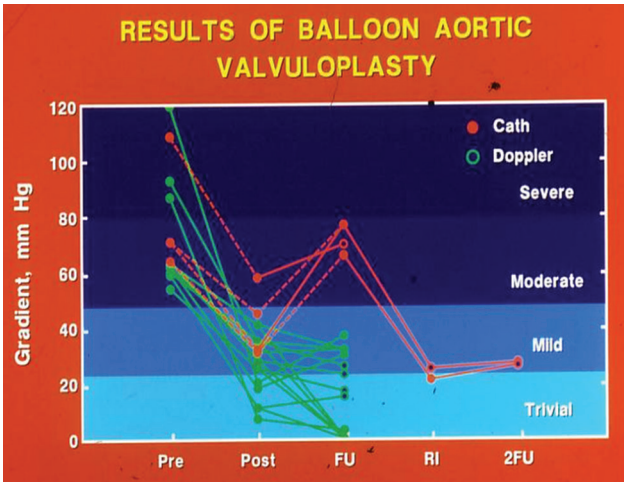

Figure 14. Display illustrating results of balloon aortic valvuloplasty (BAV). Children with good outcomes are depicted in green, while the poor outcome group is shown in orange. Solid circles indicate data secured at cardiac catheterization, while open circles indicate data obtained using Doppler interrogation. Trivial, mild, moderate, and severe gradients are marked in different color zones. In the good results group, the peak gradients across the aortic valve decreased significantly and remained decreased at follow-up (FU). In the poor results group, while the gradients fall after BAV, they increase at FU. Repeat BAV in some of these patients resulted in a fall of the gradients, which remained low at the second FU (2FU). When the magnitude of the aortic valve gradients was evaluated, the degree of obstruction declined in all children, falling from a higher to a lower category Notes: Pre: Before BAV; Post: Immediately after BAV; RI: Repeat intervention by BAV. Reproduced from Rao.[20]

investigated the causes of AI at late follow-up;[19] while the causes could not be identified, the degree of Doppler-

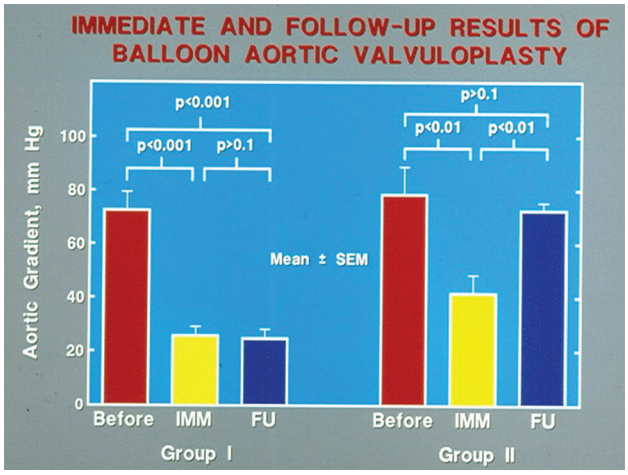

Figure 15. Graph illustrating the results of balloon aortic valvuloplasty (BAV) based on outcomes. In the favorable outcomes group (Group I), the aortic valve peak gradients fell ($p < 0.001$) immediately (IMM) after BAV and remained low ($p > 0.1$) at follow-up (FU) (left panel). In the poor results group (Group II), the aortic valve peak gradients fell ($p < 0.01$) immediately after BAV but increased ($p < 0.01$) at FU (right panel). Mean ± standard error of the mean (SEM) is indicated. Reproduced from Rao.[20]

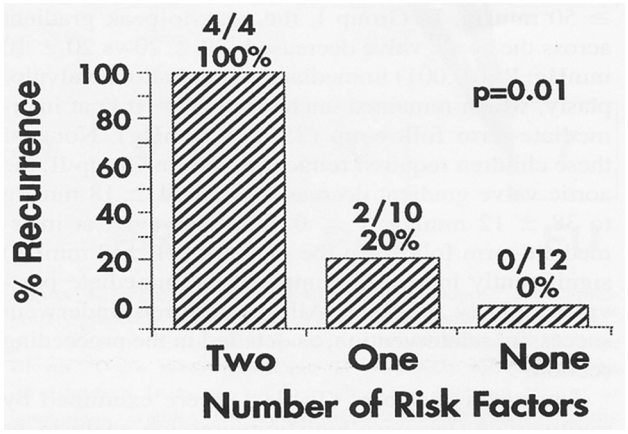

Figure 16. Chart illustrating the role of the number of risk factors on the frequency of restenosis following balloon aortic valvuloplasty. The higher the number of risk factors, the greater the likelihood of re-obstruction ($p = 0.01$). The actual number of patients and percentages are displayed on the cap of each bar. Reproduced from Rao.[11]

quantitated post-BAV AI predicts the late onset of AI (Figure 20). Large B/A ratios were thought to be producing AI both in animal models[76] and clinical cases.[76,77] Therefore, we plotted the relationship between the level of AI at long-term and B/A ratios (Figure 21) and were unable to demonstrate a positive relationship.[19]

6. Aortic stenosis in other age groups

The preceding sections provide an overview of the outcomes of BAV in children. BAV has also been proven

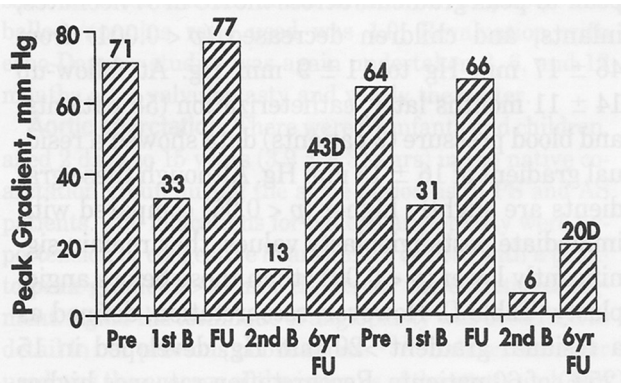

Figure 17. The role of repeat balloon aortic valvuloplasty (BAV) in two different patients. In both patients, peak aortic valve gradients fell after the first BAV (1stB), but at follow-up (FU), the gradient rose. On repeat BAV (2ndB), the pressure gradients decreased and stayed low at 6-year (6Yr) FU by Doppler (D) assessment. Note: Pre: Before BAV. Reproduced from Rao *et al.*[65]

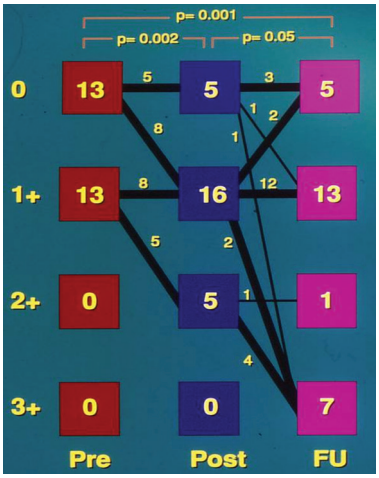

Figure 18. Diagram displaying the magnitude of aortic insufficiency (AI) evaluated in Doppler studies before (Pre), the day following (Post) balloon aortic valvuloplasty (BAV), and at long-term follow-up (FU). An increase ($p = 0.002$) in AI from pre-BAV to post-BAV was observed. At late FU, the AI increased further, with grade 3+ AI identified in seven out of 26 patients ($p < 0.02$). Reproduced from Galal *et al.*[19]

beneficial in relieving AVS in fetuses,[78-92] full-term[93-97], and premature[98-104] neonates, as well as in adolescents and young adults.[105] These age groups are not discussed further here, and interested readers may review the data in the cited references. In the elderly with calcific AVS, BAV was initially considered a useful technique.[10,106-109] However, subsequent evaluations revealed that BAV provides only temporary relief for calcific AVS in the elderly.[110,111] As a result, BAV is no longer recommended for this age group. Instead, the replacement of the aortic valve through transcatheter methodology[112] has become the procedure of choice.[112-114]

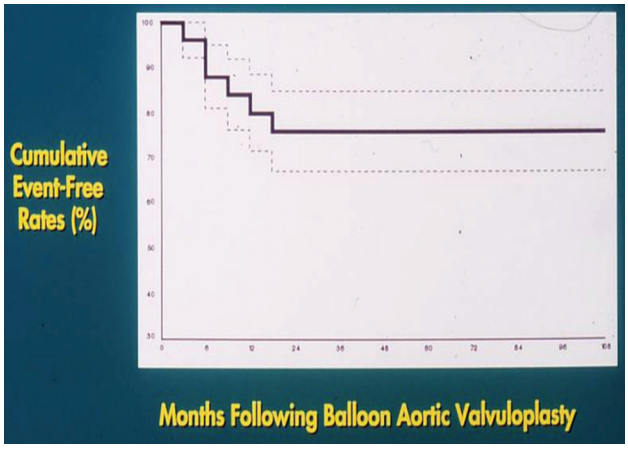

Figure 19. Graph depicting event-free rates following balloon aortic valvuloplasty. Confidence limits are set at 70%, indicated with dashed lines. Actuarial rates without intervention are 80%, 76%, 76%, and 76% at 1, 2, 5, and 9 years, respectively. Reproduced from Galal *et al.*[19]

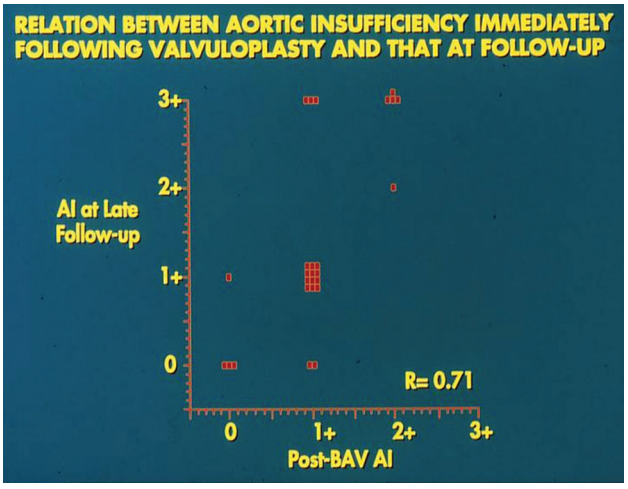

Figure 20. The magnitude of aortic insufficiency (AI) observed on the day following balloon aortic valvuloplasty (BAV) plotted against AI at long-term follow-up. There is a discernible relationship between these two parameters (R = 0.71). Reproduced from Galal *et al.*[19]

7. Summary and conclusions

Subsequent to the publication of BAV by Lababidi in 1983, the procedure has been widely adopted by cardiologists to manage AVS. The indications for BAV were similar to those utilized for surgery. The recommended B/A ratio was between 0.8 and 1.0. BAV is most frequently performed through the femoral artery; however, due to the potential for femoral artery injury in neonates, trans-umbilical arterial or trans-venous routes may be used. A decrease in aortic valve peak pressure gradients was detected immediately following BAV and in short-term follow-up. However, restenosis developed in 25% of patients, particularly in those under 3 years old and those with residual post-BAV gradients <30 mmHg. Repeat BAV emerges as a potential candidate for effectively addressing restenosis. Data from late follow-ups indicate persistent relief of aortic valve narrowing, no significant additional restenosis, and the onset of AI in 25% of patients. Actuarial rates of no re-intervention at 5 and 10 years after BAV were approximately 75% and 60%, respectively. Based on the author's own experience and that of others, it is concluded that BAV is the primary treatment choice for the relief of AVS. However, the occurrence of AI at late follow-up is significant. Methods to prevent the late onset of AI should be pursued. Given the late occurrence of AI, some cases requiring surgery, it is wise to closely adhere to guidelines for BAV intervention and to avoid BAV for milder or marginal gradients.

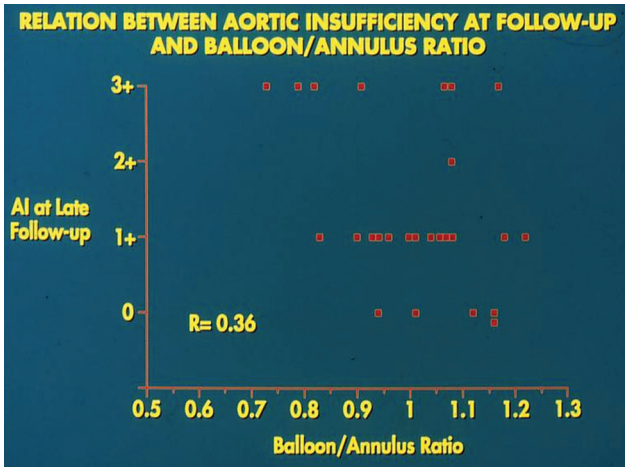

Figure 21. The magnitude of aortic insufficiency (AI) observed at late follow-up (assessed through Doppler) following balloon aortic valvuloplasty (BAV) plotted against balloon/annulus ratios used during BAV. There is an insignificant correlation between these values, with an R of 0.36, suggesting that the balloon/annulus ratios are unlikely to be a causative factor for the development of AI. It is further noted that grade 3+ AI was observed with large ranges of balloon/annulus ratios. Reproduced from Galal *et al.*[19]

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ACCSCIENCE PUBLISHING

Brain & Heart

PERSPECTIVE ARTICLE

Die-hard soccer fandom: A new cardiovascular risk factor

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Abstract

The relationship between the mind and the heart has been the subject of admiration for millennia. As early as 1500 BC, in the famous *Ebers Papyrus*, functions such as intelligence, thought, and moral conscience were attributed to the heart. While modern science later reassigned these functions to the brain, recent studies have partially vindicated the ancient Egyptians by discovering neural networks in the heart capable of learning, feeling, and perceiving autonomously, establishing a heartbrain dialog that remains largely unexplored. From the perspective of sports cardiology, recent publications have highlighted the potential cardiovascular risks associated with emotionally charged matches during events like the World Cup, particularly for fans of teams experiencing defeat. This paper presents a personal opinion based on years of research on this subject.

Keywords: Soccer; Cardiovascular risk factors; Acute coronary syndrome; Hostility; Cardiopsychology

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1. Introduction

"A heart-stopping match this weekend!"

Beyond being a typical headline in the sports press or a conversation starter, this phrase reflects a reality for many spectators of emotionally charged sports (cricket, curling, and chess enthusiasts can breathe a sigh of relief). Over the past 25 years, numerous scientific articles have highlighted the relationship between competitive sports and cardiovascular events, focusing not on the athletes but on the fans. This new approach is striking because the incidence of heart attacks is higher among fans than among players.

2. Concurrence of cardiovascular events relationship and relevant soccer games

The most relevant data comes from the soccer World Cups and national derbies. In almost all the World Cups, an increase in emergency room visits for cardiovascular causes, heart attacks, and even cardiovascular deaths has been observed. This phenomenon is most pronounced in finals decided by penalties and predominantly affects supporters of the losing team (as the saying goes, misfortune never comes alone).[1-6] An illustrative example is the penalty taken by "Crazy" Abreu during Uruguay's 2010 World Cup quarter-final match, 40 years after their past semi-final appearance, decided by a penalty shootout. He was the last one to take the spot kick and had the chance to secure victory for his

team. True to his nickname, he opted for a "Panenka style" shot (for those unfamiliar with the nuances of the "king sport"): a slow, central kick that would have been easily stopped had the goalkeeper not dived early, but it sailed into the net. It was unforgettable for those who watched it on television, but even more so for a Uruguayan family whose father suffered an acute myocardial infarction just at that moment, and unfortunately, his wife, witnessing the tragedy, experienced another type of heart attack known as takotsubo due to the sudden adrenergic discharge. Fortunately, both survived, but these incidents were important enough for the scientific community that the prestigious *New England Journal of Medicine* urged FIFA to suspend penalty shootouts for public health reasons. However, FIFA, seemingly inclined toward a "the show must go on" attitude, did not comply.

Cardiologists are not only concerned with heart attacks; arrhythmic events have also increased in these contexts, leading to more episodes of palpitation, syncope, and sudden death due to arrhythmias, ranging from benign extrasystoles to more serious conditions such as atrial fibrillation and ventricular tachycardia/fibrillation.[6] Young individuals predisposed to channelopathies face the risk of developing potentially fatal arrhythmias triggered by physical or emotional distress. This is a broad concept. For example, case reports highlight incidents such as two teenagers experiencing cardiac arrest from ventricular tachycardia while playing war video games, termed the "Fortnite phenomenon";[7] another case documented a 41-year-old woman experiencing cardiac arrest (with a primary detected rhythm of ventricular fibrillation) after a car accident. However, this was not due to the impact but rather to an emotional argument with the other driver.[8] In addition, a 25-year-old female patient was hospitalized due to syncope that occurred immediately after her first solo concert. The first emergency room electrocardiogram revealed polymorphic ventricular tachycardia, which degenerated into ventricular fibrillation requiring cardioversion.[9] These cases were subsequently diagnosed as channelopathies like catecholaminergic polymorphic ventricular tachycardia and long QT syndrome, both triggered by adrenergic stress in the absence of structural heart disease. Another reason to relativize.

3. Role of classical cardiovascular risk factors

Before readers move on to the next article or stop following their favorite team, two aspects deserve clarification. First, the emotions aroused by sports are not the only triggers for heart attacks and/or arrhythmias. Natural disasters, social conflicts, and, more recently, temporary shutdowns in social networks (which deserve urgent study) have shown their potential to induce cardiovascular incidents. Second, most of these pathologies are influenced not only by genetic factors but also by sociocultural ones. When investigating the profile of spectators most susceptible to these cardiovascular events, we typically find males over 65 years of age with a high prevalence of classic cardiovascular risk factors (high blood pressure, diabetes, hypercholesterolemia, obesity, sedentary lifestyle, or smoking habit). Moreover, these individuals often exhibit poor adherence to medication.

Interestingly, on several occasions, the patient was diagnosed with some cardiovascular risk factors during his first admission, often due to misconceptions embedded in social beliefs. High blood pressure does not necessarily cause headaches. It is possible to have diabetes without requiring insulin. Consuming a small wedge of cured cheese daily does not benefit cholesterol levels, especially when eaten excessively. Being obese is not a mere social construct. Working 8 h a day does not equate to healthy physical exercise. Contrary to popular belief, vaping is not a healthier alternative to smoking a traditional cigarette, which contains harmful substances such as tobacco, arsenic, and tar.

4. Impact of new cardiovascular risk factors

On this foundation, new cardiovascular risk factors such as environmental pollution, drug consumption, and stress come into play. Different studies have shown how negative emotions (hostility, anger, sadness, indignation, frustration, etc.) can trigger cardiovascular events.[10-14] This explains why, beyond the excitement of the soccer match itself, the outcome and the spectator's experience can significantly impact health outcomes: more heart attacks occur when their team loses and fewer when their team wins. For those academic readers interested in the mechanisms underlying this relationship, they lie beyond the scope of this perspective article. However, available data suggest that hostility has a dual effect on cardiovascular health:[15,16] Directly, through psychophysiological mechanisms linked to increased neuroendocrine and cardiovascular reactivity (altered hypothalamic-pituitary-adrenal axis leading to increased sympathetic nervous system activity, causing elevated autonomic system activity; insulin resistance; hypertension; exaggerated inflammatory response; endothelial dysfunction; atherosclerotic plaque rupture due to shear stress across the endothelial wall; platelet activation; etc.) and indirectly, by correlating with increased unhealthy behaviors (smoking, alcohol abuse, unhealthy diet, sedentary lifestyle, etc.).

Therefore, in today's world, where emotions such as fear, hostility, and frustration are gaining ground, it

Figure 1. The first team of the 11 most prevalent cardiovascular risk factors

is essential to promote cardiovascular health not only through a medical-pharmacological approach but also from a more bio-psycho-social perspective, encouraging educational campaigns for health promotion and disease prevention. Adopting healthy lifestyle habits could prevent up to 80% of cardiovascular diseases. The American Heart Association has developed "Life's Essential 8": key measures for improving cardiovascular health and lowering the risk for heart disease, stroke, and other major health problems. These include eating better, being more active, quitting tobacco, getting healthy sleep, managing weight, controlling cholesterol, and monitoring both glucose levels and blood pressure. From this perspective, the final objective is to beat the first team of the 11 most prevalent cardiovascular risk factors (Figure 1).

5. Conclusion

Finally, as a personal recommendation, when you watch a game for your favorite team, try to be prepared to prevent a heart attack from scoring against you. For this purpose: (i) when it comes to cardiovascular risk factors, "there is no small rival": avoid excesses, especially with alcohol and meals, as we rarely consume broccoli and fresh orange juice while watching the game. If you smoke, quit. It's the cancer of the team. Consider this advice as definitive: remember that "finals are not there to be played; they are there to be won." Take your necessary medication if you have high blood pressure, diabetes, high cholesterol levels, etc. Engage in regular physical exercise. I'm not suggesting you go all out from day 1, but get into the habit of moderate exercise, "match by match." (ii) Take care of your group of friends. Sometimes, a single person influences the bad habits of the group. In such a case, remember the words of Helenio Herrera: "It is better to play with 10 than with 11." (iii) When the match gets tense, practice emotion management strategies (breathing, mindfulness, or taking a "time out") and try to put things in perspective: you are playing for more than three points, and in reality, life happens between the World Cups.

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ACCSCIENCE PUBLISHING

Brain & Heart

ORIGINAL RESEARCH ARTICLE

Wine intake and 45-year mortality in middle-aged men with high alcohol consumption: The Italian rural areas of the Seven Countries Study

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Abstract

Research into the relationship between alcohol consumption and health has a long-standing history. Previous studies have revealed the beneficial effects of moderate alcohol consumption on cardiovascular disease (CVD) and all-cause mortality compared to abstainers and heavy drinkers. To study the long-term impact of wine intake on mortality, we conducted a study involving 1284 men aged 45 – 64 years in 1965, followed over a period of 45 years. We analyzed their wine-drinking habits in relation to all-cause mortality and specific causes using the Cox model. In addition, we utilized a multiple regression model with age at death as the dependent variable adjusted for age, smoking habits, body mass index, physical activity, dietary score, and comorbidity index. At baseline, 97.7% of participants were drinkers, consuming alcohol at an average of 77.4 g/day (mostly from wine). After 45 years, 98.4% of men had passed away. Our findings revealed a J-shaped relationship between alcohol intake and mortality from major CVD and all causes, while the relationship was roughly linear for cancer and liver cirrhosis. The relationship with CVD and all-cause mortality remained J-shaped, even when abstainers were excluded from the analysis, indicating potential health benefits for those consuming an average of 52 g/day (range: 47 – 70 g) and a 34% excess in mortality for those consuming an average of 176 g/day (range: 142 – 570 g). The average age at death for the reference class was 3.5 years higher compared to abstainers and 3.8 years higher compared to the upper class (average: 176 g/day). Reducing alcohol intake during the first 20 years of follow-up was beneficial in terms of life expectancy. In a lifetime follow-up, the relationship between alcohol consumption and mortality formed a J-shaped curve for CVD and all-cause mortality, even when excluding abstainers. Thus, relatively high wine consumption is more beneficial than lower intakes, especially when associated with vigorous physical activity at work – a common practice among rural men in the 1960s.

Keywords: Alcohol intake; Wine consumption; Cardiovascular disease mortality; All-cause mortality; Long-term follow-up

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1. Introduction

Research into the relationship between alcohol consumption and health has a long-standing history. Epidemiological findings consistently suggest a J-shaped relationship between alcohol consumption and cardiovascular disease (CVD) and all-cause mortality, highlighting beneficial effects among moderate drinkers compared to abstainers and heavy drinkers.[1-17] Recent contributions have specifically focused on wine rather than overall alcohol intake.[18-25] Therefore, an in-depth discussion comparing these findings to our own investigation is warranted.

Our study aimed to assess long-term CVD and all-cause mortality in a cohort of middle-aged men, nearly all of whom were habitual wine drinkers with very high alcohol consumption levels.[26,27] Notably, over two-thirds of subjects also engaged in heavy physical labor related to the rural environment approximately 60 years ago. This raises the question of whether high levels of physical activity and alcohol consumption might compensate for each other, potentially mitigating their individual impacts on survival, though the duration of this effect remains unclear. Therefore, we also investigated whether alcohol intake had differing effects on survival and age at death during the first 20 years of follow-up.

2. Methods

2.1. Population and baseline-line risk factors

The data analyzed in this study were derived from two Italian cohorts enrolled in the Seven Countries Study (SCS) of CVDs, started in 1958. This pioneering study was the first to systematically and comparably investigate multiple population samples of middle-aged men across various countries, focusing on lifestyle habits, risk factors for CVDs, and mortality in a long-term follow-up in different countries. Further details on the study design and methodology can be found elsewhere.[28]

In 1965, the population samples of the Italian rural areas of the SCS were re-examined on the fifth anniversary of their enrollment.[29] The cohort consisted of 1284 middle-aged men (aged 45 – 64). During this follow-up examination, alcohol intake was assessed using a dietary history questionnaire administered by trained and supervised nutritionists.[30] Alcohol consumption was reported in mL/day and converted to g/day based on the average alcohol content of local wine. The vast majority of participants consumed red wine, with an average alcohol content of 12%. Spirits with higher alcohol content played a minor role, accounting for only 3% of total alcohol intake. After 20 years of follow-up, an interim examination was conducted, during which alcohol consumption was reassessed following the same procedure among the survivors.

For the purpose of the analysis, several other personal characteristics were considered, including: (i) age, in years, approximated to the nearest birthday, (ii) body mass index, expressed as kg/m^2, calculated following the procedure outlined in the World Health Organization (WHO) Cardiovascular Survey Methods Manual,[29] (iii) cigarette smoking, quantified as the number of cigarettes smoked per day, derived from a questionnaire, (iv) working physical activity, classified as sedentary, moderate, or vigorous based on a questionnaire matched with reported occupation. These categories were validated using ergonometric procedures in a subgroup of subjects and calorie intake derived from the Dietary History,[31,32] and (v) dietary score, termed the Mediterranean Adequacy Index (MAI), derived from the population sample used for the SCS feasibility study. High MAI levels indicate a diet rich in vegetable-based food groups, olive oil, and fish, while low levels indicate a diet dominated by animal-based food groups, hard fats, and sugars.[33] In previous analyses, the MAI was inversely related to all-cause mortality[34] and coronary heart disease (CHD) mortality.[35] For this analysis, the MAI was recomputed, excluding wine from the vegetable food group to avoid mathematical conflicts with alcohol intake. The MAI score was transformed into its natural logarithm (lnMAI).[34,35] In addition, a comorbidity index was established by adding the number of major morbid conditions recorded during field examinations for each subject. These conditions included CHD, heart failure, arrhythmia or block of undefined origin, stroke, peripheral artery disease, chronic bronchitis, diabetes, and cancer (scores: 1 – 8).

2.2. Endpoints

The follow-up period spanned 45 years, during which the date and cause of death for all men were recorded and coded according to the WHO ICD-8 classification.[36] These codes were assigned based on defined criteria and allocated by a single reviewer. In the presence of multiple causes of death or uncertainties regarding the principal cause, a ranking system was adopted, prioritizing causes such as violence, cancer, CHD, stroke, and others in that order.

The primary end-points for analysis included mortality from all causes, major CVDs of atherosclerotic, hypertensive, and degenerative origin (CVD; ICD-8 codes 410 – 404, 427, 430 – 438, 441 – 445), cancer (ICD-8 codes 140 – 239), and liver cirrhosis (ICD-8 code 571). Moreover, age at death was expressed as the difference between the date of death and the date of birth for those who died during the 45-year follow-up period.

Baseline data were collected before the Helsinki Declaration era, with consent implied through participation in the examinations. Subsequently, verbal or written consent was obtained during follow-up data collection.

2.3. Statistical analysis

Alcohol intake was classified into six arbitrary classes, partly derived from the inspection of the interquartile distribution. Kaplan–Meier survival curves were generated based on these six classes of alcohol intake. Cox proportional hazard models were employed to assess associations with all-cause mortality and mortality from CVD, cancer, and liver cirrhosis, using alcohol intake and six confounding covariates as predictors. In addition, a multiple regression model was used with the same covariates and age at death as the dependent variable. Statistical significance was defined as $P < 0.05$.

Class 2 of wine consumption served as the reference category in multivariate models as it demonstrated the lowest risk for all-cause and cardiovascular mortality in the preliminary analyses.

Another Cox model was computed on the survivors after 20 years of follow-up, considering mortality over the subsequent 25 years as the end-point. Baseline alcohol consumption, changes in alcohol consumption over the 20-year period, and the usual confounding variables were included as covariates. Similarly, a multiple linear regression model was used to predict age at death. The reduced denominator for these models was based on 430 men.

3. Results

Out of 1284 men examined at entry, only 29 (2.3%) were not drinkers. The six classes of alcohol drinking (together with the correspondent wine equivalent) are given in Table 1, where the overall alcohol intake was very high, with an average of 76 g/day (77.4 for drinkers). Classes 2 and 3 covered almost half of the sample and had an average alcohol intake of 47 – 95 g/day, corresponding to roughly 500 – 1000 mL of wine.

During 45 years, there were 1264 deaths (98.4%), and the main causes of death were major CVDs (CVD = 44% of all deaths) and cancer (19% of all deaths), while liver cirrhosis covered 3% of all deaths.

Table 1. Alcohol and the correspondent wine consumption in the study population were divided into six arbitrary classes

Classification	*N*	%	Alcohol, g/day (range)	Wine equivalent, mL/day (range)
Class 0	29	2.3	0	0
Class 1	270	21.0	25 (0.6 – 46)	260 (6 – 483)
Class 2	332	25.9	52 (47 – 70)	543 (500 – 734)
Class 3	270	21.0	82 (71 – 95)	861 (750 – 1000)
Class 4	222	17.3	103 (95 – 134)	1085 (1001 – 1414)
Class 5	162	12.6	176 (142 – 570)	1860 (1500 – 6016)
All	1284	100	76 (0 – 570)	798 (0 – 6016)

Notes: Alcohol among drinkers only: mean=77.4 g/day; wine among drinkers only: mean=813 mL/day.

Kaplan–Meier survival curves as a function of the six classes of alcohol intake showed a large overlap and were difficult to read. Therefore, we show a simplified figure where only Class 2 and Class 5 are reported, showing a large difference, with Class 2 having the most favorable outcome (p of log-rank Chi-square 0.0006) (Figure 1). Class 2 was then chosen as a reference in the multivariate analysis, although it corresponded to four to seven drinks of wine per day.

Cox proportional hazard models with alcohol intake classes, six other covariates, and various mortality end-points as dependent variables are reported in Table 2, together with a multiple regression model using the same covariates and age at death as dependent variables. Mortality in Class 5 for all causes, CVD, and liver cirrhosis was significantly higher than in Class 2, which was used as a reference. This was not the case for mortality from cancer, and in this case, not even the difference between Class 5 and Class 0 was statistically significant, despite the presence of a weak, increasing trend from the lowest to the highest class. In the case of all causes and CVD, Class 0 had an excess of risk versus the reference class, but the difference did not reach significance. For these two endpoints, reference Class 2 carried the lowest risk. A definitely increasing mortality was recorded when liver cirrhosis was the endpoint.

Similar findings were obtained analyzing age at death, with Class 2 showing the highest level, declining for lower and higher alcohol intake classes, and a difference of 3.8 years between Class 2 and Class 5. The relationship of alcohol intake in the six classes with age at death is depicted in Figure 2.

In a correlation matrix including alcohol intake and the other covariates fed into the multivariate models,

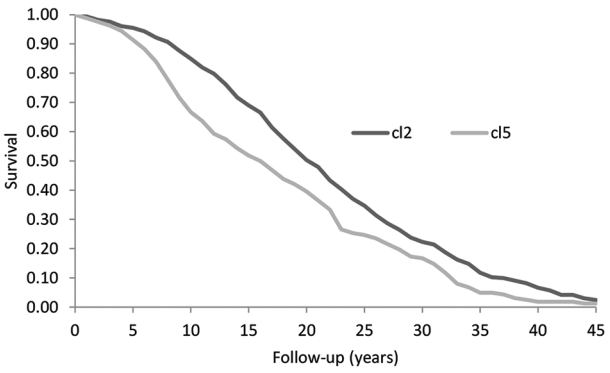

Figure 1. Kaplan–Meier survival curves for alcohol intake Class 2 (cl2) (average 52 g/day) and 5 (cl5) (average 176 g/day).

Table 2. Relationships of six classes of alcohol (wine) intake with all-cause and some specific causes of mortality and with age at death

Models and parameters	Hazard rate (95% CI [confidence interval]) for alcohol classes					
Cox models for 45-year mortality	**Class 0**	**Class 1**	**Class 2**	**Class 3**	**Class 4**	**Class 5**
All-cause	1.41 (0.97–2.07)	1.08 (0.92–1.27)	1.00 reference	1.13 (0.96–1.33)	1.08 (0.91–1.29)	1.34 (*) (1.10–1.62)
Cardiovascular disease	1.74 (1.02–2.96)	1.30 (1.03–1.65)	1.00 reference	1.12 (0.88–1.43)	1.03 (0.78–1.34)	1.34 (*) (1.00–1.82)
Cancer	0.75 (0.31–1.84)	0.87 (0.64–1.19)	1.00 reference	0.84 (0.62–1.15)	0.86 (0.62–1.18)	1.14 (0.81–1.62)
Liver cirrhosis	0.00 (n.a.)	0.73 (0.18–3.08)	1.00 reference	0.87 (0.19–3.28)	3.76 (*) (1.29–10.94)	6.43 (*) (2.23–18.52)
MLR model for age at death						
Age at death	−2.51 (−6.23 – 1.21)	−0.55 (−2.13 – 1.03)	1.00 reference	−1.12 (−2.70 – 0.46)	−0.99 (−2.67 – 0.68)	−2.85 (*) (−4.72 – [−0.98])
	Age at death (years)					
	72.7	74.7	76.2	74.1	74.2	72.4

Notes: Estimates of Cox models and multiple linear regression (MLR) are adjusted for age, BMI (body mass index), physical activity, cigarette smoking, lnMAI (natural logarithm of the Mediterranean Adequacy Index), and comorbidity index. (*) $p<0.05$ versus reference following the Cox model or MLR.

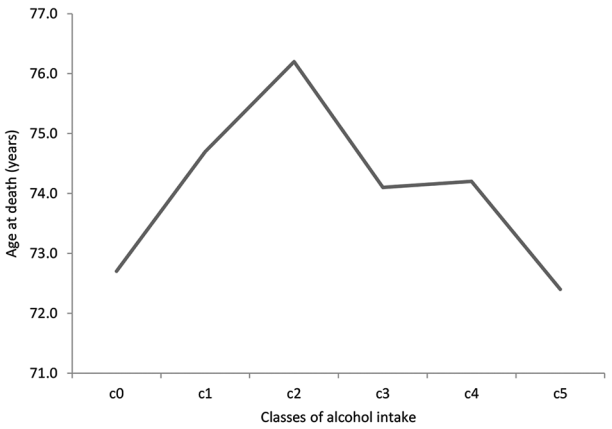

Figure 2. Age at death in six classes of alcohol intake (c0 [Class 0] through c5 [Class 5]), adjusted in a multiple linear regression model for age, physical activity, cigarette smoking, body mass index, lnMAI, and comorbidity index.

we found relatively high correlations between alcohol intake and working physical activity. In fact, average alcohol intake was 59 g/day in sedentary men, 67 g/day in men with moderate physical activity, and 84 g/day in men with vigorous physical activity. In cross-tabulation of three classes of physical activity with six classes of alcohol intake, resulting in a total of 18 cells, the highest age at death of 77.3 years was that with vigorous physical activity plus Class 2 of alcohol intake, while the lowest was 69.8 years in sedentary men plus Class 5 of alcohol consumption, i.e., a large difference of 7.5 years (Table 3). These estimates were derived from the multiple regression equation, and therefore, they were adjusted for the other covariates.

Findings related to comorbidity showed that alcohol intake Class 0 had the highest comorbidity index (0.59), while Class 1 had the lowest comorbidity index (0.36), suggesting that men who did not drink had the highest all-cause mortality, partly explained by an excess of prevalent diseases.

During the first 20 years of follow-up, alcohol consumption among survivors decreased from 73 to 44 g/day (i.e., a 29 g/day difference). The role of this time change in alcohol intake on all-cause mortality was tested in the Cox model of Table 4, where it appears that it was associated with significantly lower all-cause mortality during the next 25 years since the coefficient of change was negative and significant. The same conclusion was reached by the multiple linear regression of Table 4, where the reduction of alcohol intake was associated with a higher age at death during the next 25 years, roughly corresponding to 1 year for a reduction of 55 g of alcohol intake. In both models, the role of baseline alcohol intake was not significant.

4. Discussion

The specificity and potential interest of this analysis were bound to the following issues: (i) the universal wine intake of almost all participants; (ii) the extremely high alcohol intake that practically derived only from wine (facilitating analysis and conclusions); (iii) the extremely long follow-up that reached the quasi extinction of the cohort; and (iv) the fact that among the majority of subjects characterized by a high level of working physical activity, such a high alcohol intake was not necessarily dangerous.

Table 3. Age at death as a function of 3 classes of physical activity combined with six classes of alcohol intake. Data estimated from the multiple linear regression given in Table 2

Physical activity class	Alcohol intake class					
	0	1	2	3	4	5
	Age at death (years)					
1 (sedentary)	70.2	72.1	73.7	71.6	71.7	69.8
2 (moderate)	72.0	74.0	75.5	73.4	73.5	71.7
3 (vigorous)	73.8	75.8	77.3	75.2	75.4	73.5

Table 4. Models estimating all-cause mortality between year 20 and year 45 of follow-up and age at the death after 45 years of follow-up as a function of alcohol intake at year zero and changes in alcohol intake between year zero and year 20 of follow-up

Models and parameters	Coefficient	*t* value	*P* value
Cox model			
Alcohol intake: Year 0	−0.0020	−1.34	0.1799
Changes in alcohol intake between year 0 and year 20	−0.0027	−2.24	0.0250
MLR model			
Alcohol intake: year 0	0.0139	1.70	0.0893
Changes in alcohol intake between year 0 and year 20	0.0177	2.60	0.0098

Notes: Estimates are adjusted for entry levels of age, Mediterranean adequacy dietary index, physical activity, and cigarette consumption. Denominator=430. For the Cox model, end-point: all-cause mortality between years 20 and 45 of follow-up. For the multiple linear regression (MLR) model, the end-point is age at death after 45 years of follow-up.

In fact, 97.7% of subjects were drinkers, and this is probably a unique case. Moreover, wine consumption in the study population was very high, probably among the highest ever recorded in other studies, although precise comparisons were difficult since we expressed the intake in g/day of alcohol (and using our specific consumption classes) while consumption is frequently expressed as drinks per day. Approximately 70% of the population could be classified as heavy drinkers. The comparison of the various classes of alcohol intake with that of the nondrinkers was not promising, and therefore, we used Class 2 as a reference (about 52 g of alcohol/day), but due to the small numbers involved, the differences versus the reference class were not always statistically significant.

We were uncertain whether the few abstainers refrained from alcoholic beverages due to personal preference, experienced unpleasant reactions when drinking, or had stopped drinking due to illness or medical advice. However, their small number likely had minimal impact on our analysis. Even when excluding these abstainers, the mortality curves for total and CVD remained J-shaped. In fact, the relationship between alcohol (wine) intake showed a J-shaped pattern for both all-cause and CVD mortality, while it was direct and positive for liver cirrhosis and cancer (although with some uncertainties in the latter case).

The curvilinear relationship with age at death was even more relevant because the piece of analysis on all-cause death could have been biased by the fact that the study cohort was almost extinct. On the other hand, the age at death of the reference class was 3.5 years higher versus abstainers and 3.8 years higher versus men in Class 5. All of this was adjusted for the covariates. It is of interest that the abstainer class carried the highest comorbidity index across the six classes of alcohol consumption, partly explaining their excess mortality.

A peculiar relationship was identified between alcohol consumption and physical activity because the three classes of physical activity had an increasing intake of alcohol, from sedentary to moderate to vigorous physical activities. The consequence of this association was that everything else being equal, larger alcohol intake among very active men was still associated with a good outcome.

Changes in alcohol intake played a role in all-cause mortality and age at death starting from the 20-year mark of follow-up when another examination allowed for updates in alcohol consumption. At that point, the entry-level of alcohol intake was no longer significant, while a significant decrease over the previous 20 years was associated with lower all-cause mortality and higher age at death.

Early literature on the issue[1-10] consistently showed a J-shaped relationship between alcohol intake, CVD, and all-cause mortality. More recent meta-analyses and review articles[11-17] produced similar findings but added several exceptions and doubts. In particular, according to a review article, low alcohol consumption may be beneficial to prevent CHD but not other fatal conditions.[11] In a meta-analysis, alcohol intake of <30 g/day was protective of CHD mortality, but episodic heavy drinking carried a risk similar to that of the abstainers.[13] Moreover, the use of abstainers as reference groups seems to introduce a large bias.

In a large meta-analysis, a curvilinear relationship was identified between drinking and mortality risk, but there were uncertainties in interpretations due to large differences in the methods used to measure the exposure.[14] Another meta-analysis did not show any benefit for moderate drinking for all and some specific mortality causes.[15] In a recent meta-analysis, it was found that after adjustment for the role of abstainers and for the quality of the studies, low levels of alcohol intake did not provide benefits for all-cause mortality.[16] Finally, a meta-analysis involving only studies

that applied the Alcohol Use Disorders Identification Test score showed a dose-response with all-cause mortality, but the assessment was different depending on the population examined, and it was not always clear whether the abstainers included former drinkers.[17]

Among the references derived from the past 5 years, there were contributions explicitly dealing with wine intake that allow at least marginal comparisons with our findings. Over 500,000 participants were studied from the UK biobank cohort, and alcohol consumption was obtained from a questionnaire separating the various sources of alcohol.[18] In a median follow-up of 7 years, beer, cider, and spirits were significantly associated with an excess of all-cause mortality, CVD events, CHD, cerebrovascular events, and cancer. On the other hand, champagne, white wine, and red wine were associated with a significantly decreased risk for CHD only. The conclusion was that the type of alcoholic beverage may play a different role in its impact on health and disease. Taking as reference the debated problem of the French Paradox, the authors recognize that the J-shaped relationship of alcohol intake is related to CVD events, still fixing a limit for maximum protection of around 20 g of alcohol/day in the form of wine.[19] They invoke the use of randomized trials to clarify the situation, insisting on the fact that the protection may derive from the wine's micro-active components (such as resveratrol in red wine) and their potential action against inflammation and thrombosis.

In the Polish section of the PURE study covering over 2000 adults of both sexes, the majority of participants (55%) consumed low-alcohol drinks, 21% frequently used spirits, while the non-drinkers covered the residual 24%.[20] The current drinkers had a hazard ratio of 1.5 to develop diabetes and CVDs compared to never drinkers, while former drinkers were at excess risk of developing hypertension and CVDs.

In 16 cohorts of the WHO MONICA project, a total of over 142,000 subjects aged 50 years or older were studied for their alcohol intake and subsequent mortality risk.[21] The consumption of less than 10 g/day of alcohol, compared with abstainers, was associated with an 11% lower total mortality risk, while 20 or more g/day corresponded to an increased risk of 13%. Similar figures were found for CVD, while for cancer, no advantages were found for low intakes, but an increased 22% risk was found for an alcohol intake of 20 or more g/day. Moreover, it was shown that high-density lipoprotein cholesterol explained 2.9 and 18.7% of the association between low alcohol intake and total and cardiovascular mortality, respectively, reducing the previously estimated impact of alcohol consumption.

A narrative review[22] suggests that high alcohol intake is a risk factor for various major diseases, while low or moderate intake seems beneficial within terms of the U-shaped or J-shaped relationship, mainly in the case of major CVDs. However, it seems that low or moderate alcohol intake is frequently associated with other lifestyle behaviors. In a large meta-analysis that included 22 studies, wine intake was associated with a hazard ratio of 0.76 for CHD mortality and 0.83 for all CVD mortality, concluding that high wine intake might be harmful only in cases of advanced age, use of medications, and other diseases.[23] The review of 27 articles published between 2002 and 2020 tends to conclude that moderate wine intake (a drink of wine a day) might be helpful in reducing the risk of major CVDs and diabetes, mainly if red wine is used.[24] In another narrative review, including historical notes, the authors state that high alcohol intake might be a hazard for the incidence of cancer, liver disease, and some CVD conditions. Moreover, they express some doubts about the possible protective effect of red wine versus CHD in the absence of a final proof and recommend the use of red wine due to its high content of polyphenols.[25]

A limitation of the present study depends on the small numbers, which are still partially compensated by the extremely long follow-up and the presence of men only. Some strengths are the use of almost universal wine consumption in the study population as a source of alcohol, the limited number of abstainers, and the use of six major covariates as possible confounders, including a dietary score.

5. Conclusion

Our study suggests that over a lifetime, the relationship between wine consumption, red-one in the majority of cases and rated as alcohol intake, exhibits a J-shaped curve, particularly for CVD and all-cause mortality. This trend persists even when excluding abstainers. Moderate (or relatively high) wine consumption is more beneficial than lower intakes, especially when coupled with vigorous physical activity at work. However, recent meta-analyses have highlighted uncertainties, suggesting the need for further investigation. Notably, the potential interaction between alcohol intake and vigorous physical activity has not been adequately explored in previous studies, underscoring the relevance of our findings.

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Conflict of interest

The authors declare that they have no competing interests.

Author contributions

Conceptualization: All authors
Investigation: All authors
Writing-original draft: All authors
Writing-review & editing: All authors

Ethics approval and consent to participate

The board of directors of the various institutions involved in data collection was de facto playing the role of an ethical committee, approving the execution of the study on the basis of the local existing legislation by the date this investigation started. Baseline measurements were taken before the era of the Helsinki Declaration, and approval was implied in participation, while verbal or written consent was obtained for the collection of follow-up data.

Consent for publication

Participants gave their consent to publish their data in this study.

Availability of data

The data and computing codes are not available for replication because the original data are not publicly available, although the Board of Directors of the Study may evaluate specific requests for dedicated analyses.

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ACCSCIENCE PUBLISHING

Brain & Heart

ORIGINAL RESEARCH ARTICLE

In silico investigation of lipid-based compounds implicated in amyotrophic lateral sclerosis

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Abstract

Amyotrophic lateral sclerosis (ALS) is a fatal, currently incurable neurodegenerative disease that progressively affects the upper motor neurons in the brain and lower motor neurons in the spinal cord. A significant portion of patients exhibit abnormalities in lipid metabolism, which appear to contribute to denervation and subsequent motor neuron degeneration. This study aimed to computationally identify lipid-based compounds associated with ALS and elucidate their pathological and therapeutic roles to facilitate effective ALS diagnosis and treatment. The methodologies employed encompassed rational database searches, pharmacokinetic and target predictions, as well as docking and molecular dynamics simulations (MDS). The findings from the disease-to-lipids analysis identified 20 lipid compounds correlated with ALS, primarily categorized as potential inhibitors within the functional classes of vitamins, antibiotics, organic acids, and phytochemicals. Pharmacokinetic predictions revealed that only four compounds exhibited permeability across the blood–brain barrier, namely 4-hydroxynonenal, resveratrol, rotenone, and valproic acid. Docking simulations indicated the highest binding affinity for 4-hydroxynonenal with alkaline ceramidase 2 (–4.516 kcal/mol), resveratrol with carbonic anhydrase 6 (–7.070 kcal/mol), rotenone with cytochrome P450 2C19 (–7.378 kcal/mol), and valproic acid with glutamate carboxypeptidase 2 (–5.629 kcal/mol). Furthermore, MDS and molecular mechanics/generalized born surface area calculations demonstrated the stability and binding energies of the complexes under simulated physiological conditions. In summary, further investigation is warranted to explore the synergistic effects of resveratrol and valproic acid in ALS, the mechanisms underlying 4-hydroxynonenal adduct-assisted aggregate formation of certain enzymes in ALS, and the impact of prolonged human exposure to rotenone from marine food sources (such as fish) on the development of ALS.

Keywords: Amyotrophic lateral sclerosis; Blood–brain barrier; Resveratrol; Valproic acid; 4-Hydroxynonenal; Exposure to rotenone; Molecular targets; Docking

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1. Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal, currently incurable neurodegenerative disease that affects the upper motor neurons in the brain and lower motor neurons in the spinal cord and it progresses gradually, culminating in immobility and eventual death due to respiratory failure.[1-4] Neurons acquire monocarboxylates from the brain through monocarboxylate transporters, which are essential for energy metabolism in mitigating neurodegenerative diseases. A study indicated a significant decrease in the expression of sodium-coupled monocarboxylate transporter 1 (MCT1) and MCT1 in the motor neurons of ALS (hSOD1G93A) mice compared to wild-type mice.[5]

The brain harbors abundant lipids, and anomalies in lipid metabolism are prevalent among ALS patients.[6,7] Various factors influencing cerebrospinal fluid (CSF) dynamics in ALS patients include diminished arterial pulsations, disrupted sleep, compromised respiratory function, and abnormal aquaporin 4 expression.[8] A study has identified apolipoprotein B-100 in the CSF of sporadic ALS as a probable agent responsible for motor neuron debility and degeneration, as well as dysregulation of TDP-43 translocation.[9]

Furthermore, research indicates clear connections between metabolic components and ALS pathogenesis, suggesting the potential for additional molecular targets for therapeutic interventions.[4,10,11] Numerous studies have delved into lipid profiles in biofluids of ALS patients, pinpointing dysregulation of sphingolipids, including sphingomyelin and glycosphingolipids.[12-14] An extensive lipidomic investigation in $SOD1^{G93A}$ transgenic rats revealed minimal lipid alterations in the motor cortex, contrasting with substantial changes in the lipidome of the lumbar spinal cord.[15] In addition, a recent study highlighted significant lipid dysregulation in a $TDP\text{-}43^{Q331K}$ mouse model of ALS.[16] Herein, this study investigates lipid-based compounds linked to ALS, assessing pharmacokinetics, molecular binding affinities, and dynamics to enhance understanding of the disease and develop effective therapeutics.

2. Methods

2.1. Lipids dataset preparation

The term "Amyotrophic Lateral Sclerosis" was queried in the medical subject headings (MeSH) 2023 (www.meshb.nlm.nih.gov/search) to obtain the unique ID (D000690). This MeSH unique ID was then employed for disease-to-lipids analysis on the LipiDisease web server (www.cbdm-01.zdv.uni-mainz.de:3838/piyusmor/LipiDisease/) to extract metabolic and medicinal lipids, along with their corresponding associated *P*-values.

2.2. Target prediction analysis

The PubChem database (https://pubchem.ncbi.nlm.nih.gov/) was used to obtain the chemical structures of 20 identified lipid-based compounds in SMILES format. Subsequently, target prediction was carried out on the similarity ensemble approach (SEA) search server (http://www.sea.bkslab.org/)[17] using the SMILES notation of each compound.

2.3. *In silico* absorption, distribution, metabolism, excretion, and toxicity (ADMET) prediction

The SMILES notation of the compounds was used to evaluate the ADMET properties on the SwissADME server (www.swissadme.ch[18]) and ProTox II web server (https://tox-new.charite.de/protox_II/).

2.4. Molecular docking

The SMILES representation of the ligands was processed and saved in.mol format. Subsequently, PyMol software was employed to convert the ligand files from.mol to.pdb format. Before docking, the ligands' 3D structures were optimized using ACDLab/Chemsketch software. The 3D AlphaFold structures and subcellular locations of the proteins were directly retrieved from the UniProt database (https://www.uniprot.org). The preparation of ligand and protein docking files was performed using AutoDock Tools (ADT) v1.5.6.,[19] and the docking was implemented using AutoDock Vina v1.2.3,[20,21] following previous protocols.[22,23] Subsequently, ezLigPlot on the ezCADD web server[24] (http://dxulab.org/software) was used to visualize the binding interactions.

2.5. Molecular dynamics simulations (MDS)

Ligand-protein complexes with the highest docking binding affinity were used for this analysis. Each complex was preprocessed using Maestro's Protein Preparation Wizard, which included optimization and energy minimization. Desmond software by Schrödinger LLC was used to conduct a system simulation of 100 ns.[23,25,26] The system setup includes an OPLS-2005 force field, which is an orthorhombic box with a TIP3P water model containing 0.15 M NaCl counter ions to establish physiological conditions,[27] maintained at 300 K temperature and 1 atm pressure for the simulation. The models were relaxed before simulation, and trajectories were saved after every 100 ps. The post-simulation trajectory analysis was conducted to determine the root-mean-square fluctuation (RMSF), root-mean-square deviation (RMSD), and protein-ligand interaction profile. In addition, prime molecular

mechanics/generalized born surface area (MMGBSA) calculations were performed to determine the binding free energy (ΔG^{bind})[23,26,28] as follows:

$$\text{MMGBSA } \Delta G^{bind} = \Delta G^{Coulomb} + \Delta G^{Covalent} + \Delta G^{Hbond} + \Delta G^{Lipo} + \Delta G^{Packing} + \Delta G^{SolvGB} + \Delta G^{vdW} \quad \text{(I)}$$

3. Results

The disease-to-lipids analysis identified 20 lipid compounds that are correlated with ALS. These lipid compounds are predominantly recognized as potential inhibitors belonging to the functional class of vitamins, antibiotics, organic acids, and phytochemicals (Table 1).

Results from the pharmacokinetic prediction revealed that only four compounds are permeable through the blood–brain barrier (BBB), that is, 4-hydroxynonenal, resveratrol, rotenone, and valproic acid, while other compounds were predicted to be non-permeable through the BBB as singular entities (Table 2). These four compounds have high gastrointestinal absorption and moderate solubility, and they are not substrates for p-glycoprotein. However, resveratrol and rotenone could also inhibit various cytochromes, such as CYP2C9 and CYP3A4. The predicted toxicity for these four compounds indicated that the average lethal dose (LD_{50}) is as follows: Rotenone, 3 mg/kg; valproic acid, 670 mg/kg; resveratrol, 1560 mg/kg; and 4-hydroxynonenal 1925 mg/kg. Moreover, rotenone could potentially exert immunotoxic effects, while valproic acid has the potential for hepatotoxicity (Table 3).

Target prediction results of the four selected BBB permeant compounds (Table 4) suggest that 4-hydroxynonenal has good target precision for solute carrier organic anion transporter family member 2A1, all-trans-retinol dehydrogenase (NAD[+]) (ADH7), and alkaline ceramidase 2 (ACER2); resveratrol has good target precision for nuclear factor erythroid 2-related factor 2, amyloid-beta precursor protein, and transthyretin; rotenone has good target precision for cytochrome P450 2C19 (CYP2C19) and NADH-ubiquinone oxidoreductase chain 4 (MT-ND4); valproic acid has good target precision for glutamate-cysteine ligase catalytic subunit, mast cell carboxypeptidase A, glutamate carboxypeptidase 2 (FOLH1), and glutamate receptor ionotropic, kainate 1. The results were ranked based on *P*-value and the maximum Tanimoto coefficient (MTC),

Table 1. Lipid-based compounds obtained from the LipiDisease web server for amyotrophic lateral sclerosis (MeSH unique ID: D000690)

SN	Ligands	PubChem CID	*P*	FDR
1	4-Aminobutanoic acid	119	1.53E-25	3.35E-25
2	Minocycline	54675783	1.29E-20	2.68E-20
3	Coenzyme Q10	5281915	1.83E-14	3.55E-14
4	2S-Amino-3S-methylpentanoic acid	6306	5.84E-14	1.13E-13
5	Pyruvic acid	1060	3.23E-11	5.98E-11
6	Alpha-tocopherol	14985	7.36E-11	1.36E-10
7	4-Hydroxynonenal	5283344	8.88E-10	1.61E-09
8	Succinic acid	1110	3.78E-08	6.66E-08
9	Acetic acid	176	9.56E-08	1.67E-07
10	Malonic acid	867	3.01E-06	5.09E-06
11	Resveratrol	445154	5.16E-06	8.67E-06
12	Lipoic acid	6112	5.67E-05	9.25E-05
13	Propan-2-one	180	0.000186	0.000298
14	Rotenone	6758	0.000418	0.000662
15	Valproic acid	3121	0.000649	0.001021
16	Deoxycholic acid	222528	0.001557	0.002412
17	Prostaglandin E2	5280360	0.002854	0.004367
18	Hematoxylin	442514	0.019571	0.028625
19	Beta-carotene	5280489	0.024258	0.035251
20	Epigallocatechin 3-gallate	65064	0.029872	0.043105

Abbreviations: CID: Compound identifier; FDR: False discovery rate; MeSH: Medical subject headings; SN: Serial number.

Table 2. Absorption, distribution, metabolism, and excretion (ADME) properties from the SwissADME web server

SN	Ligands	MW	MR	TPSA (Å²)	Log P	ESOL Log S	ESOL Class	GIA	BBB permeant	P-gp	CYP inhibitor	Log Kp (cm/s)	BS	SA
1	4-Amino-butanoic acid	103.12	25.82	63.32	−0.72	1.72	Highly soluble	High	No	No	None	−9.18	0.55	1
2	Minocycline	457.48	119.14	164.63	0.23	−3.44	Soluble	Low	No	Yes	None	−8.16	0.11	5.14
3	Coenzyme Q10	863.34	280.5	52.6	15.24	−15.38	Insoluble	Low	No	Yes	None	2.22	0.85	8.28
4	2S-Amino-3S-methylpentanoic acid	131.17	35.44	63.32	−0.4	0.63	Highly soluble	High	No	No	None	−8.32	0.55	1.65
5	Pyruvic acid	88.06	18.51	54.37	−0.37	−0.11	Very soluble	High	No	No	None	−7.07	0.85	1
6	Alpha-tocopherol	430.71	139.27	29.46	8.27	−8.6	Poorly soluble	Low	No	Yes	None	−1.33	0.55	5.17
7	4-Hydroxynonenal	156.22	46.26	37.3	1.77	−1.46	Very soluble	High	Yes	No	None	−6.07	0.55	2.63
8	Succinic acid	118.09	24.89	74.6	−0.3	0	Very soluble	High	No	No	None	−7.44	0.85	1.29
9	Acetic acid	60.05	13.5	37.3	−0.09	−0.08	Very soluble	High	No	No	None	−6.82	0.85	1
10	Malonic acid	104.06	20.08	74.6	−0.65	0.16	Highly soluble	High	No	No	None	−7.51	0.85	1
11	Resveratrol	228.24	67.88	60.69	2.48	−3.62	Soluble	High	Yes	No	CYP1A2, CYP2C9, CYP3A4	−5.47	0.55	2.02
12	Lipoic acid	206.33	55.41	87.9	2.04	−1.85	Very soluble	High	No	No	None	−6.37	0.56	2.87
13	Propan-2-one	58.08	16.73	17.07	0.4	−0.17	Very soluble	High	No	No	None	−6.69	0.55	1
14	Rotenone	394.42	106.15	63.22	3.58	−4.98	Moderately soluble	High	Yes	No	CYP2C19, CYP2C9, CYP2D6, CYP3A4	−5.79	0.55	4.52
15	Valproic acid	144.21	42.34	37.3	2.12	−2.14	Soluble	High	Yes	No	None	−5.23	0.85	1.28
16	Deoxycholic acid	392.57	112.6	77.76	3.68	−4.21	Moderately soluble	High	No	Yes	None	−6.21	0.56	4.76
17	Prostaglandin E2	352.47	99.49	94.83	2.99	−3.01	Soluble	High	No	Yes	CYP2D6	−6.45	0.56	4.9
18	Hematoxylin	302.28	77.22	110.38	1.21	−2.87	Soluble	High	No	Yes	CYP1A2	−7.3	0.55	3.74
19	Beta-carotene	536.87	184.43	0	11.11	−11.04	Insoluble	Low	No	Yes	None	0.04	0.17	6.19
20	Epigallocatechin 3-gallate	458.37	112.06	197.37	1.01	−3.56	Soluble	Low	No	No	None	−8.27	0.17	4.2

Notes: Druglikeness: Bioavailability score (BS); Lipophilicity: Consensus Log P; Medicinal Chemistry: Synthetic accessibility (SA); Pharmacokinetics: Gastrointestinal absorption (GIA), blood–brain barrier (BBB), P-glycoprotein (P-gp) substrate, inhibition of cytochrome P450 (CYPs) type CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4, and skin permeation (Log Kp); Physicochemical properties: Molecular weight (MW), molar refractivity (MR), and total polar surface area (TPSA); Water solubility: Estimated solubility (ESOL) Log S and ESOL class.
Abbreviation: SN: Serial number.

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Table 3. Toxicity prediction results of the selected blood–brain barrier permeant compounds

SN	Properties	4-Hydroxynonenal	Resveratrol	Rotenone	Valproic acid
1	Predicted LD_{50} (mg/kg)	1925	1560	3	670
2	Predicted toxicity class	4	4	1	4
3	Hepatotoxicity	Inactive	Inactive	Inactive	Active
4	Carcinogenicity	Inactive	Inactive	Inactive	Inactive
5	Immunotoxicity	Inactive	Inactive	Active	Inactive
6	Mutagenicity	Inactive	Inactive	Inactive	Inactive
7	Cytotoxicity	Inactive	Inactive	Inactive	Inactive

Note: Class 1: Harmful if swallowed ($0.1 < LD_{50} \leq 5$); Class 2: Harmful if swallowed ($5 < LD_{50} \leq 50$); Class 3: Harmful if swallowed ($50 < LD_{50} \leq 300$); Class 4: Harmful if swallowed ($300 < LD_{50} \leq 2000$); Class 5: Harmful if swallowed ($2000 < LD_{50} \leq 5000$).
Abbreviations: LD_{50}: Average lethal dose; SN: Serial number.

which determines the similarity between compounds from the reference and query targets, where the highest similarity is denoted as 1.

The results of the cellular location of the targets and molecular docking binding affinity scores of the four selected BBB permeants are presented in Table 5. The results demonstrated the target proteins that are associated with the mitochondria, microtubule, cytoskeleton, postsynaptic cell membrane, lysosome, cytoplasm, cell membrane, and endoplasmic reticulum. In addition, the results indicated that 4-hydroxynonenal has the highest binding affinity for ACER2 (−4.516 kcal/mol); resveratrol has the highest binding affinity for carbonic anhydrase 6 (−7.070 kcal/mol); rotenone has the highest binding affinity for CYP2C19 (−7.378 kcal/mol); and valproic acid has the highest binding affinity for FOLH1 (−5.629 kcal/mol). The molecular binding interaction poses presented for some of the target binding affinities of 4-hydroxynonenal are presented in Figure 1; resveratrol (Figure 2); rotenone (Figure 3); and valproic acid (Figure 4).

The results of MDS studies are depicted in Figure 5. For the resveratrol-P23280 complex, the RMSDs of the protein and ligand are 8.5 and 13.5 Å, respectively, across the 0 – 100 ns timeframe (Figure 5A). RMSF analysis revealed a maximum fluctuation at the N- and C-terminal amino acid residues of P23280 (Figure 5B). The protein-ligand interactions involved specific amino acid residues, including ILE121, GLU167, VAL168, ASN170, GLU248, and GLU298 (Figure 5C). In the valproic acid-Q04609 complex, the RMSDs of the protein and ligand are 10.0 and 6.00 Å, respectively, across the same timeframe (Figure 5D). RMSF analysis indicated a maximum fluctuation at the N-terminal amino acid residues of Q04609 (Figure 5E). The protein-ligand interactions involved various amino acid residues, such as LEU261, ASN262, GLY263, ALA264, GLY265, GLY427, GLU522, THR687, and LYS699 (Figure 5F). For the rotenone-P03905 complex, the RMSDs of the protein and ligand are 2.4 and 24.0 Å, respectively, across the 0 – 100 ns timeframe (Figure 5G). RMSF analysis displayed a maximum fluctuation specifically for P03905 amino acid residues (Figure 5H). The protein-ligand interactions involved amino acid residues such as LYS3, LEU39, LEU40, ASN43, PHE50, LEU181, and PHE457 (Figure 5I). Overall, the protein-ligand interaction profile validated the amino acid residues present in the docking interactions. The MMGBSA results (Table 6) indicated a binding energy ΔG^{bind} (Total) of −34.372 and −52.740 kcal/mol for the resveratrol-P23280 complex at 0 and 100 ns, respectively; for the valproic acid-Q04609 complex, the values were −47.477 and 326.070 kcal/mol; for the rotenone-P03905 complex, the values were −53.558 and −32.824 kcal/mol, respectively. These calculations suggest that the resveratrol-P23280 complex was the only stable and energetically favorable complex under the simulated physiological conditions.

4. Discussion

Medicinal plants are being investigated as a rich source of phytochemicals with potential for treating ALS.[22,29] In this study, 20 lipid-based compounds have been identified, including antibiotics (minocycline), intermediary metabolites (pyruvic acid, malonic acid, and succinic acid), and functional antioxidants (e.g., lipoic acid, coenzyme Q10, beta-carotene, and alpha-tocopherol). These antioxidants, along with Vitamin E, carotenes, resveratrol, epigallocatechin gallate, coenzyme Q10, and melatonin, have been explored for their potential in managing ALS by modulating the oxidative stress pathway, including activating the KEAP1-NRF2 system.[30,31] Compounds such as glutathione, Vitamin E (alpha-tocopherol), and propyl gallate have demonstrated the ability to prevent the impairment of glucose and glutamate transport in the NSC-19 cell line with a motor neuron phenotype.[32]

Table 4. Target prediction results of the selected blood–brain barrier permeant compounds

Compound	Target gene code	Target description	*P*	MTC
4-Hydroxynonenal	SLCO2A1	Solute carrier organic anion transporter family member 2A1	2.288e-41	0.35
	ADH7	All-trans-retinol dehydrogenase (NAD[+]) ADH7	1.921e-39	0.31
	ACER2	Alkaline ceramidase 2	9.362e-36	0.28
	ADH1C	Alcohol dehydrogenase 1C	1.652e-26	0.38
	PDCD4	Programmed cell death protein 4	1.194e-24	0.30
	ADH1A	Alcohol dehydrogenase 1A	9.197e-23	0.38
	ADH1B	All-trans-retinol dehydrogenase (NAD[+]) ADH1B	3.546e-21	0.38
	OXER1	Oxoeicosanoid receptor 1	8.999e-19	0.48
	PTGFR	Prostaglandin F2-alpha receptor	1.11e-16	0.35
Resveratrol	NFE2L2	Nuclear factor erythroid 2-related factor 2	6.382e-54	1.00
	APP	Amyloid-beta precursor protein	1.581e-31	1.00
	TTR	Transthyretin	5.249e-25	1.00
	CA3	Carbonic anhydrase 3	6.151e-25	1.00
	NQO2	Ribosyldihydronicotinamide dehydrogenase [quinone]	1.887e-22	1.00
	CA5B	Carbonic anhydrase 5B, mitochondrial	2.56e-20	1.00
	CA6	Carbonic anhydrase 6	1.939e-19	1.00
	CA5A	Carbonic anhydrase 5A, mitochondrial	2.297e-19	1.00
	CYP1B1	Cytochrome P450 1B1	8.018e-19	1.00
	PTGS1	Prostaglandin G/H synthase 1	4.92e-17	1.00
	CA14	Carbonic anhydrase 14	1.11e-16	1.00
	TUBB1	Tubulin beta-1 chain	2.082e-47	0.38
	ALDH5A1	Succinate-semialdehyde dehydrogenase, mitochondrial	1.973e-43	0.34
	ABAT	4-aminobutyrate aminotransferase, mitochondrial	6.58e-28	0.34
	RELA	Transcription factor p65	7.873e-20	0.41
	AKR1B10	Aldo-keto reductase family 1 member B10	1.221e-15	0.46
Rotenone	CYP2C19	Cytochrome P450 2C19	0.9217	1.00
	MT-ND4	NADH-ubiquinone oxidoreductase chain 4	4.162e-69	0.60
Valproic acid	GCLC	Glutamate-cysteine ligase catalytic subunit	3.594e-67	0.38
	CPA3	Mast cell carboxypeptidase A	1.377e-54	0.31
	FOLH1	Glutamate carboxypeptidase 2	7.041e-32	0.42
	GRIK1	Glutamate receptor ionotropic, kainate 1	9.514e-32	0.62
	GRIK2	Glutamate receptor ionotropic, kainate 2	4.445e-27	0.62
	MME	Neprilysin	1.33e-27	0.41
	CPA1	Carboxypeptidase A1	4.052e-18	0.38

Abbreviation: MTC: Maximum Tanimoto coefficient.

From the 20 identified lipid-based compounds, four of these compounds (i.e., 4-hydroxynonenal, resveratrol, rotenone, and valproic acid), predicted to be BBB permeants, were examined further. The capacity to penetrate the BBB is crucial in drug discovery due to its impact on drug access to the brain (upper motor neurons) and central nervous system (lower motor neurons). High gastrointestinal absorption is dependent on the high solubility and low lipophilicity of the compound, subsequently defining the bioavailability and the pharmacological or toxicological effects. The toxicity prediction test, utilizing the ProTox-II online tool, categorizes compounds into six toxicity classes (1 – 6) with predicted LD_{50} (mg/kg) based on available online databases.[33] Ligand-based target prediction is a reliable and useful method in the drug discovery process for target identification and assay development. The SEA

Table 5. Cellular location of the targets and molecular docking binding affinity scores

Compound	Target	Target description	UniProt ID	Amino acid length	Subcellular location	Binding affinity (kcal/mol)
4-Hydroxynonenal	SLCO2A1	Solute carrier organic anion transporter family member 2A1	Q92959	643	Cell membrane, cytoplasm, lysosome	−4.142
	ADH7	All-trans-retinol dehydrogenase (NAD[+]) ADH7	P40394	386	Cytoplasm	−3.747
	ACER2	Alkaline ceramidase 2	Q5QJU3	275	Golgi apparatus membrane	−4.516
	ADH1C	Alcohol dehydrogenase 1C	P00326	375	Cytoplasm	−3.863
	PDCD4	Programmed cell death protein 4	Q53EL6	469	Cytoplasm, nucleus	−3.510
	ADH1A	Alcohol dehydrogenase 1A	P07327	375	Cytoplasm	−4.363
	ADH1B	All-trans-retinol dehydrogenase (NAD[+]) ADH1B	P00325	375	Cytoplasm	−4.295
	OXER1	Oxoeicosanoid receptor 1	Q8TDS5	423	Cell membrane	−3.980
	PTGFR	Prostaglandin F2-alpha receptor	P43088	359	Cell membrane	−4.313
Resveratrol	NFE2L2	Nuclear factor erythroid 2-related factor 2	Q16236	605	Cytoplasm, nucleus	−6.733
	APP	Amyloid-beta precursor protein	P05067	770	Cell membrane, cytoplasm, nucleus	−6.245
	TTR	Transthyretin	P02766	147	Amyloid, cytoplasm, secreted	−5.435
	CA3	Carbonic anhydrase 3	P07451	260	Cytoplasm	−5.947
	NQO2	Ribosyldihydronicotinamide dehydrogenase (quinone)	P16083	231	Cytoplasm	−6.093
	CA5B	Carbonic anhydrase 5B, mitochondrial	Q9Y2D0	317	Mitochondrion	−6.894
	CA6	Carbonic anhydrase 6	P23280	308	Secreted	−7.070
	CA5A	Carbonic anhydrase 5A, mitochondrial	P35218	305	Mitochondrion	−6.128
	CYP1B1	Cytochrome P450 1B1	Q16678	543	endoplasmic reticulum membrane, microsome membrane, mitochondrion	−6.715
	PTGS1	Prostaglandin G/H synthase 1	P23219	599	endoplasmic reticulum membrane, microsome membrane	−6.214
	CA14	Carbonic anhydrase 14	Q9ULX7	337	Cell membrane	−6.167
	TUBB1	Tubulin beta-1 chain	Q9H4B7	451	Cytoplasm, cytoskeleton, microtubule	−5.958
	ALDH5A1	Succinate-semialdehyde dehydrogenase, mitochondrial	P51649	535	Mitochondrion	−6.775
	ABAT	4-Aminobutyrate aminotransferase, mitochondrial	P80404	500	Mitochondrion	−6.924
	RELA	Transcription factor p65	Q04206	551	Cytoplasm, nucleus	−5.980
	AKR1B10	Aldo-keto reductase family 1 member B10	O60218	316	Lysosome, secreted	−7.007

(Cont'd...)

Table 5. ***(Continued)***

Compound	Target	Target description	UniProt ID	Amino acid length	Subcellular location	Binding affinity (kcal/mol)
Rotenone	CYP2C19	Cytochrome P450 2C19	P33261	490	Endoplasmic reticulum membrane, microsome membrane	−7.378
	MT-ND4	NADH-ubiquinone oxidoreductase chain 4	P03905	459	Mitochondrion inner membrane	−7.279
Valproic acid	GCLC	Glutamate-cysteine ligase catalytic subunit	P48506	637	Cytosol, mitochondrion	−4.603
	CPA3	Mast cell carboxypeptidase A	P15088	417	Cytoplasmic vesicle	−4.703
	FOLH1	Glutamate carboxypeptidase 2	Q04609	750	Cell membrane	−5.629
	GRIK1	Glutamate receptor, ionotropic kainate 1	P39086	918	Postsynaptic cell membrane	−4.410
	GRIK2	Glutamate receptor, ionotropic kainate 2	Q13002	908	Postsynaptic cell membrane	−4.447
	MME	Neprilysin	P08473	750	Cell membrane	−4.535
	CPA1	Carboxypeptidase A1	P15085	419	Secreted	−4.162

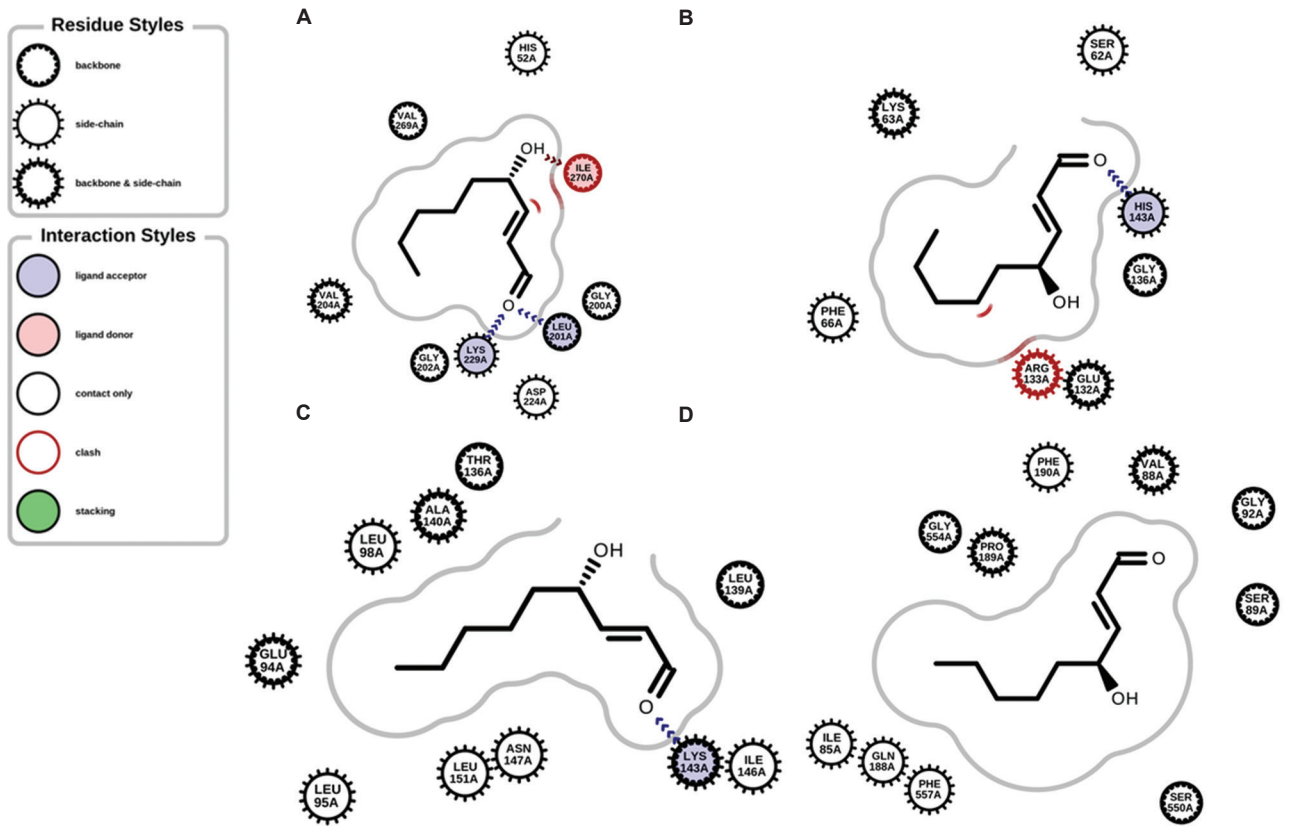

Figure 1. Interaction of the binding poses: (A) 4-hydroxynonenal and P07327, (B) 4-hydroxynonenal and P43088, (C) 4-hydroxynonenal and Q5QJU3, and (D) 4-hydroxynonenal and Q92959

Search Server was utilized to explore potential molecular targets for organic compounds, generating a list of MTC and *P*-values for result interpretation.[17] MTC ranges from 0 to 1, with higher values indicating greater similarity than lower ones, but the value does not necessarily connote that the compounds are identical to those curated in the database.

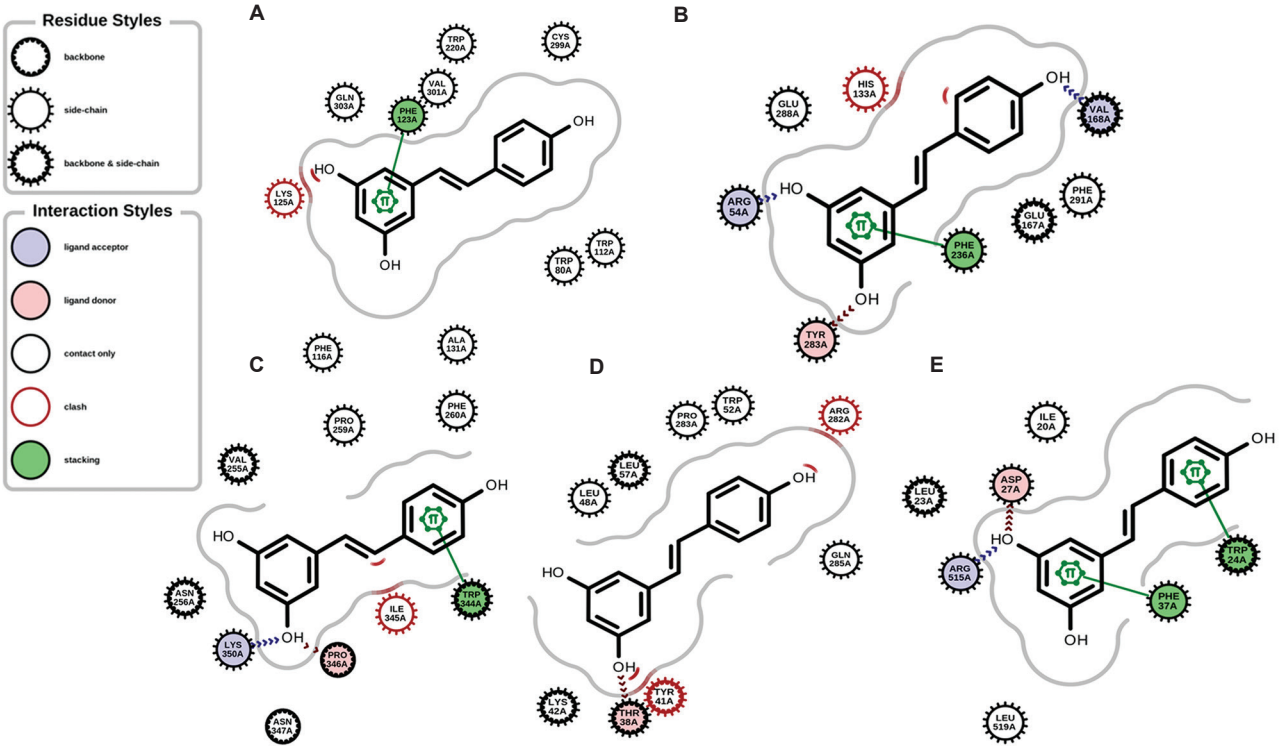

Figure 2. Interaction of the binding poses: (A) resveratrol and O60218, (B) resveratrol and P23280, (C) resveratrol and Q9H4B7, (D) resveratrol and Q9Y2D0, and (E) resveratrol and Q16236

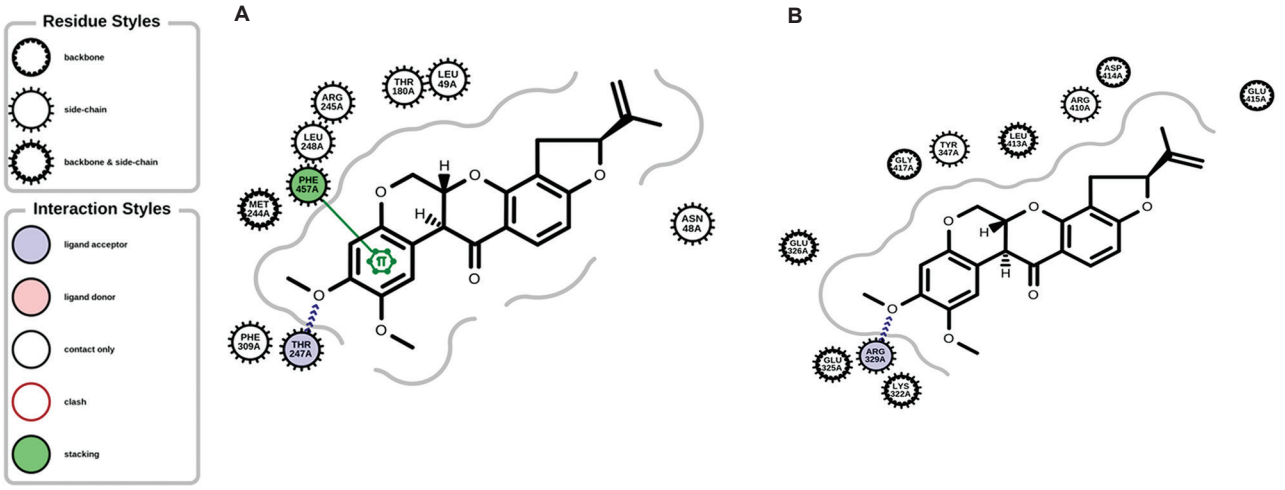

Figure 3. Interaction of the binding poses: (A) rotenone and P03905 and (B) rotenone and P33261

Resveratrol, a polyphenol found in various plants, exhibits potential neuroprotective activity by affecting processes such as nuclear factor-kappa B (NF-κB) activation and activator protein-1 DNA binding.[29,34] The administration of resveratrol has displayed protective effects on rat cortical motor neurons against the toxicity of CSF in ALS patients.[2] In addition, oral intake of resveratrol at 160 mg/kg/day improved motor abilities and prolonged the lifespan of ALS mice.[35] Furthermore, in the SOD1^{G93A} mouse model of ALS, a resveratrol-enriched diet demonstrated protective effects associated with increased expression and activation of sirtuin 1 and AMPK in the ventral spinal cord.[36] Delivery of resveratrol to the brain through nanocarriers has been suggested to optimize its pharmacokinetics and effectiveness in preventing and treating neurological disorders.[37] However, recent research

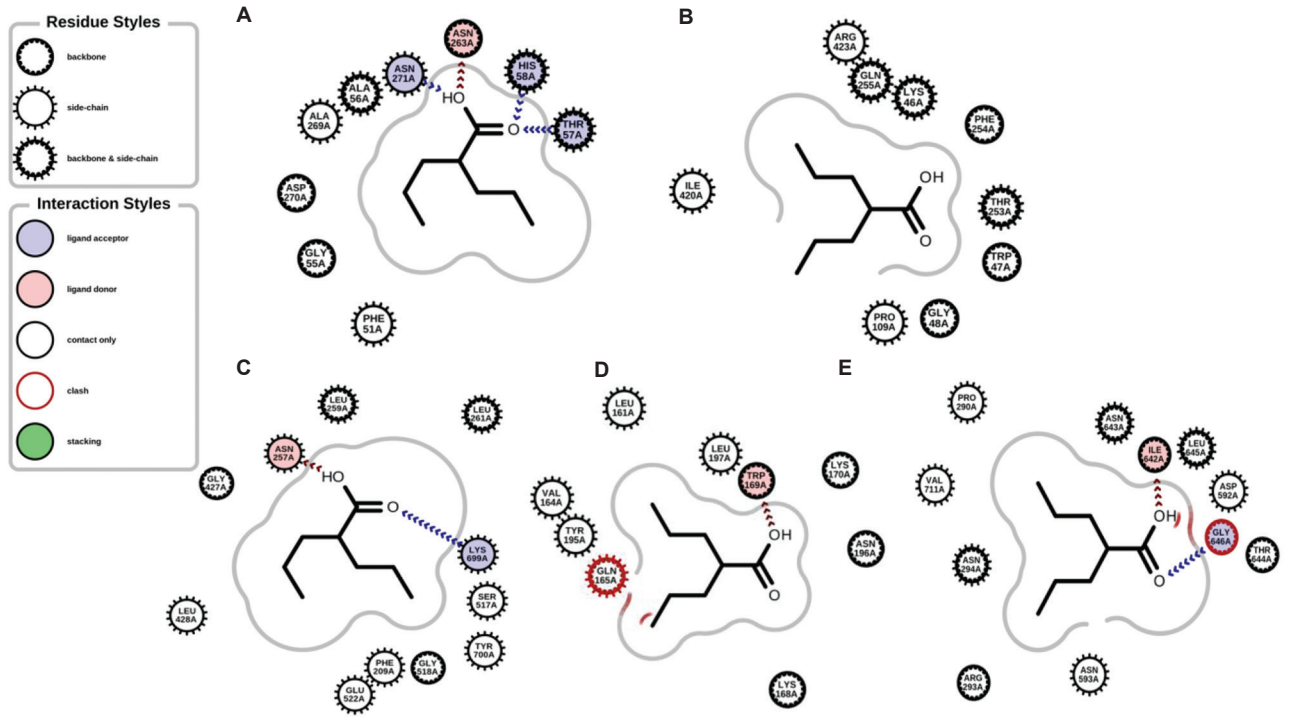

Figure 4. Interaction of the binding poses: (A) valproic acid and P15088, (B) valproic acid and P48506, (C) valproic acid and Q04609, (D) valproic acid and Q13002, and (E) valproic acid and P08473

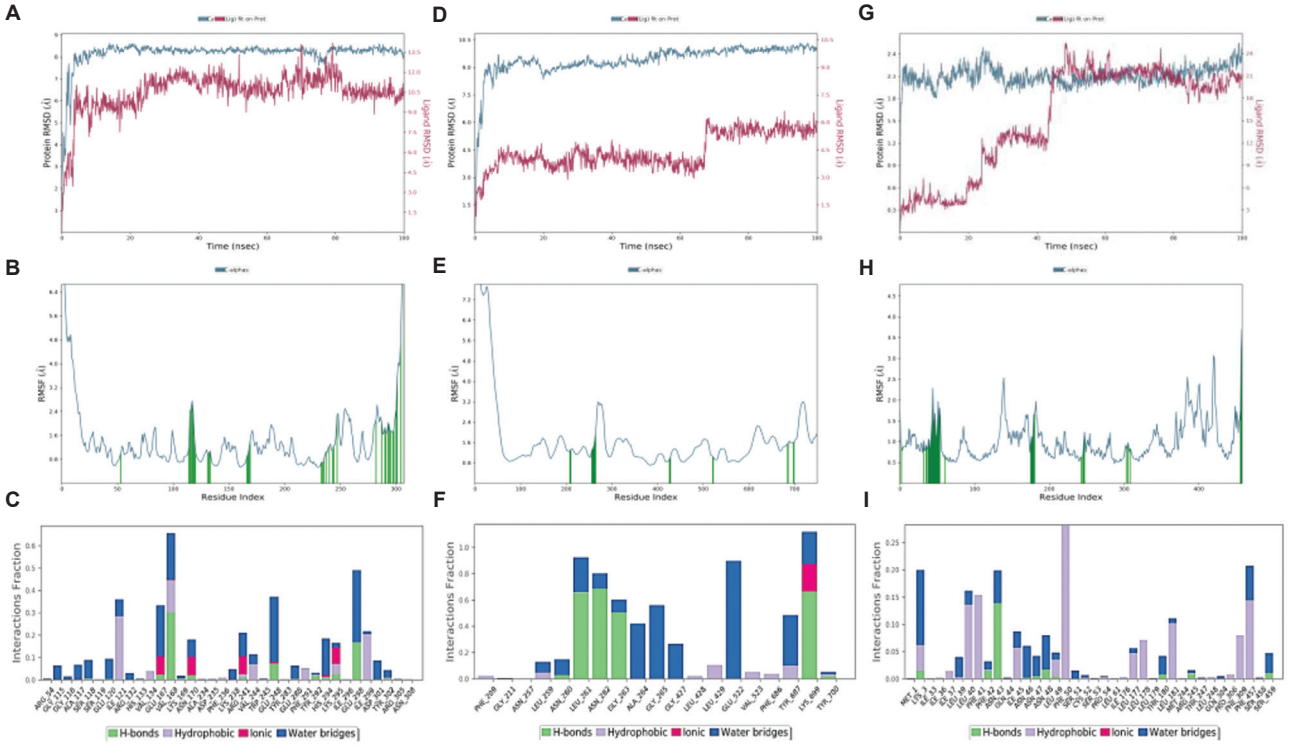

Figure 5. Protein-ligand complex simulation results. (A) Root-mean-square deviation (RMSD) of resveratrol and P23280. (B) Root-mean-square fluctuation (RMSF) of P23280. (C) Interaction profile of the contact between resveratrol and P23280. (D) RMSD of valproic acid and Q04609. (E) RMSF of Q04609. (F) Interaction profile of the contact between valproic acid and Q04609. (G) RMSD of Rotenone and P03905. (H) RMSF of P03905. (I) Interaction profile of the contact between Rotenone and P03905.

Table 6. Prime molecular mechanics/generalized born surface area (MMGBSA) binding energy (Δ^{Gbind}) of interactions of the complex before and after molecular dynamics simulation

Complex	Simulation time (ns)	MMGBSA, ΔG (kcal/mol)							
		Coulomb	Covalent	Hbond	Lipo	Packing	Solv_GB	vdW	ΔG^{bind} (Total)
Resveratrol-P23280	0	−9.006	1.617	−1.019	−17.711	−1.861	21.954	−28.344	−34.372
	100	−22.164	2.003	−0.705	−17.041	−2.442	21.699	−34.091	−52.740
Valproic acid-Q04609	0	−33.447	2.300	−5.125	−23.496	0	40.797	−28.505	−47.477
	100	−34.038	162.038	−4.788	−33.272	0	33.971	202.158	326.070
Rotenone-P03905	0	−4.659	1.396	−0.639	−21.817	−3.544	14.299	−38.593	−53.558
	100	−6.560	1.560	−0.744	−10.654	−0.381	8.925	−24.970	−32.824

Abbreviations: vdW: Van der Waals energy; Solv GB: Generalized born electrostatic solvation energy; Packing: Pi-pi packing correction; Lipo: Lipophilic energy; Hbond: Hydrogen bonding energy; Covalent: Covalent binding energy; Coulomb: Coulomb energy; Total: Total energy (prime energy).

found that resveratrol did not provide beneficial effects in treating ALS at a dose of 120 mg/kg/day.[38] A study suggested that the pathological acetylation state of NF-κB/RelA and histone 3 observed in the $SOD1^{G93A}$ murine model of ALS could be corrected by the synergistic combination of low doses of resveratrol, an activator of the AMPK-sirtuin 1 pathway, with histone deacetylase inhibitors MS-275 (entinostat) or valproate.[39]

Valproic acid, commonly prescribed for epilepsy, bipolar disorder, and migraine prophylaxis, affects various signaling pathways, including ERK and Wnt/beta-catenin pathways, and interferes with the metabolism of arachidonate and inositol. Valproic acid could modulate the expression of genes crucial for transcription regulation, cytoskeletal modifications, signal transduction, ion homeostasis, and cell survival.[40] Studies have demonstrated that chronic valproic acid treatment slows down motor neuron degeneration but does not significantly extend the lifespan in G93A mice.[41] In a rat spinal cord injury model, valproic acid increased the expression of nestin and SOX2, markers for neural stem/progenitor cells.[42] The pharmacokinetics of valproic acid can be influenced by cytochrome activity and metabolic enzyme gene polymorphisms, such as CYP2C19*2 and UGT1A6 variants, which may require dosage adjustments to achieve target plasma concentrations.[43]

The study highlighted the significance of 4-hydroxynonenal, a biomarker of lipid peroxidation, which has been found elevated in the CSF of patients with sporadic ALS, potentially affecting motor neuron function.[9] 4-Hydroxynonenal can activate stress response pathways and prompt the expression of antioxidant enzymes, while also downregulating cholinergic markers and impairing glucose and glutamate transport in motor neurons, thereby contributing to excitotoxic cell degeneration.[40,44-47] The biological importance of 4-hydroxynonenal-protein adducts, detected by mass spectrometry, includes inactivation of protease activity, decrease in kinase activity, impairment of mitochondrial function, and disruption of cellular structures.[48] 4-Hydroxynonenal has displayed high toxicity in mammalian cells, leading to inactivation of mitochondrial respiration, protein synthesis, and glycolytic enzymes.[49,50] In addition, 4-hydroxynonenal treatment has been linked to the insolubilization, phosphorylation, and partial cytosolic localization of TDP-43.[51]

Rotenone, a natural toxin, acts as a highly specific metabolic poison by inhibiting mitochondrial electron transport, leading to cellular aerobic respiration blockade.[52,53] Although it is highly toxic to fish and other aquatic life, rotenone has low toxicity to birds and mammals and is rapidly broken down in the environment.[54,55] Long-time exposure to rotenone could cause brain neurotoxicity through sufficient BBB permeation.[56,57] Therefore, exposure to rotenone may lead to progressive deterioration of brain function characteristic of neurodegenerative diseases, as it decreases intracellular aldehyde dehydrogenase activity.[58] This effect could be ameliorated by bioactive compounds such as naringenin and luteolin.[59,60]

Moreover, some of the identified non-BBB permeant compounds have therapeutic or pathologic linkage to neurodegenerative diseases. Studies have reported that acetic acid could activate the AMPKα signaling pathway, leading to the inhibition of acetyl-CoA carboxylase. This activation promotes lipid oxidation and limits lipid synthesis[61,62] Dysregulation of acetic acid, succinic acid, malonic acid, citric acid, and other compounds related to lipid metabolism has been reported for ALS.[63] Studies have also indicated that epigallocatechin gallate could prevent glutamate excitotoxicity by inhibiting glutamate transporter, thereby offering neuroprotection against neurodegenerative diseases such as ALS.[64,65]

Deoxycholic acid has not been extensively studied in ALS, but its derivative, tauroursodeoxycholic acid, has been found promising against ALS.[66] Research suggests that dysregulation of bile acids, such as deoxycholic acid produced by gut microbiota, plays a key role in the pathogenesis of neurodegenerative diseases.[67]

Molecular docking and MDS play crucial roles in predicting ligand binding sites and evaluating protein-ligand complex stability, providing insights into their interactions and potential therapeutic efficacy.[23,68] A binding affinity score of ≤−5.00 kcal/mol indicates good ligand-protein interaction.[69] RMSD values >7 Å indicate suggest high flexibility, less compactness, and conformational divergence of protein structural ensembles during simulation.[23] These computational methods, along with prime MMGBSA analysis, contribute to understanding the energetics of ligand-receptor interactions.[23,68]

5. Conclusion

This study has identified several lipid-based compounds that exhibit varying effects on ALS pathology. Among these compounds, four were found to permeate the BBB: Two displayed therapeutic potential (resveratrol and valproic acid), while the other two were associated with detrimental effects (4-hydroxynonenal and rotenone). Further investigation is warranted to explore the synergistic effects of resveratrol and valproic acid in ALS treatment, elucidate the mechanisms underlying 4-hydroxynonenal involvement in the formation of enzyme aggregate in ALS, and assess the potential impact of long-term human exposure to rotenone from marine food on ALS development. These avenues of research could provide valuable insights into ALS pathogenesis and facilitate the development of effective therapeutic interventions. Moreover, our future research endeavors will prioritize classifying potential contributors to ALS, including genetic factors, environmental toxicity, and autoimmune diseases that target the nervous system, potentially leading to motor neuron damage. This classification could provide valuable insights into disease etiology and guide personalized treatment approaches.

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Conflict of interest

The authors declare that they have no competing interests.

Author contributions

Conceptualization: Toluwase Hezekiah Fatoki
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Availability of data

Data are available from the corresponding author on reasonable request.

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ACCSCIENCE PUBLISHING

Brain & Heart

CASE REPORT

Chronic systemic thrombosis of unknown etiology with concurrent essential thrombocythemia and recurrent stroke: A case report

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Abstract

Here, we report a male patient under hypercoagulable state of unproven etiology, who used to present with recurrent thrombotic episodes since young age after he stopped taking anticoagulant. At the age of 73, he was detected to have essential thrombocythemia while grappling with recurrent strokes. On both occasions, he presented with a basilar thrombus. His National Institutes of Health Stroke Scale (NIHSS) score improved from 18 to 2 on the first occasion and from 17 to 2 sixteen days later on the second occasion. Thrombolysis was done on both occasions, but endovascular therapy was not done on either occasion due to chronicity of the thrombus and remarkably improved NIHSS post-thrombolysis. For patients with recurrent thrombotic events of undetermined etiology, an anticoagulant should be indicated. Repeat thrombolysis can be performed without endovascular therapy within a brief gap of 16 days, even in stroke patients with moderate to high NIHSS.

Keywords: Acute stroke; Stroke thrombolysis; Basilar thrombus

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1. Background

Treating patients under a prothrombotic state of unproven etiology and presenting bizarre coagulation cascade are common in clinical settings. Unfortunately, only a small fraction of patients with these conditions have an unpredictable response to thrombotic agents. Thus, managing these patients has become more challenging if they present with acute stroke since it is critical to strike a balance between inducing clot lysis and controlling hemorrhagic risk. We report on a patient with an unknown hypercoagulable state, presenting with recurrent stroke and a large intracranial thrombus, and how the patient was managed.

2. Case presentation

This case report revolves around a 73-year-old man with a history of hypertension, diabetes, hypertrophic cardiomyopathy, and peripheral artery disease of unknown etiology, who had been taking aspirin, cilostazol, and acenocoumarol for acute limb ischemia for 17 years. He had stopped taking antithrombotics, except aspirin, due to a lower limb hematoma. He was presented to our emergency room a year after the acute onset of severe dysarthria, expressive aphasia, right-sided hemiplegia, and left-sided hemiparesis, and on the day, he detected manifestation of varying symptoms. His

National Institutes of Health Stroke Scale (NIHSS) score was 18 during the symptom presentation.

The magnetic resonance imaging (MRI) of the brain (the time interval between the onset of symptoms and the MRI was 5 h) revealed a left-sided acute evolving infarct in the central pons, with restriction in diffusion-weighted imaging (DWI) without the corresponding changes in fluid-attenuated inversion recovery (FLAIR), accompanied by old infarcts at the right centrum semiovale and putamen. There was an abrupt cut-off of the proximal part of the basilar trunk with blooming thrombus in the proximal and mid-basilar trunk with the reformation of the distal basilar trunk, bilateral superior cerebellar, and posterior cerebellar arteries (Figure 1). Based on the DWI-FLAIR mismatch results, he was given thrombolytic agent tenecteplase, and afterward, his condition improved dramatically, with the NIHSS score improving from 18 to 2.

A computed tomography angiogram (CTA) which was done after thrombolysis revealed the same result as in the magnetic resonance angiography, which was done just before the thrombolysis during this presentation with acute stroke. The CTA further unveiled eccentrically calcified plaques in both carotid bulbs, cavernous, supra-clinoid segments of both internal carotid arteries, and the V4 segment of the right vertebral artery with no hemodynamic stenosis. Considering the pros and cons of the current NIHSS, we did not proceed with administering endovascular therapy in the patient as we reasoned that the occlusion could be chronic in view of the dramatically improved NIHSS even with such a big thrombotic occlusion, which implied sufficient collaterals engaging in compensatory mechanisms. With a low NIHSS, we were worried that an attempt for endovascular therapy might dislodge the clot (with the intact proximal and distal flow) and cause an unnecessary deterioration. Thus, we kept the patient under close scrutiny and provided immediate interventions in case of any new deficit. When his NIHSS improved to 1 (MRS of two), the patient was discharged from the hospital since he was capable of walking independently despite the presentations of mild dysarthria and ataxia.

The patient was diagnosed with essential thrombocythemia (CALR positive in genetic test) along with beta-thalassemia trait and was found to be positive for *MTHFR* gene mutation. In the beginning, hematologist prescribed him hydroxyurea (1 g once daily). The coagulation parameters, markers of hypercoagulability, and vasculitis markers of the patient were within limits and he showed no angiographic evidence of arterial dissection. Transesophageal echocardiography was conducted to look specifically for any left atrial appendage thrombus, but the test ended up with negative finding. Repeated prolonged cardiac monitoring was also performed to detect occurrence of atrial fibrillation (AF), but the results were not remarkable. It is worthy to mention that hypertrophic cardiomyopathy, with or without AF, is an important cause of stroke. In a study on 32,206 patients with isolated hypertrophic cardiomyopathy, 38.8% of them had AF and 7.7% had ischemic stroke.[1] To further unravel in this direction, we performed MR vessel wall imaging on the patient (Figure 2), but the procedure did not yield significant or meaningful findings that would be of benefit to the diagnosis. Thus, in this case, we regarded stroke mechanism as the possible pathogenic pathway contributing to intracranial atherosclerosis (ICAD). The patient was discharged home with aspirin 75 mg, clopidogrel 75 mg, and 80 mg of atorvastatin per day; he was reminded that he had heightened the risk for vascular pathology and multiple atherosclerotic plaques.

Sixteen days later, he suffered from another episode of acute dysarthria, right-sided hemiplegia, and transient hearing impairment (NIHSS of 17 at our emergency room, 2 h after the ictus). MRI revealed evolving acute infarct at the left paramedian pons and right lateral aspect of pons next to the cerebellar hemisphere (no corresponding change in FLAIR) (Figure 3). We injected the patient with tenecteplase and his NIHSS score improved to 2.

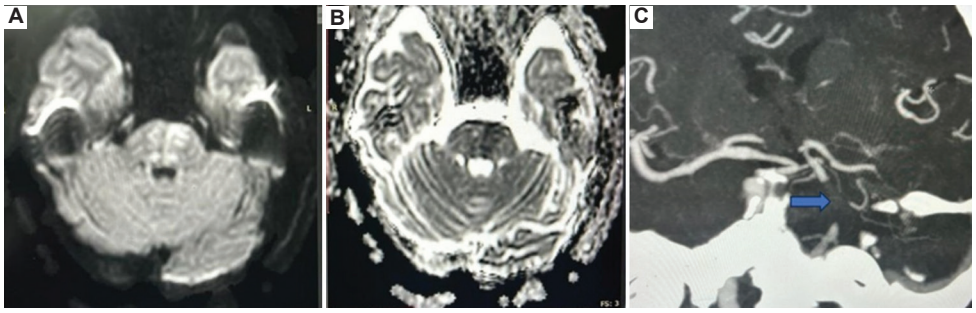

Figure 1. Magnetic resonance imaging of the brain revealed acute evolving infarct in the central pons. (A and B) (more inclined to left-sidedness) with an abrupt cut-off of the proximal part of the basilar trunk with non-opacification of proximal and mid-basilar trunk due to the thrombus (marked with blue arrow). (C) Reformation of the distal basilar trunk, bilateral superior cerebellar and posterior cerebellar arteries.

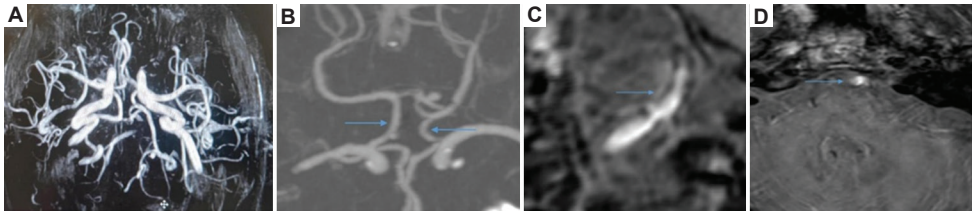

Figure 2. Computed tomography (CT) and magnetic resonance angiogram including vessel wall imaging. (A and B) CT angiogram showing good collaterals and robust bilateral posterior communicating arteries (blue arrows). (C and D) Magnetic resonance imaging showing thrombus in the proximal basilar artery (blue arrows) but no significant finding in the vessel wall imaging.

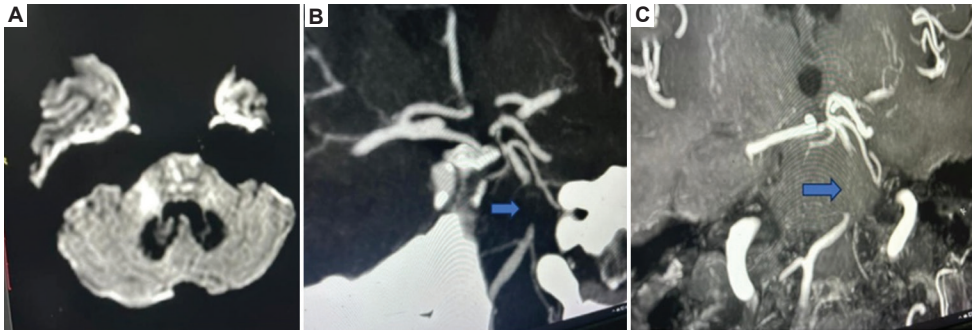

Figure 3. Magnetic resonance imaging revealed evolving acute infarct at left paramedian pons and right lateral aspect of pons adjacent to cerebellar hemisphere. (A) Magnetic resonance angiography; (B) computed tomography angiogram; (C) revealed the site of basilar thrombus (marked by blue arrows).

The angiogram this time revealed the same findings as during the previous admission. A repeat MRI of the brain revealed the new infarct at the pons (at the left paramedian pons and right lateral aspect of the pons next to the cerebellar hemisphere, where it was evolving during this presentation) with no evidence of bleeding. Despite the mild fluctuations in clinical symptoms, with an obvious speech impediment, his NIHSS score remained at two, showing stable vitals. A 48-h Holter monitoring and echocardiography did not yield remarkable findings. Thus, considering the possibility of antiplatelet resistance, we then replaced clopidogrel with ticagrelor (90 mg twice daily) while maintaining the same medications, such as aspirin and high-dose statin for the patient. On the 4th day, the patient was given enoxaparin 40 mg twice daily (at a lower dose to avoid bleeding episode) along with ticagrelor and statin (aspirin was stopped), to dissolve the thrombus; his condition was put on close monitoring. We did an MRA with vessel wall imaging but found no enhancement of the vessel wall or any additional findings. During follow-up, his condition remained stable with apixaban 5 mg twice daily (replacement of enoxaparin) and ticagrelor and atorvastatin 80 mg.

Sixteen days after the 1st stroke, the 2nd stroke happened, which was suspected through the ICAD detection and based on the failure of the conservative treatment administered. Basilar stenting was considered for treating the patient, but the treating physicians and his family decided against it as his condition showed improvements. The following variables were used to help distinguish ICAD in the current case: location of thrombus in proximal basilar artery (classic for ICAD-related occlusion), persistent distal basilar artery filling because of good posterior communicating artery (Figure 2), the 2nd stroke in middle cerebellar peduncle (classic location of watershed infarct in posterior circulation secondary to ICAD), and rapid improvement in both occasions (despite failed recanalization of occluded basilar artery), which pointed toward transient collateral failure because of hemodynamic compromise, which was possible in this situation.

In both occasions, the patient showed improvements post-thrombolysis, concurrent with an NIHSS score drop from almost 18 to 2, without any hemorrhagic transformation. There were intact flows proximal and distal to the thrombus, which indicate a possible chronicity of the thrombus. In light of this, we did not proceed with endovascular therapy in any occasion, and fortunately, the patient successfully recovered afterward. After 8 months of follow-up, he experienced no further deterioration. The findings of a repeat MRI of the brain with vessel wall imaging remained unchanged (Figure 2).

3. Discussion

Essential thrombocythemia is a myelodysplastic syndrome and represents a rare cause of stroke where both the

number of platelets and their function are associated with stroke occurrence. This disorder is typically comorbid with thrombocytosis and megakaryocytic hyperplasia in the bone marrow, with the former specifically contributing to the risks for thrombosis and hemorrhage.[2]

The current case is particularly distinctive because no definite prothrombotic etiology could be identified after conducting repeated tests over many years, despite the recurrent thrombotic events, until he was admitted to our institute due to acute strokes. This is probably because minor hematological derangements in the peri-stroke period are sometimes overlooked, causing a lack of proper follow-up. These aberrations may later prove to be a critical stroke etiology and a "harbinger of havoc" in the future. The platelet counts during our patient's 1st admission (in our institute) were $9 \times 10^5/\mu L$, and during the 2nd stroke, it was $8 \times 10^5/\mu L$. Interestingly, based on his previous medical records, we learned that his baseline platelets used to be borderline high, at around $4 \times 10^5/\mu L$. Rarely, essential thrombocythemia is diagnosed in patients with high normal platelet counts (between 350,000 and 600,000 platelets per microliter of blood) that are persistent over a long period of time and has relevant genetic components that warrant a supportive bone marrow examination, which is performed to rule out possibilities other than essential thrombocythemia. In fact, if a patient is aged over 60 years with over 1,50,000 platelets per microliter and has a history of thrombosis, he falls under the high-risk category for essential thrombocythemia that necessitates strict follow-up.[3] Hence, if this kind of information was available, we could have been able to detect essential thrombocythemia in our patient at a much earlier phase, even before he presented with acute stroke. Another uniqueness of our case was that our patient had recurrent thrombotic events but never exhibited common vasomotor symptoms of thrombocythemia, such as headache, nausea, vomiting, light-headedness, transient visual disturbances, atypical chest pain, acral paresthesia, livedo reticularis, and erythromelalgia.

Patients who had a minor stroke in the initial presentation, and improved significantly post-thrombolysis, can be considered for re-thrombolysis if a new stroke strikes within 3 months after the initial minor stroke.[4] In the present case, although not a minor stroke, we administered re-thrombolysis as a treatment for the patient. The back-to-back thrombolysis sessions were administered too close to each other, with only 16 days apart. The rather small timeline gap in giving multiple thrombolytic treatments stands as a unique point of this case, and fortunately, the patient recovered without presenting any catastrophic bleeding. In hindsight, we should have anticoagulated him during the 1st presentation to dissolve any new, evolving thrombus, so as to minimize the chances, he would develop a new stroke. The basilar thrombus most likely was secondary to an atherosclerotic plaque rupture (an acute event on a chronic one). It is possible that an eddy current of blood flow might have formed at the end of the large thrombus, causing a secondary hypercoagulable state. To tackle this, we treated him with long-term anticoagulation with a plan to dissolve the whole thrombus or address the eddy current.

As the patient had a history of peripheral arterial disease and was stable with anticoagulation, there might be an underlying hypercoagulable state that contributed to the formation of basilar thrombus and a prothrombotic state, despite the negative results for thrombophilia. Since we did not have the facility to perform round-the-clock monitoring of partial thromboplastin time, in which case unfractionated heparin is applied,[5] we chose to use low-molecular-weight heparin to dissolve the intraluminal thrombi. It has been found that anticoagulation therapy in a subgroup of embolic stroke of undetermined source patients with abnormal serum d-dimer, thrombin-antithrombin complex, prothrombin fragment, and fibrin monomer or having severe left atrial enlargement was associated with a reduced rate of recurrent stroke.[6] Thus, in the case when it is not possible to pinpoint a hypercoagulable state or cardiac etiology in stroke cases, anticoagulation may be warranted in special cases. In the current case, our patient had been on acenocoumarol for 17 years due to a lower limb ischemic incident and reported no significant clinical event. He had a stroke secondary to a big basilar thrombus, which occurred a few years after he stopped taking anticoagulant because of an incident of lower limb hematoma. In the 2nd occasion, he had a recurrent stroke while not taking any anticoagulants and his condition had since remained stable after he resumed taking anticoagulant.

Due to the non-availability of relevant facility at our center, we did not conduct CYP2C19 testing for clopidogrel resistance. However, we presumed that this patient had clopidogrel resistance, since Asian populations have high prevalence of clopidogrel resistance with nearly 70% in some communities, and he experienced a recurrent stroke while on clopidogrel.[7]

4. Conclusion

In conclusion, prescribing anticoagulants for stroke patients who have experienced recurrent strokes of unknown etiology while on antiplatelet, and who are in an unknown prothrombotic state, should be considered, after deliberating over the pros and cons of such strategy with their family. Endovascular therapy is not mandatory for all cases of basilar thrombus with acute stroke, because thrombolysis may be sufficient in special situations like in this case, considering the clinical picture and natural history

of the clot. Back-to-back thrombolysis may be executable within a brief gap of 16 days, even in stroke patients with moderate-to-high NIHSS scores. Genetic tests for patients under prothrombotic state of unknown etiology and bone marrow examination for doubtful cases should be carried out to provide a complete clinical picture, ensuring that a coveted hematological disorder that might precipitate the occurrence of thrombotic events is not overlooked during the investigation process. However, many patients are reluctant to undertake bone marrow examination because it is a painful procedure and, especially, when no new event occurs after the index thrombotic event. Despite the testing efficacy, genetic tests are not widely available or entail considerable costs. These limitations likely put a constraint in the detection of essential thrombocythemia when the suspected cases do not have a classical presentation.

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Conflict of interest

The authors declare that they have no competing interests.

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Ethics approval and consent to participate

The Ethics committee of the Apollo Multispeciality Hospitals, Kolkata, had acknowledged the verbal and written consent obtained from the patient.

Consent for publication

Verbal and written consent has been obtained from the patient.

Availability of data

Not applicable.

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ACCSCIENCE PUBLISHING

Brain & Heart

CASE REPORT

Perioperative cardiopulmonary resuscitation in a prone position: A case report

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Abstract

Post-cardiac arrest brain injury remains a major cause of mortality in perioperative cardiac arrest, although intraoperative events are uncommon due to pre-procedural preparations, risk-benefit anticipation, and a controlled working environment. Herein, we describe the successful cardiopulmonary resuscitation of a 57-year-old male with a C2-C3 schwannoma who experienced intraoperative cardiac arrest due to massive blood loss during a C2-C3 laminectomy and excision of tumor while in a prone position. The intricacies and effectiveness of resuscitation in a prone position, as well as neuroprotective strategies in the perioperative setting to optimize functional outcomes, will be discussed.

Keywords: Post-cardiac arrest brain injury; Intraoperative resuscitation in a prone position; Intraoperative cardiac arrest; Neuroprotection; Intraoperative massive blood loss

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1. Background

Cardiac arrest is one of the leading causes of morbidity and mortality worldwide. Intraoperative cardiac arrest has been recorded as high as 5.7/10,000 procedures, increasing the predictability of inpatient mortality and significantly higher hospital costs.[1] Many clinical interventions have been established to enhance the return of spontaneous circulation (ROSC). Optimizing post-cardiac arrest resuscitation is as important as the advancements in basic and advanced life support for the best chance of survival with good neurological function.[2]

Global ischemia occurs immediately after ROSC, leading to consequential insults that aggravate multi-organ injury during and after reperfusion.[3,4] Post-cardiac arrest brain injury has been identified as the main cause of mortality in 68% and 23% of cases after out-of-hospital and in-hospital cardiac arrest, respectively,[5] due to poor awareness and low implementation of neuroprotective efforts following successful cardiopulmonary resuscitation (CPR).[6]

Critical care monitoring, early hemodynamic optimization, oxygenation and ventilation, circulatory support, normoglycemia, seizure control and prevention, and neuroprotective pharmacology are all essential neuroprotective strategies to reduce brain injury post-cardiac arrest.[2]

Herein, we describe our experience in the successful delivery of perioperative CPR on a patient who was at risk for cardiac arrest due to intraoperative hypovolemic shock.

In addition, we discuss the intricacies and effectiveness of resuscitation in a prone position and neuroprotective strategies in the perioperative setting to optimize long-term functional outcomes.

2. Case presentation

A 57-year-old male, weighing 70 kg, with a history of mitral valve prolapse (MVP), hypertension, dyslipidemia, asymptomatic bronchial asthma, and a previous cerebrovascular infarct, presented with a 5-month history of nape pain and numbness in the upper and lower extremities. Spine magnetic resonance imaging revealed progressive spinal cord compression classified as American Spinal Injury Association Impairment Scale C from a C2-C3 Schwannoma, which was consistent with the patient's quadriparesis.

2.1. Pre-operative course

The patient was hemodynamically stable and oriented to three spheres, with a Glasgow Coma Scale score of 15. Manual muscle testing results were as follows: C5-T1 was 4/5 on the right and 0/5 on the left; L2-S2 was 2/5 on the right and 4/5 on the left. The last normal sensory level was noted at C2, and proprioception was intact on all extremities. Pre-operative blood tests were all within normal range. A 12-lead electrocardiogram showed regular sinus rhythm and normal axis. A 2D echocardiogram showed MVP with moderate mitral regurgitation, good cardiac wall motion and contractility, normal cardiac chamber dimensions, and adequate left ventricular systolic function, with aortic sclerosis and a 64% ejection fraction.

2.2. Intraoperative course

Induction and intubation were well tolerated, and general anesthesia was maintained with a target-controlled infusion of remifentanil and propofol, along with sevoflurane at 0.5 minimum alveolar concentration. End-tidal carbon dioxide ($EtCO_2$) was maintained at 35 – 38 mmHg, with controlled ventilation. Bispectral index (BIS) values were maintained at 40 – 60, and pulse pressure variations were sustained at 12 – 15%. Brief hypotensive episodes were noted as the patient was shifted to the prone position, eventually requiring low-dose norepinephrine to maintain a mean arterial pressure >85 mmHg. Two hours into the surgery, a sudden massive blood loss of approximately 2 L was noted and was simultaneously addressed with fluid resuscitation and blood transfusion. The patient continued to be hemodynamically unstable despite resuscitative efforts. Oxygen saturation decreased to 80% with sinus bradycardia, which eventually converted to pulseless ventricular fibrillation. CPR was started in the prone position during surgical closure, in preparation for resuscitation in the supine position. $EtCO_2$ was maintained at 20 – 25 mmHg, and propofol 1 mg/kg and midazolam 0.05 mg/kg IV were administered. Advanced cardiac life support (ACLS) then proceeded in the supine position, and ROSC was achieved after 3 min. BIS was noted at 51 – 55. The assessment at this time was hypovolemic shock. Propofol and remifentanil infusions were continued throughout the intraoperative course. The patient was shifted to the supine position, and central access was secured through the left subclavian vein for inotropic support, fluid resuscitation, and blood transfusion. Once stable, the patient was shifted back to the prone position for hemostasis and surgical closure of the C1-C3 partial left and complete right laminectomy and excision of dumbbell schwannoma. Total anesthesia time was 8 h, with an estimated blood loss of 3 L, which was replaced.

2.3. Post-operative course

At post-anesthesia care unit (PACU), the patient was fully awake and responsive, hemodynamically stable, and tolerated a trial of extubation well. The patient developed hospital-acquired pneumonia during his admission, requiring ventilatory support. He was eventually discharged on the 46th post-operative day with a tracheostomy tube in place.

3. Discussion

Post-cardiac arrest brain injury is the main cause of death and disability in resuscitated patients. As its oxygen reserve is limited and 65% of body glucose consumption mainly occurs in the brain, the brain's tolerance to ischemia and hypoxia is low.[6,7] Brain viability strongly depends on a consistent supply of oxygen and energy substrates, and cessation of cerebral blood flow results in an immediate interruption of brain activity. The mechanism of brain injury during cardiac arrest and resuscitation after ROSC are complex and include excitotoxicity, disrupted calcium homeostasis, free radical formation, pathological protease cascades, and neuronal apoptosis.[8-10] Selective neuronal damage increases with longer ischemic duration, especially in areas of higher vulnerability, such as subcortical regions. This consequential cascade of secondary insult occurs over hours to days, suggesting a broad therapeutic window for neuroprotective strategies post-cardiac arrest.[2,11-16] Cerebral blood flow decreases to 50% as the cerebral metabolic rate of oxygen and oxygen extraction fraction are both decreased within 24 – 72 h.[17] Post-resuscitative neuroprotective efforts should aim to achieve cerebral autoregulation, ensuring that derangements in temperature, blood pressure, oxygenation, ventilation, and fluid status are addressed.[16] In our patient, fluid resuscitation and blood transfusion, as well as correction of electrolyte and arterial blood gas

parameters, were already being undertaken before the cardiac arrest. As massive blood loss was identified as the cause of the arrest, aggressive resuscitative efforts were geared toward blood replacement, adequate perfusion, and oxygenation, potentially averting or forestalling the need for ACLS.

Numerous studies on neuroprotective modalities for global cerebral ischemia have been conducted on animal models, proving to be greatly valuable in improving neurologic status and functional outcomes in post-cardiac arrest patients despite statistical limitations. Pharmacologic approaches that diminish secondary injury from ischemia and reperfusion after ROSC aim to mitigate excitotoxicity, improve neuronal metabolism, limit mitochondrial injury, and reduce neurologic inflammation.[16] Pharmacologic sedation for neuronal silence in the early phase of recovery after hypoxic-ischemic brain injury permits damaged neurons to shut down before primary cellular dysfunction becomes irreversible. Modulation of slow-wave activity may be suggestive of metabolic suppression, allowing quiescence of neuronal activity amidst insult. Propofol, a known GABA-receptor agonist, has recently been recognized for its neuroprotective role in brain ischemia/reperfusion injury through the induction of heme oxygenase-1 expression,[18] reduction of hypoxic-induced hippocampal neuronal injury, improvement of alterations in neuronal structure,[19] attenuation in cortical and hippocampal caspase-3 activation to improve cognitive function through GABA-A receptor action,[20] and improvement of cerebral perfusion.[21] Its early post-arrest initiation has led to metabolic suppression, attenuation of cerebral hyperemia, and modulation of slow-wave electroencephalogram activity. Recent reports demonstrate the neuroprotective effects of midazolam through the suppression of intracellular reactive oxygen species accumulation and its radical scavenging activities, thereby preventing apoptosis.[22] In our patient, maintenance of propofol and interrupted doses of midazolam were administered intraoperatively as neuroprotection during resuscitation and post-ROSC. Despite the expected brain insults during cardiac arrest, our patient was immediately fully awake and appropriately responsive in the PACU.

The patient's position was another concern as to our approach to ACLS. Most providers are trained to perform CPR in the supine position, and reluctance to perform CPR in the prone position poses challenges due to a relative lack of knowledge and experience. For patients with cardiac arrest undergoing neurosurgery, the 2014 United Kingdom Resuscitation Council recommends starting CPR without changing their position from prone to supine if chest compressions are adequate based on $EtCO_2$ and capnograph waveforms.[23] Shifting positions from prone to supine raises concerns due to time constraints, the need for 3 – 4 operators, the surgical risk associated with open wounds and the Mayfield clamp, and the potential dislodgment of airway devices. Initiating CPR in the prone position has advantages, such as reducing the time of brain ischemia and improving the efficiency of chest compressions. This efficiency is due to the stiffness of the costovertebral joint, which allows for increased force delivery to the ventricles, and rapid reduction of intrathoracic volume due to the hard surface placed over the sternum.[24] However, complications such as pressure injuries and accidental dislodgments of the advanced airway may occur during prone CPR, necessitating a shift to the supine position, which can affect the quality of CPR. In our case, cardiopulmonary arrest was recognized early, and immediate high-quality CPR was commenced in the prone position during surgical closure. $EtCO_2$ was maintained at 20 – 25 mmHg, indicating high-quality CPR and optimal brain perfusion.

4. Conclusion

Brain injury is recognized as the main cause of morbidity and mortality after cardiac arrest. Early recognition and aggressive resuscitation are crucial to prevent subsequent ischemic reperfusion injury. As demonstrated in our patient, high-quality CPR, maintenance of $EtCO_2$ above 20 mmHg, and the implementation of neuroprotective efforts prove to be promising strategies for successful resuscitation and improved functional outcomes post-ROSC.

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Conflict of interest

The authors declare that they have no competing interests.

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Ethics approval and consent to participate

This study is based on chart review, and no direct communication or experiment involved in the study subject, rendering ethics approval and informed consent not relevant. The authors have implemented the necessary measures to ensure the anonymity of the study subject.

Consent for publication

Not applicable.

Availability of data

Data used in this work is available from the corresponding author upon reasonable request.

Further disclosure

Part of the findings was presented in Neuroanesthesia Symposium Anesthesia Updates (NAS-AU) on July 7 – 9, 2023, in Kuching, Sarawak, Malaysia.

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ACCSCIENCE PUBLISHING

Brain & Heart

CASE REPORT

L2-hydroxyglutaric aciduria presenting with learning disability and cerebellar signs: A case report

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Abstract

L-2-hydroxyglutaric aciduria (L2HGA) is a rare, autosomal recessive neurometabolic disorder due to variants in the *L2HGDH* gene, which encodes L-2-hydroxyglutarate dehydrogenase (L2HGDH), an enzyme involved in tricarboxylic acid cycle. Deficiency of L2HGDH causes leukoencephalopathy predominantly affecting the cerebellum. This case presents L2HGA in a 12-year-old Sri Lankan boy born to third-degree consanguineous parents, who also showed learning disability and bilateral cerebellar signs. Magnetic resonance imaging (MRI) of the brain revealed T2/FLAIR hyperintensities in bilateral symmetrical subcortical white matter involving the cerebellum. A diagnosis of 2-hydroxyglutaric aciduria was made after a massive peak of 2-hydroxyglutaric acid (2HG) was observed in the analysis of urine organic acids (UOA) using gas chromatography/mass spectrometry. Genetic variant analysis revealed two heterozygous pathogenic variants in the *L2HGDH* gene, confirming the genetic diagnosis of autosomal recessive L2HGA. Parental genetic testing confirmed the trans phase of the variants in the index patient and their carrier status of a pathogenic *L2HGDH* variant. Despite typical clinical features and classical MRI findings, initial clues toward the diagnosis are mainly derived from the UOA analysis. In conclusion, this case underscores the paramount importance of analyzing the UOA profile in the process of identifying rare metabolic causes, such as L2HGA, that contribute to learning disability with neurological involvement in children.

Keywords: L2-hydroxyglutaric aciduria; Urine organic acids; Gas chromatography/mass spectrometry

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1. Background

2-hydroxyglutaric aciduria (2HGA) is a rare autosomal recessive neurometabolic disorder characterized by slow progression.[1] Since the first description of this disorder by Duran *et al.*,[2] up to 300 cases have been reported worldwide thus far.[1]

The three major types of 2HGA are D-2-hydroxyglutaric aciduria (D2HGA), L-2-hydroxyglutaric aciduria (L2HGA), and D, L-2-hydroxyglutaric aciduria (DL2HGA).[3] L2HGA is caused by variants in L-2-hydroxyglutarate dehydrogenase gene (*L2HGDH,* OMIM®: 609584) gene located on chromosome 14q21.3. It encodes the L2HGDH enzyme, which is a flavin adenine dinucleotide (FAD)-linked mitochondrial enzyme known as a metabolite repair enzyme.[1]

Alpha-ketoglutarate (αKG), a metabolite of the tricarboxylic acid cycle, is converted to L-2-hydroxyglutaric acid (L2HG) by the action of mitochondrial enzyme malate dehydrogenase. L2HG is then irreversibly converted back into αKG by the L2HGDH to prevent the accumulation of L2HG (Figure 1).[1]

Thus, the deficiency of L2HGDH causes the accumulation of L2HG, which is toxic to myelin, causing leukodystrophy in subcortical white matter and basal ganglia. Elevated L2HG level also has a carcinogenic effect resulting in brain tumors such as medulloblastoma, oligodendrocytoma, and gliomas, probably due to the demyelinating and remyelinating process leading to overproduction of mitogenic growth factors. Several case reports in the published literature describe the cases of patients diagnosed with L2HGA developing brain tumors.[5,6]

L2HGA usually presents with psychomotor retardation, macrocephaly, cerebellar ataxia, seizures, pyramidal and extrapyramidal signs. In addition, seizures and growth stunting may occur. The age of onset of the disease varies from 3 to 35 years. Short attention span and hyperactivity have been reported in children aged <10 years of age. Cerebellar involvement usually becomes prominent around 12 years of age. The disease course of L2HGA resembles that of static encephalopathy. According to the literature, acute encephalopathy is rarely reported in children affected by L2HGA.[7] Although autism does not typically fall within the phenotypic spectrum of the disease, a case featuring severe autistic features in a 3-year-old child has been reported.[8] D2HGA presents early with delayed development, seizures, hypotonia, cerebral abnormalities, and cardiomyopathy.[4] DL2HGA presents with severe neurodevelopmental dysfunction in early infancy and intractable seizures associated with respiratory distress.[4]

Diagnosis of L2HGA is made based on clinical and biochemical findings, as well as characteristic findings in MRI. The main biochemical finding contributing to L2HGA diagnosis is the elevated 2HGA levels in urine, cerebrospinal fluid, and, to a lesser extent, plasma, which are detected through organic acid analysis by gas chromatography/mass spectrometry (GC/MS). Another prime feature in the biochemical results for L2HGA is, other than the elevated 2-hydroxyglutaric (2-OHG) level, the L form accounts for >90% of the isoforms. Absolute configuration of D and L forms of 2HG was performed through capillary gas-liquid chromatography as described by Duran *et al.*[2] In addition, magnetic resonance spectroscopy shows reduced levels of N-acetyl aspartate and elevated choline and myoinositol peaks in the case of L2HGA.[4] Characteristic MRI findings of the L2HGA are bilaterally symmetrical, centripetal, subcortical white matter T2 hyperintensities of the dentate nucleus, globus pallidus, putamen, and caudate nucleus. The deep white matter of the periventricular region, corpus callosum, internal capsule, and brain stem are preserved.[9]

The mainstay of management for L2HGA is through supportive and symptomatic measures such as L-carnitine supplementation and rehabilitation, which is best achieved through a multidisciplinary team approach. Genetic counseling and prenatal diagnosis through the measurement of L2HG levels in amniotic fluid are also employed to detect the risk of giving birth to infants with L2HGA, a significant part of the overall management of this disorder. It has been demonstrated that treating patients with riboflavin, which acts as a FAD precursor, is therapeutically effective in a subset of cases.[10] Since they are at increased risk of developing brain tumors, patients with L2HGA may need follow-up sessions, with periodic MRI brain examination. In addition, the disease condition is monitored annually using urine L2HG testing, as the disease activity is consistent with the L2HG level.

2. Case presentation

A 12-year-old boy born from a third-degree consanguineous marriage presented with a learning disability, bilateral cerebellar signs, and tonic extensor spasms in his limbs. He also had difficulty in buttoning and unbuttoning his shirt and in writing. Based on historical records, the patient had an uncomplicated perinatal and postnatal period with a birth weight of 3.4 kg. He underwent normal development up to the age of 4 years, but he had been complained of his poor school performance and attention deficits by teachers at preschool.

On examination, the patient had relative macrocephaly with an occipito-frontal circumference of 55 cm, which is between 75th and 85th percentile for the age and sex; tonic extensor spasms in all four limbs; and positive cerebellar signs bilaterally. The scale for the assessment and rating of ataxia (SARA) score was 13.5. He clearly had an abnormal gait and was unable to perform tandem walking for more than 10 steps. Based on the results from the Test of Nonverbal Intelligence, Third Edition (TONI–3), we

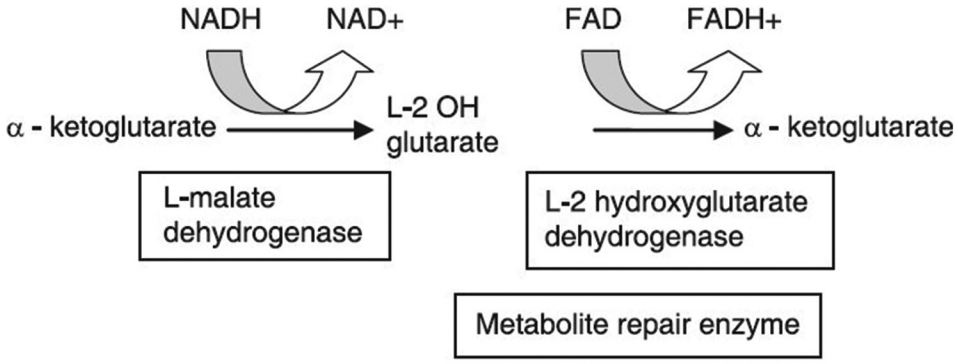

Figure 1. Schematic representation of the action of L-2-hydroxyglutarate dehydrogenase (L2HGDH). Taken from Canda *et al.*[4]

determined that he had a moderate to severe learning disability, having the intelligence equivalent to a 5 – 6-year-old school-aged child.

2.1. Examination of the external appearance and biochemical findings

Figure 2 shows the external appearance of the patient, who showed relative macrocephaly.

The results of his general biochemical and hematological investigations were mostly normal, except for a mild elevation of creatine phosphokinase (Table 1) (237 U/L; reference range: 30 - 350 U/L).

2.2. Brain MRI examination

Brain MRI revealed T2/FLAIR hyperintensities in bilateral symmetrical subcortical white matter involving the cerebellum and bilateral basal ganglia (Figure 3). On the other hand, a spine MRI yielded normal results.

2.3. Urine organic acid chromatogram

The urine organic acid chromatogram based on an analysis by GC/MS revealed a massive peak of 2HG (Figure 4).

2.4. Genetic testing

To confirm the diagnosis, the entire codon region and highly conserved exon-intron splice junctions of *L2HGDH*, *D2HGDH*, *IDH2,* and *SLC25A1* genes were analyzed using next-generation sequencing (NGS). Two heterozygous pathogenic variants (class 1) were identified in the *L2HGDH* gene: a missense variant in exon 3, c.293A>G p. (His98arg), and a nonsense variant in exon 7, c.829c>T p.(Arg277*), confirming the diagnosis of autosomal recessive L2HGA due to compound heterozygosity. No clinically relevant variants were identified in other genes. The classification was done according to the recommendations of Centogene and ACMG and the latest ACMG/AMP and ClinGen guidelines. Parental genetic testing confirmed the trans phase of the variants in the index patient and their status as carriers for a pathogenic *L2HGDH* variant. The pathogenic nonsense variant was maternally inherited, whereas the pathogenic missense variant was inherited from the paternal side (Sanger sequencing was performed to verify in this regard).

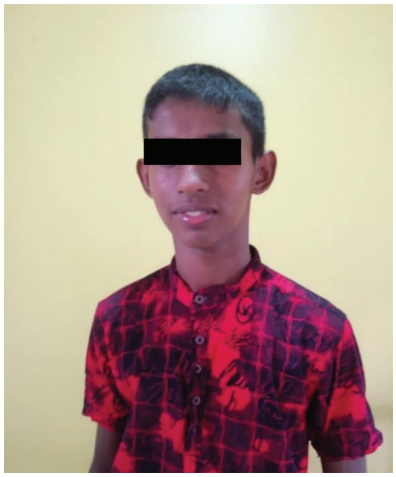

Figure 2. External appearance of the patient showing relative macrocephaly

2.5. Management and treatment

The patient was managed through a multidisciplinary approach that also encompasses periodic follow-up, rehabilitation, and genetic counseling. The patient was treated with riboflavin, carnitine, FAD, and coenzyme Q for improving his motor and intellectual disability. UOA analyses (GC-MS) performed 1 month before and after the treatment with riboflavin similarly revealed the distinct peak of 2HG on the chromatogram. However, the patient was less compliant with the treatment and defaulted to follow-up. At the time of writing this paper, the patient was studying at grade eight in school, with very poor academic performance. He was also unable to do daily living

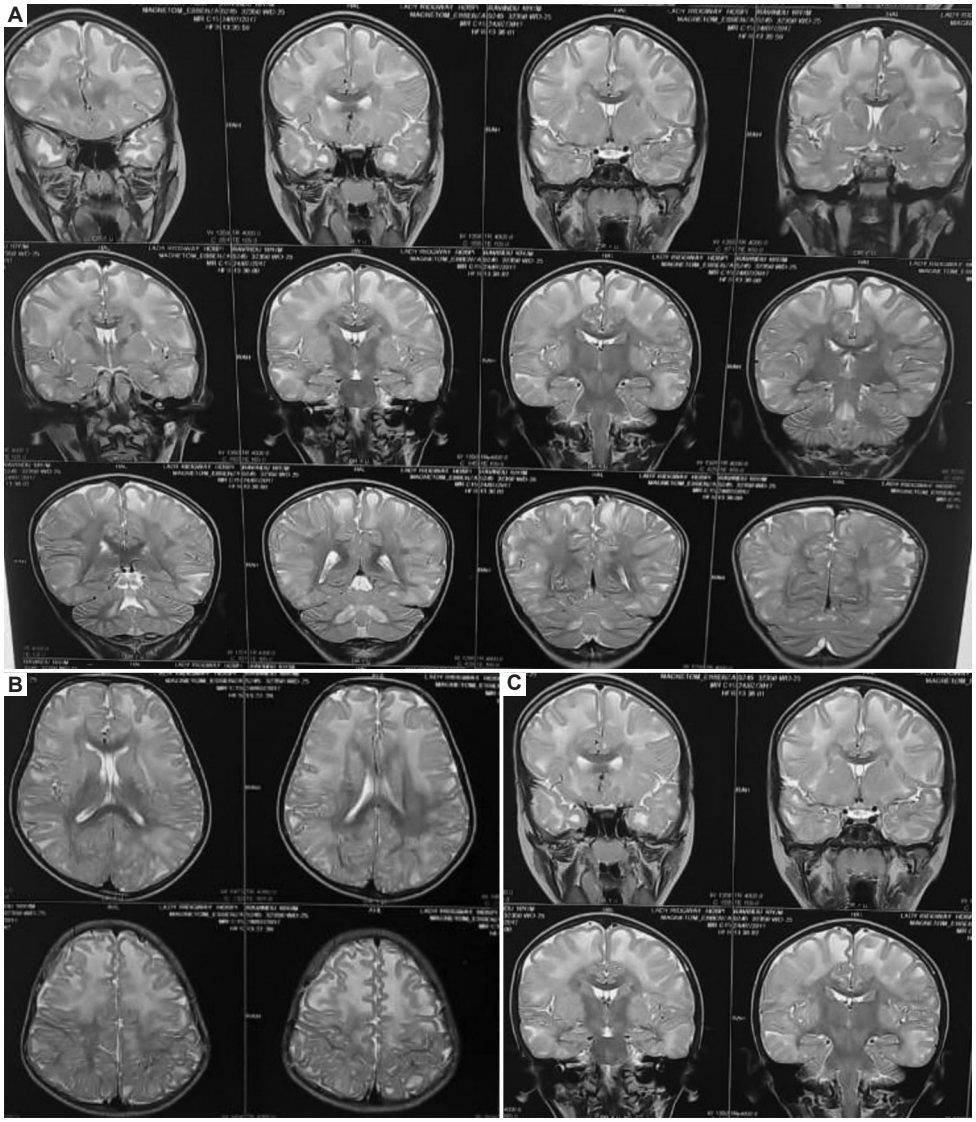

Figure 3. Brain magnetic resonance imaging results of the patient

activities such as cleaning and grooming all by himself due to the disease.

3. Discussion

L2HGA is a rare, autosomal recessive neurometabolic disorder, characterized by slow progression. This neurometabolic disorder is caused by the variants in the *L2HGDH* gene encoding the enzyme L2HGDH. The absence of the L2HGDH enzyme leads to the accumulation of L2HG in the brain cells, thereby causing leukoencephalopathy.[1] This case report presents the first case of L2HGA reported in Sri Lanka.

In this case, the patient had a static clinical picture without acute decompensating episodes, which is the classical pattern observed in L2HGA. A range of manifestations, such as learning disability and poor attention span detected at 4 years of age, macrocephaly, cerebellar involvement presenting at 12 years of age, and the pyramidal signs are the clinical features indicative of an L2HGA diagnosis. The diagnosis of L2HGA—an autosomal recessive disorder—is also supported by the fact that he is a child born from a consanguineous marriage.

The brain MRI scan for the patient revealed T2/FLAIR hyperintensities in bilateral symmetrical subcortical white matter involving the cerebellum and bilateral basal ganglia, a finding consistently characteristic of the classical pattern seen in L2HGA. Despite the classical clinical picture and MRI findings obtained, however, confirming the diagnosis of L2HGA is rather challenging at the outset, given the rarity of the disease. Thus, differential diagnosis was

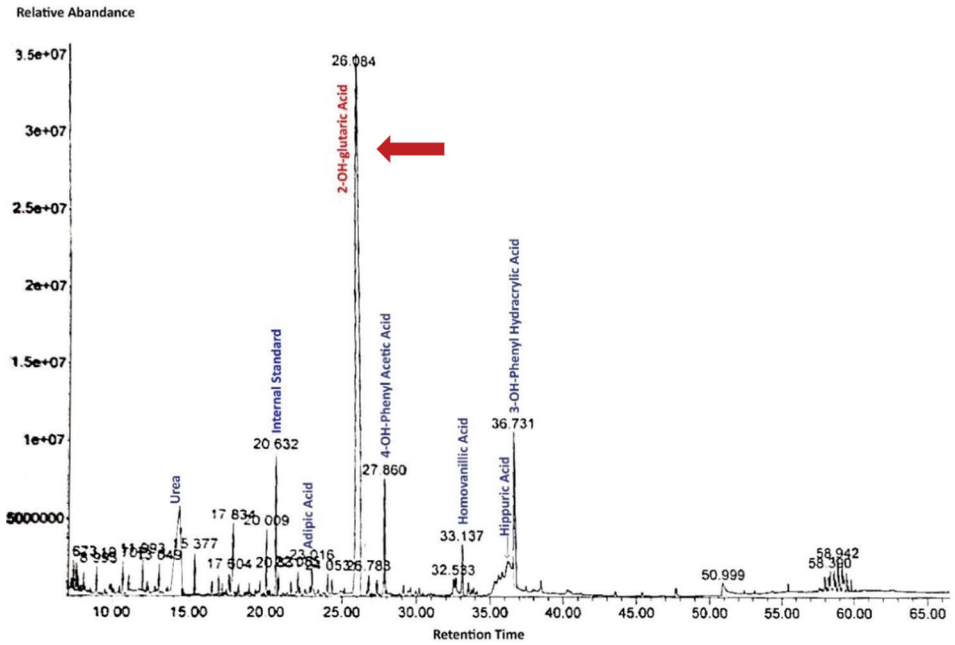

Figure 4. Urine organic acid chromatogram depicting a massive peak corresponding to 2-hydroxyglutaric acid (red arrow)

Table 1. General biochemical and hematological investigations

Analyte	Result	Reference range	Unit
Full blood count			
WBC	10.9	4,000 - 1,100	$\times 10^3/\mu L$
Neutrophils	41%	N/A	N/A
Hemoglobin	12.4	11 – 16	g/dL
Platelets	260	150 – 350	$\times 10^3/\mu L$
AST	51	9 – 48	U/L
ALT	14	10 – 40	U/L
ALP	237	60 – 425	U/L
Sodium	134	135 – 145	mmol/L
Potassium	5.5	3.5 – 5.3	mmol/L
Creatinine	53	40 – 60	µmol/L
Urea	4.2	1.8 – 6.4	mmol/L
Uric acid	215	119 – 327	µmol/L
CPK	237	30 – 150	U/L
Total cholesterol	4.4	3.6 – 5.7	mmol/L
Triglycerides	0.5	0.4 – 1.8	mmol/L

Abbreviations: ALP: Alkaline phosphatase; ALT: Alanine transaminase; AST: Aspartate transaminase; CPK: Creatine phosphokinase; WBC: White blood cells; N/A: Not applicable.

performed to rule out other disorders, such as juvenile Canavan disease, which is a neurodegenerative disorder characterized by macrocephaly, abnormal tone, and neurodevelopmental delay.[11]

In this case, the detection of a massive peak corresponding to 2HG in the GC/MS-based UOA chromatogram stood as the first clue for the diagnosis of L2HGA. At the time of writing this paper, facilities to perform biochemical tests for detecting the absolute configuration of D and L forms were not available in Sri Lanka; therefore, we were facing challenges during the diagnostic process. In children presenting with learning disability and neurological involvement, performing UOA analysis will be helpful to avoid missing out on diagnosis of rare diseases like 2-hydroxyglutaric aciduria (2-OHGA) because such diseases necessitate early therapy before significant neurological damage is established.

The genetic analysis in this case was performed by CENTOGENE GmbH, Germany, which identified two heterozygous pathogenic variants in the *L2HGDH* gene: a missense variant in exon 3, c.293A>G p.(His98Arg), which causes an amino acid change from His to Arg at position 98; and a non-sense variant in exon 7, c.829c>T p.(Arg277*), which creates a premature stop codon. These findings confirmed the diagnosis of autosomal recessive L2HGA following the verification of compound heterozygosity in parental testing. Several cases of L2HGA due to compound heterozygosity have already been reported in the literature. No clinically relevant variants were identified in other genes.

Topcu *et al.* reported that the *L2HGDH* gene consists of 70 variants, including 18 recurrent variants occurring

in some families.[12] Twelve families were found to possess c.293A>G p.(His98Arg) gene variant. Two families were identified with homozygous variants in the same gene. In one family, the affected child was presented, at 23 months of age, with generalized tonic-clonic seizures, relative macrocephaly, slight global psychomotor retardation, and subcortical leukodystrophy at 4 years of age. In another family, the affected child was presented with psychomotor retardation, global developmental delay, and cerebellar signs at the age of 6 years, and with subcortical leukodystrophy at the age of 8 years. Therefore, these findings indicate that individuals carrying the c.293A>G p.(His98arg) gene mutation seem to demonstrate consistent phenotype. Six families were identified to carry the c.829c>T p.(Arg277*) gene variant. One affected child carrying this variant presented with developmental delay, loss of milestones, cerebellar ataxia, and extrapyramidal signs with no macrocephaly or behavioral problems at 8 months of age. Therefore, this gene variant also correlates, to a certain extent, with the phenotype of the mutation carriers.

The patient was initially indicated to be managed through a multidisciplinary approach, but he was less compliant with the medical management and defaulted to follow-up. During the 1st month of follow-up, the same 2HG peak remained in the UOA chromatogram, probably due to the delay in disease diagnosis and treatment where neuronal damage had already been resulted.

4. Conclusion

This case report underscores the importance of incorporating UOA analysis in the diagnostic process for a patient presenting with learning disability together with neurological involvement, to evaluate the possible involvement of rare neurometabolic disorders such as L2HGA.

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Conflict of interest

All authors declare no conflicts of interest.

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Writing – review & editing: Maththage H.K. Amarasekara, Arndt Rolfs, Christian Beetz, Sabine Schroder, Pyara Rathnayake, Eresha Jasinge

Ethics approval and consent to participate

Informed written consent was obtained before the participation from the patient's father.

Consent for publication

Informed written consent was obtained from the patient himself and the parents for publication of the images of investigation reports in an international journal.

Availability of data

Data are available from the corresponding author upon reasonable request.

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ACCSCIENCE PUBLISHING

Brain & Heart

CASE REPORT

Adenosine-induced cardiac standstill in intracranial aneurysm surgery: A case report

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Abstract

Surgical clipping of complex intracranial aneurysms can pose intraoperative challenges due to difficulty in anatomic exposure and uncontrolled intraoperative rupture, leading to significant morbidities or mortalities. This report presents a case of a 61-year-old Asian male with an unruptured saccular basilar tip aneurysm and an unruptured right middle cerebral artery aneurysm. The patient underwent a left temporal craniotomy and clipping of an unruptured basilar tip aneurysm, successfully achieved through adenosine-induced cardiac standstill. This technique reduces perfusion pressure and decreases the turgor of the aneurysm, thereby facilitating clip ligation. Our experience demonstrated that thorough, careful patient selection, optimal anesthetic management, and proper communication between neurosurgeon and anesthesiologist are important for achieving successful surgical outcomes.

Keywords: Adenosine-induced cardiac standstill; Complex intracranial aneurysm; Aneurysm clipping anesthesia; Adenosine cardiac arrest; Flow arrest in intracranial aneurysm

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1. Introduction

Intracranial aneurysms pose a significant global health warning, leading to debilitating morbidities and mortalities, with an overall worldwide incidence of approximately 6.1/100,000[1] and a global prevalence of 8.09 million cases.[2]

A comprehensive analysis of 24 distinct studies between 1984 and 2018 described three approaches indicating cardiac standstill for aneurysm surgery: (i) adenosine-induced cardiac arrest (AiCS) for aneurysms with wide necks, thin walls, and restricted surgical visibility; (ii) rapid ventricular pacing to diminish ventricular contractility; and (iii) profound hypothermic circulatory arrest.[3] AiCS is especially beneficial for cases involving giant or complex aneurysms, which are strongly linked to higher rates of perioperative morbidity and mortality.

Limited case studies have explored the use of AiCS in intracranial aneurysm surgery. Luostarinen *et al.*[4] reported on 16 patients who received single or multiple boluses of adenosine, totaling 12 – 29 mg IV (intravenous). All patients were hemodynamically

stable after 10 min of administration, although 13 patients required vasoactive drugs. Bebawy *et al.*[5] reported 23 patients who were given adenosine at 0.3 – 0.4 mg/kg IV to facilitate temporary clipping of internal carotid artery aneurysms with complex anatomical structures. Two patients developed atrial fibrillation; otherwise, they were hemodynamically stable. In another study, Guinn *et al.*[6] described 27 patients undergoing elective intracranial aneurysm surgery in the anterior circulation, with adenosine doses ranging from 3 to 60 mg IV. The study demonstrated that AiCS effectively decompresses intracranial aneurysms for safer and easier clip ligation where a temporary clip is not feasible. However, one patient experienced prolonged hypotension, leading to asystole after rapid re-dosing, with a return of spontaneous circulation (ROSC) after 3 min.

Locally, this is the first reported use of adenosine for flow arrest in intracranial aneurysm surgery. We describe our experience with AiCS to facilitate surgical clipping of a complex cerebral aneurysm. This case report details the dosage, route of administration, duration of flow arrest, and perioperative status of our patient.

2. Case presentation

2.1. Pre-operative course

A 61-year-old Asian male, weighing 70 kg (body mass index: 28.4) and with a history of gouty arthritis, presented with a month-long history of dull headaches, disorientation, and progressive weakness of the lower extremities. The placement of a right frontal external ventricular drain (EVD) improved his neurologic status. He was then transferred to our institution for definitive management of unruptured saccular basilar tip and right middle cerebral artery (MCA) aneurysms.

Pre-operative blood tests were unremarkable. A chest X-ray revealed reticulonodular densities in both upper lung fields, which suggested either pneumonia or pulmonary tuberculosis, and an atherosclerotic aorta. A computed tomography (CT) angiogram identified two aneurysms: (i) a saccular aneurysm at the tip of the basilar artery and (ii) an aneurysm in the M2 segment of the right MCA (Figure 1). In addition, a CT scan revealed hydrocephalus and dilatation of the lateral ventricles (Figure 2).

Due to the presence of a giant unruptured basilar tip aneurysm with expected high turgor, thrombosis, and severe atherosclerosis, the patient was scheduled for a left temporal craniotomy with aneurysm clipping, potentially utilizing AiCS under general anesthesia.

2.2. Intraoperative course

Pre-induction vital signs were within the normal range. General anesthesia was maintained with propofol,

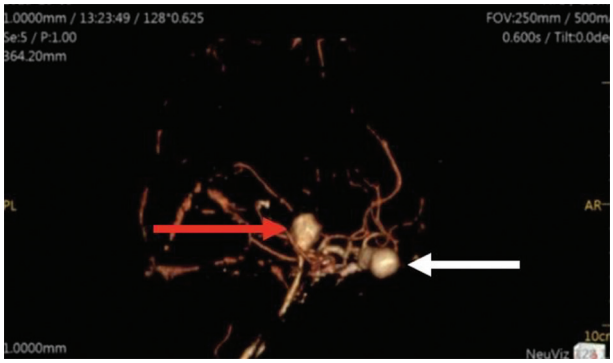

Figure 1. Computed tomography angiogram revealing: (i) basilar tip aneurysm measuring 1.9 × 1.7 × 2.5 cm (indicated by the red arrow) and (ii) M2 segment aneurysm of the right measuring 2.2 × 1.2 × 1.4 cm (indicated by the white arrow)

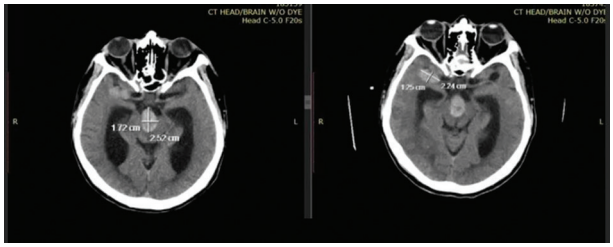

Figure 2. Computed tomography scan revealing hydrocephalus and dilated lateral ventricles

remifentanil, and sevoflurane at 0.5 minimum alveolar concentration. Standard monitoring, including invasive blood pressure monitoring and cerebral oximetry, was employed. Cardiac defibrillator pads were attached in case intraoperative defibrillation or pacing was required. Central venous access was secured through the left subclavian vein. Stable vitals and neuroprotective measures were ensured, including intermittent cerebrospinal fluid drainage via the EVD. The following parameters were observed: $EtCO_2$ 30 – 35 mmHg, bispectral index (BIS) 40 – 60, bilateral rSO_2 80 – 85%, with normoglycemia and normovolemia.

Given the complexity of both aneurysms and the increased risk for perioperative morbidities, the surgical approach prioritized the surgical clipping of one aneurysm at a time, particularly the unruptured basilar tip, due to its symptomatic characteristics. A subtemporal craniotomy was performed as the low-riding basilar tip was situated slightly below the dorsum sella, which did not necessitate a tentorial incision.

The initial attempt at clipping was unsuccessful due to technical difficulty and anatomical complexity. A second attempt was made after administering a rapid bolus of adenosine (0.4 mg/kg IV) through central access, followed by a flush with 20 cc of normal saline IV. Within 5 s, the

electroencephalography was isoelectric, BIS dropped to 1, and rSO_2 values were 84 (left) and 82 (right). The clipping of the basilar tip aneurysm was then successful. The ROSC was noted after 45 s, with vital signs ($EtCO_2$, BIS, and rSO_2) returning to baseline. After 45 min of skin closure, the patient had a Glasgow Coma scale (GCS) score of 6 (E1VtM4), with spontaneous regular breathing and pupils (3/3 mm) fixed and non-reactive, an expected early ocular complication due to the proximity of the aneurysm to the brainstem. The patient's arterial blood gas was at a normal level.

2.3. Post-operative course

At the intensive care unit, the patient was stable with a GCS score of 8 (E1VtM6) and was on synchronized intermittent mandatory ventilation, eventually requiring a tracheostomy. The EVD was then removed. Post-operative CT angiography revealed the absence of basilar tip aneurysm, which was replaced by intact metallic densities from the clipping procedure, minimal residual subacute hemorrhage in the third and lateral ventricles, a slight regression of the hydrocephalus, and the presence of right MCA saccular aneurysm at its bifurcation (Figure 3).

A repeat CT scan revealed an acute infarct in the left thalamus and a lacunar infarct of indeterminate age on the left side of the pons. A decrease in the overall density of the basilar tip aneurysm, an apparent increase in the subdural effusion, and a regression of the obstructive hydrocephalus were noted. Clinically, there were no signs of increased intracranial pressure. The patient was discharged after a month with a GCS score of 9 (E2V1M6). At discharge, the

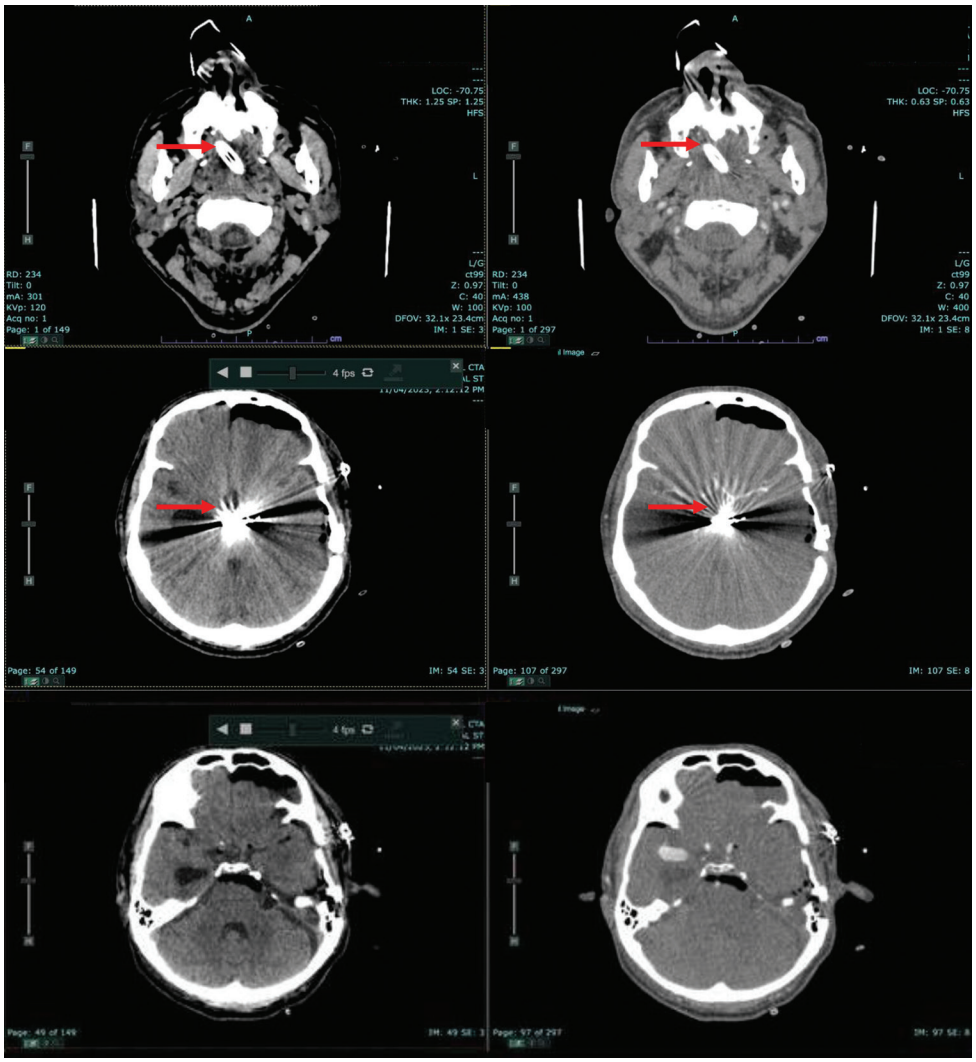

Figure 3. Post-operative computed tomography angiogram revealing the absence of basilar tip aneurysm with intact metallic densities (red arrow) in its place

patient's pupils were 3/3 mm in diameter, equally reactive to light, and able to tolerate room air.

3. Discussion

AiCS describes a brief flow arrest technique to reduce perfusion pressure and decrease aneurysm turgor, thereby facilitating clip ligation. Adenosine is efficacious and safely administered in combination with remifentanil + propofol + low-dose volatile anesthetic.[7] Patient selection for AiCS depends on the complexity of the aneurysm and the patient profile. In our patient, challenges related to aneurysm include the location and depth of basilar tip aneurysm with limited surgical exposure, a 2.5 cm giant aneurysm with a wide neck, thin walls, and expected high turgor, and the possibility of thrombosis and severe atherosclerosis. The successful clipping of the basilar tip aneurysm in this case was primarily attributed to the use of AiCS.

AiCS offers easy administration without advanced preparation or complex logistical coordination with cardiovascular surgery. This technique also provides maximal surgical field space to facilitate permanent clip ligation without obstruction from temporary clips and perforators, leading to decreased flow toward the aneurysm without the risk of intraoperative rupture and better collapse of the aneurysm through global hypotension, essentially negating the risk of bleeding.[7] As compared to other techniques for cardiac standstill, particularly rapid ventricular pacing, which enforces ventricular tachycardia to consequently reduce stroke volume and cardiac output, AiCS provides rapid onset, offset, and high predictability in providing a brief period of profound systemic hypotension with a low side-effect profile, making it a valuable tool in cerebrovascular surgery.[8] The timing and expected duration of flow arrest are carefully coordinated with the neurosurgeon for adequate aneurysm dissection and clip placement. Careful monitoring and neuroprotective strategies were applied to optimize cellular integrity during this period through continuous propofol infusion, adequate fluid hydration, normoglycemia, normoxemia, and normothermia.

Two approaches for AiCS have been described in the earlier section of this paper. The dose-escalation technique starts with 6 – 12 mg IV of adenosine, titrated on demand, while the dose-estimation technique requires a single dose of adenosine computed at 0.24 – 0.42 mg/kg IV to achieve flow arrest expected to last within 30 – 60 s.[5,6] Although both approaches are deemed safe, the dose-estimation technique is favored as the efficacy of repeated adenosine doses in asystolic duration is unpredictable.[9] In our case, a single dose of 0.4 mg/kg IV was administered at the neurosurgeon's request, effectively facilitating permanent clip placement. This outcome is consistent with the study conducted by Bebawy *et al*.,[5] which found that initial doses of adenosine provide an anticipated momentary interval of cardiac pause approximating 45 s. Normotension, sinus rhythm, normoxemia, normocarbia, and normothermia are all aimed at once ROSC is achieved. As with our patient, vital signs spontaneously returned to baseline, with BIS and rSO_2 remaining within the normal range, for which any silent episodes of cerebral ischemia have been ruled out.

Differences in dose-response times may be attributed to factors such as race, underlying vascular disease, site of administration, and interactions with other medications.[10-15] In our patient, adenosine was administered through left subclavian central access to account for its short half-life (<10 s) and total body clearance of about 30 s.

Adenosine, a known potent systemic vasodilator, may cause persistent hypotension, leading to arrhythmia followed by asystole. The application of external defibrillator pads is recommended for all patients who receive adenosine to provide external pacing in the event of prolonged bradycardia or asystole, or for cardioversion in cases of hemodynamically unstable atrial fibrillation.[16]

Adenosine induces vasodilation in healthy coronary arteries but does not affect atherosclerotic vessels. In patients with cardiac ischemia, the vasodilation of healthy coronary arteries can lead to a paradoxical coronary vascular steal involving an increase in blood flow away from non-ischemic tissue.[17] This may cause significant intraoperative ST depression, followed by sustained ventricular tachycardia and atrial flutter, particularly in patients with a known history of myocardial infarction.[18] Consequently, it is recommended to avoid adenosine administration in patients with severe left main coronary artery stenosis or extensive multi-vessel coronary artery disease.[5]

Cardiac conduction abnormalities may predispose patients to post-administration cardiac arrhythmia and prolonged cardiac arrest, with an incidence of 1%. A study has described a patient with pre-operative premature atrial contractions who developed atrial fibrillation after adenosine administration, accompanied by a prolonged duration of cardiac asystole.[9]

Adenosine acts on A2B adenosine receptors in bronchial smooth muscles, thereby causing bronchoconstriction.[4-7,19,20] Several reports have noted bronchospasm after adenosine administration in patients diagnosed with severe reactive airway diseases.[5,21,22]

Finally, the breakdown and uptake of adenosine are inhibited by dypiridamole,[23] nimodipine,[12] and

methylxanthines,[24] potentially leading to elevated levels of adenosine. As such, these agents are considered relative contraindications when used in conjunction with adenosine administration.

4. Conclusion

In our experience, AiCS at recommended doses effectively and safely facilitated optimal surgical conditions for the clipping of intracranial aneurysms. With careful patient selection, proper communication between the neurosurgeon and anesthesiologist, and optimal anesthetic management, this technique mitigated the risk of intraoperative aneurysmal rupture during surgical dissection.

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Conflict of interest

The authors declare that they have no competing interests.

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Writing – review & editing: Geraldine Raphaela B. Jose, Cristina C. Arcinue-Gomez

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Data are available from the corresponding author upon request.

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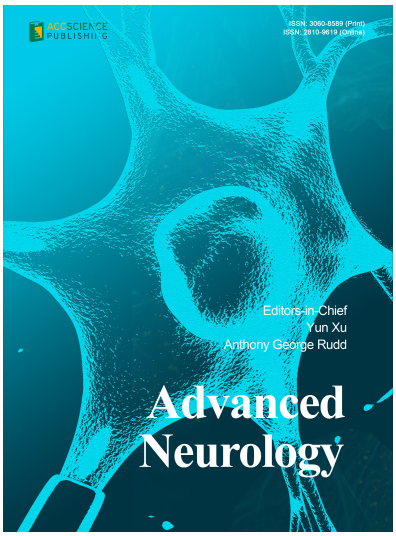

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