Chapter 2

ISCHEMIC STROKE

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Ischemic Stroke (IS) is a severe medical condition resulting from the interruption of blood supply to a part of the brain, leading to brain tissue injury. This event is triggered by various mechanisms,

including oxidative stress, acidosis, excitotoxicity, calcium overload, mitochondrial dysfunction, inflammation, and programmed cell death. Additionally, recent studies have shown that autophagy may be related to the development of the condition. The initial interruption results in a reversible loss of neuronal function, followed by irreversible damage if blood flow is not quickly restored. The acute nature of IS requires an urgent medical response to minimize brain damage and improve clinical outcomes for patients (Feske, 2021; Wang *et al.*, 2021).

Stroke is one of the leading causes of mortality, severe disability, and hospitalization due to neurological diseases worldwide. Responsible for approximately 9% of all global deaths, stroke is a critical case in the field of Clinical and Surgical Emergencies. Although preventing strokes is the most effective strategy to reduce their occurrence and improve patient health, the time window between the onset of symptoms and irreversible tissue injury is crucial. This interval, which can range from minutes to many hours, offers a vital opportunity for urgent interventions that can restore blood flow. This condition underscores the fundamental importance of rapid action and understanding the underlying mechanisms of stroke to develop effective therapies (Feske, 2021; Wang *et al.*, 2021).

IS accounts for 60-80% of all strokes. In the United States, the prevalence of stroke in 2016 was 2.5%, with nearly 800,000 events and 150,000 deaths. Important risk factors include advanced age, female sex, ethnicity, diabetes mellitus, hyperlipidemia, smoking, and cardiovascular diseases. In addition to genetic factors, moderate alcohol consumption and regular physical activity have been found to have protective effects against stroke. Acting on the prevention of these risk factors has led to a decrease in stroke incidence since 1999. However, the risk has increased over the years due to population aging. Furthermore, the importance of continuous advancements in emergency techniques, which have revolutionized the treatment of acute IS, is highlighted to optimize patient health outcomes (Feske, 2021; Wang *et al.*, 2021).

For an immediate diagnosis, brain imaging is used, i.e., imaging diagnosis is performed. Through non-contrast cranial computed tomography (CT), the presence of hemorrhage, which may appear as hyperdensity, is verified. In CT, it is essential to observe the following points: presence of hemorrhage or other diagnoses, signs of infarction, and identification of the location of vascular injury. When the stroke is recent, the cranial CT result usually does not show hemorrhage, making it possible to perform magnetic resonance imaging (MRI) or magnetic resonance angiography (MRA) (Feske, 2021; Wang *et al.*, 2021).

The autophagy process is the body›s response to stroke, alleviating cellular stress by removing damaged organelles. However, it is not sufficient to contain the damage caused by the stroke. Thus, therapeutic manipulation is necessary and is related to early reperfusion of the at-risk tissue with intravenous thrombolysis and/or endovascular thrombectomy and optimization of the hemodynamic state through the management of fluid volume, blood pressure, and cardiovascular status. The use of anticoagulants is also pertinent in the

treatment as a way to prevent a second stroke. Warfarin is an example of a well-known anticoagulant medication used to prevent venous thrombosis and blood clots (Feske, 2021; Wang *et al.*, 2021).

It is worth noting that current trends in stroke management aim to use advanced neuroimaging, which allows for faster diagnosis crucial for immediate intervention. One of the innovative treatments used is the use of thrombolytic drugs to dissolve cerebral clots, allowing blood flow to be quickly restored and minimizing permanent damage. Emphasis on early rehabilitation, including physical therapy, speech therapy, and occupational therapy, is increasing. This integrated approach helps in the recovery of motor, linguistic, and cognitive functions, improving the quality of life and functional outcomes for patients. Regarding prevention and education, it is vital to raise public awareness about the signs of stroke and the importance of rapid intervention. Prevention also involves strict control of risk factors such as hypertension, diabetes, and unhealthy lifestyle habits (Pinto *et al.*, 2023).

EPIDEMIOLOGY

Stroke is the second leading cause of death and the third most common cause of disability worldwide (Feske, 2021). It is a multifactorial disease influenced by genetic, epigenetic, and environmental factors (Jia *et al.*, 2022). About 85% of strokes are ischemic, and 15% are hemorrhagic. Occlusion of the middle cerebral artery leads to damage to the brain parenchyma and a neuroinflammatory response caused by the interruption of cerebral blood flow, potentially resulting in permanent neurological deficits, dementia, or death (Feske, 2021).

Among the pathology›s risk factors, 90% of stroke cases are associated with behavioral factors (poor nutrition, low physical activity, smoking) and metabolic factors (diabetes, obesity, hyperglycemia, hypertension). Additionally, the aging population increases the negative influence of stroke (Feske, 2021). Studies indicate that 15-18% of ischemic strokes (IS) occur in young adults, a number equivalent to 7 per 100,000 inhabitants. It is suggested that the causes of IS in young people differ from those in the older population, but few studies elucidate these differences. However, it is important to emphasize that the impact on the quality of life of younger IS patients is more severe, as they will have to live with the condition for a longer period. An observational study in a hospital in Fukuoka, Japan, conducted with 15,860 patients aged 18 to 50 years, suggested that the history of IS and hemorrhagic stroke (HS) was defined as one of the main risk factors, as well as hypertension, diabetes mellitus, dyslipidemia, alcohol consumption, smoking, and obesity (Lee; Mohd Ismail and Wei, 2021).

Regarding stroke risk factors, it is worth emphasizing metabolic causes, such as Diabetes Mellitus (DM), which generates the production of reactive oxygen species and inflammatory processes, mechanisms that accelerate atherosclerosis and increase

thrombus formation, leading to ischemic stroke. DM is a chronic disease characterized by hyperglycemia and affects 537 million adults globally, with a prevalence of 10.5% among adults aged 20 to 79 years. Among IS and HS patients, 33% and 26% are diabetic, respectively. A meta-analysis of 102 studies showed that diabetes doubles the risk of stroke recurrence and increases the likelihood of death, disability, cognitive impairment, and dementia after an ischemic stroke (Wang *et al*., 2021).

It is noteworthy that there is a relationship between cerebral ischemia, immune cells, intracranial atherosclerosis, and gut microbiota. Gut microbiota can be a risk factor and influence the prognosis after a stroke. The brain and intestine are connected by a neuronal network, forming a gut-brain axis with bilateral interactions. Post-stroke intestinal dysbiosis is common and can affect the production of short-chain fatty acids, such as butyrate, which has anti-inflammatory functions and is essential for maintaining intestinal barrier integrity and inhibiting pro-inflammatory cytokines. Up to 50% of post-stroke patients have gastrointestinal complications, such as constipation, dysphagia, bleeding, and fecal incontinence, negatively affecting treatment outcomes, increasing mortality, and neurological deficits. Studies indicate that the removal of gut bacteria worsens the post-stroke prognosis, while the composition of cecal microbiota changes after focal cerebral ischemia. High levels of Trimethylamine N-oxide (TMAO) are associated with poor treatment outcomes in ischemic brain injuries and increased risk of thrombosis. Modifying TMAO levels could be a promising therapeutic approach. Thus, gut microbiota plays a crucial role in stroke development and treatment, and chronic systemic inflammation post-stroke may be an important therapeutic target to improve clinical outcomes (Feske, 2021).

As mentioned above, stroke is also influenced by epigenetic factors, which can be hereditary but also undergo changes during life in response to lifestyle and environmental exposure. Epigenetic mechanisms include DNA methylation, post-transcriptional histone modifications, changes in chromatin and nucleosome. Some of these processes, such as DNA methylation and post-transcriptional histone modifications, play a crucial role in cellular response and recovery after stroke, as these mechanisms affect chromatin structure and gene transcription, promoting tissue repair and reorganization of damaged tissue cells, influencing neural recovery. DNA methylation, performed by DNA methyltransferase (DNMT), is regulated by factors such as diet, gender, race, and inflammation, which can influence gene expression and is associated with genomic instability and stroke development risk. It is noteworthy that methylation varies throughout life and tends to decrease with age, reflecting environmental risk factors related to aging. Histone modifications such as acetylation and deacetylation are typically physiologically regulated by histone acetylases (HAT) and histone deacetylases (HDAC), respectively. However, in stroke, these alterations have a disturbed balance. Deacetylation and acetylation can generate abnormal histone modifications, leading to smooth muscle cell proliferation, contributing to stroke risk. Non-coding RNAs (ncRNAs) also regulate gene expression and chromatin structure. Subclasses

of ncRNAs, such as tRNA, rRNA, miRNA, snRNA, and lncRNA, are involved in DNA methylation, acetylation, transcriptional and translational regulation, alternative splicing, post-transcriptional modification, and chromatin structure alteration. Enzyme complexes, chromatin modifications, and hypomethylation of long interspersed nuclear element-1 (LINE-1) are associated with a higher risk of stroke due to changes in lipid profiles and atherosclerotic plaque formation. Hyperhomocysteinemia is also a risk factor for stroke and atherosclerosis and occurs when the CBS enzyme is silenced by hypermethylation, preventing homocysteine from being metabolized into cysteine, resulting in high levels of homocysteine, which in turn cause hypomethylation and increased stroke risk. These epigenetic modifications are reversible, offering potential biomarkers for diagnosis and new therapeutic targets. Studies show that epigenetic interventions, such as DNA methyltransferase (DNMT) and histone deacetylase (HDAC) inhibitors, can reduce ischemic injuries and promote tissue recovery. RNA-based therapies and DNMT and HDAC inhibitors are being investigated as promising therapeutic options for stroke treatment (Jia *et al*., 2022).

An important variable to highlight in the epidemiology of Ischemic Stroke (IS) is the circadian moment data at stroke onset. Studies indicate that the progression of Stroke varies according to the circadian cycle, which influences its severity, progression, and long-term outcomes. The study involved 17,461 consecutive patients with witnessed ischemic stroke within 6 hours of onset. The time of stroke onset was divided into two groups (daytime onset [06:00 to 18:00] versus nighttime onset [18:00 to 06:00]) in 4-hour intervals. Preclinical data indicate that stroke initiated during the inactive phase (nighttime for humans) results in greater cell death and infarct growth. In other words, it has greater initial neurological severity, worse functional outcomes at 3 months, and a higher likelihood of early neurological deterioration (END), defined as any new or worsening neurological signs or symptoms within 72 hours after stroke onset. The study›s analysis revealed that nighttime strokes were more frequent among males, young people, and smokers, with a lower prevalence of hypertension. Patients with stroke onset between 18:00 and 02:00 had a higher risk of END.

DIAGNOSIS

Recent studies have highlighted that early identification, emergent intervention, and treatment are factors that can substantially decrease stroke-related fatalities. In cases of ischemic stroke, treatment efficacy depends on early identification followed by immediate administration of reperfusion methods. Early detection includes predicting the likelihood of occurrence based on the extent of risk factors and diagnosing at an early stage with subtle symptoms (Yang *et al*., 2024).

Each year, there are about 12 million new ischemic stroke patients worldwide. This condition is a significant burden on society due to its high incidence, mortality, and sequelae

rates. Therefore, effective diagnosis and treatment are indispensable. Currently, the primary method for diagnosing ischemic stroke is through brain imaging tests, which show the location and extent of brain injury and help distinguish between ischemic and hemorrhagic stroke (Gao *et al.*, 2024).

The most common imaging tests are computed tomography (CT) and magnetic resonance imaging (MRI). CT is more common and readily available, offering advantages such as speed, ease of use, and low cost. Additionally, CT can rule out hemorrhagic stroke and detect other diagnoses with stroke-like symptoms. However, its disadvantages are related to low sensitivity and specificity for early-stage ischemic stroke, along with patient exposure to radiation. MRI is more advanced and detailed for ischemic stroke, with high sensitivity and specificity for ischemic etiology, capable of showing small and deep lesions unlike CT. Moreover, MRI provides information such as the age, size, and type of stroke and the area of potentially salvageable brain tissue. Its disadvantages are high cost, longer execution time, and less availability, with potential contraindications such as patients with metallic implants, pacemakers, or claustrophobia. These imaging tests are expensive and require extensive equipment for large hospitals. Despite excellent diagnostic results in revealing the ischemic area, they do not offer information on disease progression or patient prognosis (Gao *et al.*., 2024).

Thus, identifying body fluids with biomarkers that are easily measured and highly specific and sensitive can address this issue. In a study by Tao et al., low expression levels of MRPS11 and MRPS12 were found in the peripheral blood of ischemic stroke patients, suggesting that MRPS11 and MRPS12 could be biomarkers for this etiology. Additionally, elevated concentrations of neurofilament light chain (NFL) and glial fibrillary acidic protein (GFAP) were found in the serum of ischemic stroke patients, indicating that NFL and GFAP could also serve as biomarkers. However, further studies on biomarkers are still needed (Gao *et al.*, 2024).

Typical symptoms of a stroke include sudden severe headache; unilateral weakness, numbness, or paralysis of the face, arms, or legs; blindness or double vision in one or both eyes; slurred, incoherent, or difficult-to-understand speech; loss of balance or coordination; and vertigo (Gao *et al.*., 2024).

In addition to common etiology symptoms, symptom variation may occur due to monogenic disorders, which are specific genetic alterations following a Mendelian inheritance pattern and can lead to stroke. Monogenic ischemic strokes occur in only 7% of patients, but it is necessary to know and diagnose the most common syndromes (Ekkert *et al.*, 2021).

Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL) is the most common cause of monogenic ischemic stroke. Clinical symptoms include recurrent strokes in young or middle-aged adults, migraine with aura, progressive dementia, apathy, and psychiatric disorders of small cerebral vessels.

Other manifestations of this disease include premature alopecia (about 90%), early vascular dementia, and severe back pain with lumbar disc herniation (Ekkert *et al*., 2021).

Fabry Disease, caused by a mutation in the GLA gene, manifests in early age, causing neuropathies and acroparesthesias. Other symptoms include chronic abdominal pain, angiokeratoma, renal dysfunction, cardiac arrhythmias, and hypertrophy. Mitochondrial Encephalopathy, Lactic Acidosis, and Stroke-like Episodes (MELAS) differ from the cited disorders as it is caused by a mitochondrial DNA (mtDNA) variant inherited from the mother. Typical symptoms include seizures, migraine-type headache, ataxia, hearing impairment, diabetes, muscle weakness, and myopathy. Retinal Vasculopathy with Cerebral Leukodystrophy (RVCL) is an autosomal dominant disease caused by a mutation in the TREX1 gene. RVCL affects small vessels, causing progressive vision loss, migraine, psychiatric abnormalities, cognitive impairment, seizures, and ischemic strokes (Ekkert *et al*., 2021).

Other similar but clinically different conditions include Vascular Ehlers-Danlos Syndrome, manifesting with vascular, urinary, intestinal, and reproductive system fragility, and Sickle Cell Disease, an autosomal recessive disease caused by a mutation in the β-globin subunit (HBB), characterized by acute pain crises, chest syndrome, stroke, and other chronic complications. Homocystinuria, caused by a mutation in the cystathionine β-synthase gene, is characterized by corresponding enzyme deficiency, presenting clinical manifestations involving retinal, skeletal, vascular, and neurological pathology (Ekkert *et al*., 2021).

To diagnose ischemic stroke (IS), various diagnostic methods are used, including physical, laboratory, and imaging tests. In the physical exam, the physician conducts a detailed neurological assessment, which involves reflex tests, muscle strength evaluation, coordination, sensitivity, and visual function. Additionally, the patient›s medical history is crucial to identify risk factors such as hypertension, diabetes mellitus, smoking, and a family history of vascular diseases, which may contribute to the development of IS.

In laboratory tests, a complete blood count is performed to detect possible abnormalities in blood cells, a lipid profile to assess cholesterol and triglyceride levels, and blood glucose levels to identify diabetes. Coagulation testing is essential to evaluate blood clotting capacity, providing important information about the risk of clot formation that can lead to IS. Among imaging tests, CT and MRI stand out. CT is used to visualize the brain and identify areas of ischemia or hemorrhage, while MRI provides detailed images that allow identification of small ischemic areas and assessment of the extent of brain damage. Carotid artery Doppler ultrasound complements the diagnosis by evaluating blood flow in the carotid arteries, identifying obstructions that may predispose to IS. Cerebral angiography allows direct visualization of cerebral arteries, enabling identification of areas of narrowing or occlusion that may cause or contribute to IS (Yang *et al*.., 2021; Gao *et al*.., 2024).

Beyond these conventional methods, radiomics is emerging as a promising technique. Recent studies have explored radiomics for advanced analysis of medical

images. Radiomics is a rapidly developing research field focusing on extracting measurable metrics, called radiomic features, from medical images. These features represent tissue and pathological attributes, such as heterogeneity and shape, and can be effectively used together. Compared to traditional clinical radiographic assessments, radiographic imaging offers benefits such as non-invasiveness, non-destructiveness, cost-effectiveness, rapid analysis, and easy serialization. Radiomics involves several steps. Initially, radiologists and specialists delineate regions of interest in medical images. Next, radiomic features are derived from these regions, encompassing characteristics such as intensity, shape, and texture. The most significant features are then selected to build the foundational model. The growing adoption of radiomics is mainly due to its remarkable compatibility with artificial intelligence, simplifying automated image segmentation and result production. This integration aids healthcare professionals in quick and accurate diagnosis and other clinical assessments (Yang *et al*., 2024; Steliga *et al*., 2020).

This approach allows extraction of quantitative and qualitative information from CT and MRI images, identifying subtle and complex patterns that may not be evident in traditional visual analysis. The application of radiomics in IS diagnosis can significantly improve diagnostic and prognostic accuracy, aiding in the selection of the best therapeutic strategies and predicting patient clinical outcomes (Yang *et al*., 2021; Gao *et al*.., 2024).

However, it is essential to consider the need for standardization of imaging protocols and overcoming technical challenges to ensure the reproducibility and reliability of results in different clinical and research scenarios.

The differential diagnosis of IS is crucial to distinguish this condition from other conditions with similar symptoms. One of the main challenges is to differentiate IS from conditions such as Transient Ischemic Attack (TIA), where brief episodes of neurological dysfunction occur without permanent infarction, usually resolving within 24 hours and without leaving visible damage on imaging tests. Additionally, migraine with aura can present temporary neurological symptoms followed by characteristic headache, distinguished by a history of migraines and the absence of ischemia signs on imaging tests. Other conditions to consider in the differential diagnosis include hypoglycemia, which can mimic neurological symptoms due to low blood glucose levels, quickly confirmed by glucose measurement. Seizures, in turn, can manifest focal symptoms similar to IS, with differentiation aided by EEG and a history of epilepsy. Brain tumors, by compressing brain structures, can cause progressive neurological symptoms identified by imaging tests such as CT and MRI. Finally, multiple sclerosis, characterized by variable symptoms related to demyelinating lesions in the central nervous system, requires a combined analysis of clinical history, imaging tests, and cerebrospinal fluid analysis for proper differential diagnosis (Yang *et al*.., 2021; Gao *et al*.., 2024)..

A systematic review examined cerebrospinal fluid biomarkers for the diagnosis and prognosis of acute ischemic stroke, highlighting the importance of distinguishing this

condition from other conditions with similar symptoms. The discussion emphasizes the need for specific criteria to differentiate between different types of stroke and other neurovascular pathologies. Biomarkers such as specific proteins, neurofilaments, and inflammatory markers have shown significant potential in accurately differentiating between ischemic and hemorrhagic stroke, as well as other neurological conditions that may present similar symptoms. Incorporating these biomarkers into diagnostic protocols not only facilitates early and accurate diagnosis but also allows risk stratification and selection of more appropriate therapies for each patient, representing a promising research area to enhance clinical care and improve patient outcomes (Naik *et al.*, 2021).

The inability to accurately diagnose a patient in the early phase of ischemic stroke can affect up to 30% of cases. The use of diagnostic tests based on biomarker panels is rationally justified in cases of: lack of characteristic symptoms, initial assessment by non-specialists, normality in conventional imaging exams, inaccessibility of equipment such as MRI or angiography, and cases where disease characterization is incomplete (absence of patient history, unknown onset of symptoms, incomplete clinical examination). Therefore, there is a gap for the introduction of simple, inexpensive, and reliable diagnostic methods, including those based on the use of biomarkers (Steliga *et al.*, 2020). Rapid and economically accessible molecular biomarkers have proven indispensable in managing specific pathologies. Identifying biomarkers for the accurate diagnosis of ongoing or imminent cerebral ischemia and for predicting clinical outcomes represents a significant shift in clinical decision-making (Niak *et al.*, 2023).

The gold standard and the goal in the search for the ideal biomarker for ischemic stroke involve a sensitive, measurable substance that is objectively evaluated as an indicator of specific physiological and pathological processes associated with a particular type of stroke. This biomarker should show an increase in concentration during a defined period after symptom onset, reflecting responses to therapeutic interventions. The biomarker›s utility also depends on its availability for diagnostic purposes, which is related to its presence in physiological fluids such as blood or cerebrospinal fluid (CSF). Due to the CSF›s proximity to the microenvironment of acute ischemic stroke, biomarkers collected from CSF may offer greater sensitivity compared to other diagnostic tools, thus providing more reliable and early identification (Steliga *et al.*, 2020).

Three of the most frequently used CSF markers are S100beta (S100B), a specific indicator produced by astrocytes that envelop blood vessels in the brain, and the two inflammatory markers interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α). Another commonly cited factor is free fatty acids, a measure of lipid and cell degradation. Other biomarkers have diagnostic importance in different phases of ischemic stroke, such as Glial Fibrillary Acidic Protein (GFAP) and Neuron-Specific Enolase (NSE) (Steliga *et al.*, 2020).

S100β is present in astrocytes, oligodendrocytes, and Schwann cells and acts as a marker of neuroprotective or neurotoxic function. Elevated concentrations of S100β promote

necrotic and apoptotic cell death. It can be used to assess malignant and hemorrhagic stroke transformation and to exclude stroke mimics. There is a correlation between serum levels of S100β and levels in CSF, as well as infarct volume. S100β leakage from astrocytes begins about 4 hours after ischemia onset and depends on reduced cerebral blood flow and tissue destruction. S100β levels in CSF are positively correlated with stroke severity. Additionally, S100β undergoes early upregulation in the course of brain tissue damage and is a good predictor of injurious events. Increased S100β concentration can provide an early warning signal before it becomes visible on CT imaging in the early phase of stroke. This biomarker can be useful for predicting the development of malignant stroke, increased infarct volume, or excluding stroke mimics (Steliga *et al.*, 2020; Niak *et al.*, 2023).

IL-6 is a pro-inflammatory cytokine secreted by microglia or astrocytes, depending on whether it is the acute or subacute phase of ischemic stroke, respectively. Its serum levels increase during ischemic stroke and can be used as a predictor of stroke prognosis along with assessing infarct extent. Elevated IL-6 concentrations after 6 hours and increased NIHSS scores on admission promote early deterioration within 48 hours or death up to 72 hours after ischemic infarction onset. IL-6 concentration also correlates with S100β levels and the extent of tissue damage. Changes in serum IL-6 concentration are not exclusive to ischemic strokes and have also been observed during subarachnoid hemorrhage and hemorrhagic stroke. In severe strokes (NIHSS 21-42), IL-6 levels are elevated and persist in chronic changes (>1 week) after stroke. Additionally, TNF-α and nitric oxide cannot be considered independent predictors of stroke outcome or severity (Steliga *et al.*, 2020; Niak *et al.*, 2023).

GFAP is produced in astrocytes and functions to signal cellular integrity and reactive gliosis. Its production increases in ischemic and hemorrhagic stroke, as well as in traumatic brain injury. GFAP distinguishes between ischemic and hemorrhagic stroke. GFAP is not exclusive to a particular type of stroke and is also activated during traumatic brain injury. In hemorrhagic stroke, the increase in expression is more pronounced than in ischemic infarction. GFAP can aid in differentiating between hemorrhagic and ischemic stroke from 2 to 6 hours after stroke onset. For hemorrhagic stroke, the highest serum levels usually occur between 2 and 6 hours after onset, while in ischemic stroke, serum concentration begins to rise after 24 hours and peaks between the second and third day. In hemorrhagic stroke, there is a connection between lesion size and GFAP serum concentration, but not in ischemic stroke, except after 2 hours from onset. This glycolytic enzyme exhibits expression changes in the acute phase of ischemic stroke. There is a reported correlation between serum NSE levels and the volume of ischemic infarction, making it an indicator of hemorrhagic transformation from an ischemic infarction (Steliga *et al.*, 2020).

In summary, the difficulties in finding suitable biomarkers for ischemic stroke diagnosis arise from the diversity of this clinical condition. Despite the continuous development of new diagnostic methods, identifying cerebral ischemic infarction, particularly in its early stage,

remains highly challenging. Although there are various markers under study, the diagnostic efficacy is still insufficient, especially for multiple types of stroke. This justifies an approach focused on selecting a panel of potential markers with acceptable sensitivity and specificity, linked to the acute or subsequent phases of stroke (Steliga *et al.*, 2020).

The Neurovascular Unit (NVU) is a conglomerate of different elements: neurons, astrocytes, microglia, oligodendrocytes, vascular endothelial cells, perivascular cells, vascular smooth muscle cells, basement membrane, and extracellular matrix. The NVU plays a significant role in the onset and progression of IS. Extracellular Vesicles (EVs) originating from the NVU can act as biomarkers for IS. Compared to bioactive substances in the blood, bioactive substances transported by NVU-derived EVs are more likely to be used as diagnostic and prognostic markers for IS (Gao *et al.*, 2024; Steliga *et al.*, 2020).

Extracellular vesicles are small vesicles surrounded by a lipid bilayer released by various cells through endocytosis. These small vesicles contain different types of molecules, including proteins, lipids, and nucleic acids. They are categorized based on their size as exosomes, microvesicles, or apoptotic vesicles. EVs play a vital role in facilitating material exchange and information transfer between cells, transporting a wide variety of bioactive substances. Based on this, NVU-derived EVs indicate the physiological and pathological condition of the brain cells that produce them. EVs demonstrate the molecular and cellular changes occurring in the brain, which can be detected in minimally invasive procedures, such as blood or CSF samples, facilitating the diagnosis, prognosis, and therapeutic response of diseases such as Alzheimer›s disease (AD), Parkinson›s disease (PD), and multiple sclerosis, besides being biomarkers for ischemic stroke. EVs can also be used to distinguish different locations of ischemic stroke, as the contents of EVs released from different brain regions vary (Gao *et al*., 2024).

MicroRNAs are small molecules that regulate gene expression by binding to messenger RNAs and inhibiting their translation into proteins. They are used as biomarkers for ischemic stroke and modify the expression of genes involved in neuronal survival, inflammation, and vascular function (Gao *et al.*, 2024). Non-coding RNAs (ncRNAs) have important functions in controlling gene expression and physiological cellular processes. These ncRNAs are abundantly present in mammalian brains, and their alterations appear to have a significant impact on cerebral ischemia and recovery after a stroke. miRNAs are remarkably stable in various biological samples, such as plasma, serum, urine, and cerebrospinal fluid, and can be used as diagnostic, prognostic, or therapeutic biomarkers for various diseases. A significant group of these miRNAs, known as «HypoxamiRs,» is especially upregulated in hypoxic conditions. Among them, miRNA-210 (miR-210) is one of the most prominent, being activated by hypoxia-inducible factor 1 (HIF-1) (Rahmati; Ferns and Mobarra, 2021).

Levels of miR-15a, miR-100, miR-339, and miR-424 in circulating EVs are lower in patients with cortico-subcortical ischemic stroke than in patients with subcortical stroke.

Additionally, EVs can be used as markers to distinguish different stages of cerebral ischemia. Plasma exosomal levels of miR-21-5p in the subacute and recovery phases of stroke were significantly higher than those in other phases. The brain-specific miRNAs miR-9 and miR-124 can be detected in serum EVs. The increase in circulating exosomal levels of miR-122-5p, miR-300-3p, and miR-450b-5p is related to transient ischemic attack (TIA). Both miR-9 and miR-124 are positively associated with National Institutes of Health Stroke Scale (NIHSS) scores, serum IL-6 levels, and infarct volume (Gao *et al.*, 2024).

Patients with IS exhibit higher serum levels of HIF-1α and lower levels of miR-210 than the upper limit of normal. miR-210 was considered a weak diagnostic biomarker upon admission, while HIF-1α was deemed an acceptable diagnostic marker for IS. HIF-1, a crucial component of the hypoxia response, belongs to hypoxia-inducible factors. Under hypoxic conditions, the alpha subunit of HIF-1 (HIF-1α) is stabilized and upregulated. Serum levels of miR-210 significantly changed between admission and 3 months post-IS, whereas serum levels of HIF-1α did not vary significantly during this time interval (Rahmati; Ferns and Mobarra, 2021).

Circulating microRNAs and disease-related blood proteins assist in distinguishing between affected and unaffected patients and predicting associated outcomes (Rahmati; Ferns and Mobarra, 2021). The detection of miR-9 and miR-124 in serum extracellular vesicles is a promising diagnostic approach for ischemic stroke, allowing early diagnosis and assessment of the extent of brain injury (Gao *et al.*, 2024). miR-210 is not viable as a diagnostic marker due to its limited performance, whereas serum HIF-1α may be a useful marker, presenting acceptable sensitivity and specificity (Rahmati; Ferns and Mobarra, 2021). The use of NVU-derived extracellular vesicles represents an emerging field that tends to optimize diagnostic efficiency in the context of ischemic cerebrovascular accidents (Gao *et al.*, 2024).

The candidate gene approach is commonly used to find the genetic reasons for stroke, especially in cases of recurrent events or younger-than-usual age of onset. In this method, genetic variations in a gene known to cause a specific condition are identified. If the condition›s characteristics are not well defined or there are no typical features, the wrong candidate gene may be selected. A limitation of this method is the inability to discover new genetic variations (Ekkert *et al.*, 2021).

Genome-Wide Association Studies (GWAS) present an innovative genetic approach that demonstrates significant efficacy. By genotyping more than a million polymorphisms across the genome simultaneously, GWAS adopts a different method compared to the candidate gene method. GWAS is not limited to a single gene but explores the entire genome, allowing the discovery of new connections between chromosomal loci and diseases. The current difficulty in identifying heritability in specific disease subtypes using GWAS, such as in ischemic stroke, may be attributed to rare and low-frequency variants (Ekkert *et al.*, 2021).

NGS technologies can gather information about entire genomes—whole genome sequencing (WGS)—or protein-coding sequences—whole exome sequencing (WES). The primary goal of NGS is to detect uncommon genetic variations that may not be revealed by GWAS. Its relatively low cost and rapid implementation make it a promising tool not only for research but also for the routine diagnosis of hereditary conditions associated with stroke (Ekkert *et al.*, 2021).

Using weighted gene coexpression network analysis (WGCNA) to validate stroke-associated genetic modules, searching for genes that change over time in stroke progression, and employing different machine learning algorithms (LASSO, SVM-RFE, and Boruta) to discover specific genes involved in stroke, it was found that in the early stages of a stroke, the damaged part of the brain is populated by activated microglia. Highly activated microglia exhibit distinct markers such as Spp1, Lpl, Lgals3, and Cst7. In stroke-related microglia, there is an increase in the expression of Spp1, Lgals3, and Cst7, and diagnostic intervention based on identifying genes attributed to stroke development proved more effective due to the nomogram constructed using machine learning (Song *et al.*, 2023). These innovative techniques provide a new perspective on stroke diagnosis and treatment, enhancing the precision and efficacy of currently used methods.

TREATMENT

Ischemic stroke continues to pose a significant public health challenge due to the scarcity of effective medications and treatment methods. Stroke is the fifth most prevalent cause of mortality worldwide, with 80% of strokes being ischemic events, and an incidence rate of 7.63 million in 2019 (Kamal *et al.*, 2023). Before the 1990s, treatment options for ischemic stroke were limited, primarily focusing on symptomatic management, secondary prevention, and rehabilitation (Herpich; Rincon, 2020). Since then, the field has been transformed by two crucial innovations. The first was the FDA approval of intravenous tissue plasminogen activator (IV-tPA) in 1995, following a seminal study by the National Institute of Neurological Disorders and Stroke (NINDS), marking a significant milestone in the acute treatment of stroke.

For approximately two decades, IV-tPA served as the cornerstone of treatment until advanced clinical trials in the 2010s demonstrated robust results for endovascular therapy (EVT). EVT, which includes mechanical thrombectomy, emerged as an effective intervention to remove larger thrombi, timely restoring cerebral blood flow (Herpich; Rincon, 2020).

Pre-hospital interventions, such as early activation of specialized teams and the use of mobile stroke units equipped with advanced diagnostic technology, have significantly reduced time to treatment, thereby improving clinical outcomes. Moreover, updated guidelines from the American Stroke Association (ASA) and the European Stroke Organization have guided clinical management, ensuring that patients benefit from treatments like alteplase within the therapeutic window of 4.5 hours after symptom onset (Zubair; Sheth, 2021).

Given that ischemic stroke is a devastating condition requiring urgent therapeutic intervention to minimize brain damage and improve clinical outcomes, in addition to thrombolytic and endovascular therapies, there are also studies that include:

- Neuroprotective Agents: Compounds that protect brain cells against ischemia and inflammation-induced damage, such as antioxidants, anti-inflammatory agents, ion channel blockers, among others. However, the efficacy of these agents is limited by their ability to cross the blood-brain barrier (Xu *et al.*, 2023).
- Stem Cell Therapy: Emerging as a promising regenerative strategy, including bone marrow mononuclear cells, adipose tissue-derived stem cells, neural stem cells, and others. These cells have the potential to functionally improve post-stroke recovery, but regulatory challenges and costs remain important considerations (Kawabori *et al.*, 2020).
- Biomarkers: Molecular markers such as GFAP, IL-1β, MMP-9, and miRNAs are used for diagnosis, prognosis, and treatment guidance. They reflect the extent of brain damage, inflammatory response, and blood-brain barrier integrity, providing valuable insights for personalizing therapy and monitoring disease progression (Gao *et al.*, 2024).
- Acetylsalicylic Acid (ASA): Known as aspirin, ASA is used in the secondary prevention of ischemic stroke. It works by inhibiting the production of thromboxane, a pro-coagulant agent, thus reducing the risk of new clot formation and helping to prevent future events (Szczuko *et al.*, 2021).

These therapeutic approaches represent significant advances in managing ischemic stroke, aiming not only to restore cerebral blood flow but also to protect neuronal tissue and improve long-term outcomes for patients affected by this serious and potentially debilitating condition.

Currently, systemic thrombolytic therapy remains the most common therapeutic option in managing ischemic stroke. Based on the goal of recanalizing and reperfusing ischemic areas, this therapeutic method can be adopted up to 4.5 hours after the onset of the ischemic event (Zhao; Zhang; Chen and Wei, 2022). The drug used in systemic thrombolytic therapy is intravenous alteplase at a dose of 0.9 mg/kg, with a maximum dose of 90 mg. Ten percent of the total dose is administered as a bolus, while the remaining 90% is administered over the next hour. Although this is the most used therapeutic method, several patient groups have contraindications to its use. These groups include people with a history of hemorrhagic stroke at any time in life or severe traumatic brain injury or ischemic stroke in the last 3 months, patients with coagulopathies, hypoglycemia, hyperglycemia, severe hypertension (SBP > 185 mmHg or DBP > 110 mmHg), or active intracranial hemorrhage (Zubair; Sheth, 2021). Additionally, the isolated use of alteplase has poor efficacy on occlusions of proximal arteries, such as the internal carotid artery or the proximal portion of the middle cerebral artery (Zhao; Zhang; Chen and Wei, 2022).

The treatment of ischemic stroke faces several challenges that limit the effectiveness of current therapeutic approaches. Endovascular and thrombolytic therapy, although fundamental, faces significant limitations due to the restricted time window and the complexity of managing the blood-brain barrier. Further advances in imaging techniques, such as enhanced perfusion scanning and new high-resolution MRI modalities, have the potential to significantly improve early diagnosis and personalized treatment of stroke. Recent studies have demonstrated that portable low-field MRI can be viable for real-time monitoring in intensive care units, promoting rapid and precise interventions (Zubair; Sheth, 2021).

Additionally, a fundamental challenge lies in the limited understanding of the mechanisms by which circRNAs contribute to ischemic stroke. These circular RNAs play crucial roles in post-transcriptional regulation and modulation of key pathophysiological pathways such as apoptosis, autophagy, and inflammation. However, their exact translation of function is not yet fully elucidated, and it is essential to develop innovative delivery mechanisms that can overcome the restrictions imposed by the blood-brain barrier. The inflammatory response triggered by cell death induced by cerebral ischemia presents itself as a promising therapeutic target (Xu *et al.*, 2023).

Despite the innovations of recent decades that have expanded access to acute stroke treatment and improved clinical outcomes, the stroke mortality rate has stabilized and even increased in certain regions. This phenomenon is attributed to the increase in the prevalence of modifiable risk factors, such as diabetes, hypertension, and hyperlipidemia. Therefore, a renewed focus on educational and preventive strategies is crucial to reduce the incidence of severe or fatal stroke in the future (Herpich; Rincon, 2020).

REFERENCES

EKKERT, Aleksandra, *et al.* Ischemic stroke genetics: what is new and how to apply it in clinical practice?. **Genes**, v. 13, n. 1, p. 48, 2021.

FESKE, Steven K. Ischemic stroke. **The American journal of medicine**, v. 134, n. 12, p. 1457-1464, 2021.

GAO, Xiangyu, *et al.* Revolutionizing Ischemic Stroke Diagnosis and Treatment: The Promising Role of Neurovascular Unit-Derived Extracellular Vesicles. **Biomolecules**, v. 14, n. 3, p. 378, 2024.

HERPICH, Franziska; RINCON, Fred. Management of acute ischemic stroke. **Critical care medicine**, v. 48, n. 11, p. 1654-1663, 2020.

JIA, Junqiu, *et al.* The role of microglial phagocytosis in ischemic stroke. **Frontiers in immunology**, v. 12, p. 790201, 2022.

KAMAL, Fatima Zahra, *et al.* The role of potential oxidative biomarkers in the prognosis of acute ischemic stroke and the exploration of antioxidants as possible preventive and treatment options. **International Journal of Molecular Sciences**, v. 24, n. 7, p. 6389, 2023.

KAWABORI, Masahito, *et al.* Clinical trials of stem cell therapy for cerebral ischemic stroke. **International journal of molecular sciences**, v. 21, n. 19, p. 7380, 2020.

LEE, Yee Teng; MOHD ISMAIL, Nor Ismaliza; WEI, Loo Keat. Microbiome and ischemic stroke: A systematic review. **PLoS One**, v. 16, n. 1, p. e0245038, 2021.

NAIK, Anant, *et al.* Cerebrospinal fluid biomarkers for diagnosis and the prognostication of acute ischemic stroke: a systematic review. **International journal of molecular sciences**, v. 24, n. 13, p. 10902, 2023.

PINTO, S.; HOGAN, C.; BERKHEMER, O.; SHAFAAT, M.; MAZUREK, A. Manejo diagnóstico e terapêutico do acidente vascular cerebral isquêmico no departamento de urgência. **Revista de Fisioterapia**, 2023.

RAHMATI, Mina; FERNS, Gordon A.; MOBARRA, Naser. The lower expression of circulating miR-210 and elevated serum levels of HIF-1α in ischemic stroke; Possible markers for diagnosis and disease prediction. **Journal of Clinical Laboratory Analysis,** v. 35, n. 12, p. e24073, 2021.

SONG, Jingwei, *et al.* Integrative Analysis of Machine Learning and Molecule Docking Simulations for Ischemic Stroke Diagnosis and Therapy. **Molecules**, v. 28, n. 23, p. 7704, 2023.

STELIGA, Aleksandra, *et al.* Neurovascular unit as a source of ischemic stroke biomarkers—limitations of experimental studies and perspectives for clinical application. **Translational stroke research**, v. 11, p. 553-579, 2020.

SZCZUKO, Małgorzata, *et al.* The role of thromboxane in the course and treatment of ischemic stroke. **International Journal of Molecular Sciences**, v. 22, n. 21, p. 11644, 2021.

WANG, Xiaoyu, *et al.* An updated review of autophagy in ischemic stroke: From mechanisms to therapies. **Experimental Neurology**, v. 340, p. 113684, 2021.

XU, Guangchen, *et al.* Circular RNAs: Promising Treatment Targets and Biomarkers of Ischemic Stroke. **International Journal of Molecular Sciences**, v. 25, n. 1, p. 178, 2023.

YANG, Jie, *et al.* Application of radiomics in ischemic stroke. **Journal of International Medical Research**, v. 52, n. 4, p. 03000605241238141, 2024.

ZHAO, Yunfei, *et al.* Neuronal injuries in cerebral infarction and ischemic stroke: From mechanisms to treatment. **International journal of molecular medicine**, v. 49, n. 2, p. 1-9, 2022.

ZUBAIR, Adeel S.; SHETH, Kevin N. Emergency care of patients with acute ischemic stroke. **Neurologic Clinics**, v. 39, n. 2, p. 391-404, 2021.