

REVIEW ARTICLE

Unveiling the complexity of acute ischemic stroke through imaging and neuropathological insights

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Abstract

Effective management of acute ischemic stroke requires timely recanalization of occluded cerebral vessels, restoration of perfusion to the ischemic penumbra, and minimization of infarct volume. At present, clinicians rely heavily on various imaging modalities to evaluate the ischemic penumbra and core. However, the current imaging techniques often fail to accurately reflect the underlying cellular and histopathological changes associated with ischemia and lack standardized assessment criteria. This may lead to inconsistencies in diagnosis and treatment, ultimately compromising patient outcomes. Consequently, there is an urgent need to refine these imaging methods to better reflect the dynamic pathophysiology of cerebral ischemia. This review addresses these critical issues by first examining the shortcomings of current imaging approaches in assessing the ischemic penumbra and core. It highlights the discrepancies in these assessments and their implications for clinical practice. Furthermore, it proposes that integrating quantitative measures of salvageable ischemic regions with imaging-based molecular markers can enhance the precision of delineating the ischemic penumbra and core, thereby facilitating improved therapeutic decision-making. Finally, this review explores emerging biomarkers identified through the analysis of molecular and cellular mechanisms triggered by ischemia and hypoxia, underscoring their potential role in future diagnostics. Future imaging methods should strive to bridge the gap between microscopic cellular and molecular events and reliable imaging markers, ultimately enabling the identification of ischemic regions through biomarkers.

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1. Introduction

In 2018, the DAWN and DEFUSE-3 trials demonstrated that imaging-guided selection not only expanded the eligibility criteria for endovascular treatment (EVT) in acute ischemic stroke (AIS) patients but also substantially improved treatment outcomes.^{1,2} Consequently, stroke care has ushered in the “Tissue is Brain” era, where treatment decisions are guided by direct imaging of brain tissue viability after stroke onset. The restoration of the ischemic penumbra is a critical focus of EVT for AIS. Therefore, the extent of the ischemic penumbra is primarily assessed through imaging examinations in

clinical practice, which serves as the clinical gold standard for diagnosis. Recently, six trials have shown that patients with large ischemic core infarction exhibit a significantly higher proportion of favorable outcomes following EVT compared to medical therapy alone, without penumbra selection.³⁻⁸ Moreover, an exploratory analysis of the SELECT-2 suggested that the volume of imaging penumbra did not modify the treatment effect of EVT.⁹ Besides, numerous studies suggest that current penumbra imaging modalities may not be sufficient to precisely capture the wide spectrum of pathophysiological changes within the ischemic penumbra post-stroke¹⁰⁻¹⁵—including energy metabolic dysfunction, immuno-inflammatory responses, and cytotoxicity.¹⁶ In addition, a study by Goyal *et al.*¹⁷ on the “ghost infarct core,” which means that baseline computed tomography perfusion (CTP) may overestimate core size especially under conditions of complete reperfusion after short ischemia, also underscores the shortcomings of sole reliance on conventional imaging to map biologically meaningful penumbra—an area that is more accurately defined by the underlying pathophysiological processes and biomolecular changes within penumbral neurons. Collectively, these studies indicate that the existing imaging-neuropathological gap may be far wider than previously perceived and worth further study.

In this context, we provide a comprehensive overview of the current knowledge gap between imaging biomarkers of salvageable ischemic regions and their histopathological characteristics through systematic review of cutting-edge research, with particular focus on exploring synergistic applications of biological and radiological markers, thereby offering new perspectives and therapeutic strategies for AIS.

2. Imaging-defined ischemic penumbra: Definition and challenges

The question of how to define the ischemic penumbra has long been a focal point of research in AIS. Various conceptualizations of the penumbra have emerged, including the “pharmacologic penumbra,” which refers to tissue that is salvageable by pharmacological intervention, and the “metabolic penumbra,” which describes tissue with compromised blood flow but preserved energy metabolism.^{18,19} These definitions approach the ischemic penumbra from distinct perspectives. However, it remains challenging to efficiently convey the clinical status of the ischemic penumbra, particularly in emergency situations. Given the considerable variability in ischemic vulnerability across individuals and brain regions, imaging modalities have been highly anticipated, as they represent the most accessible snapshots of the pathophysiological changes

associated with stroke. Furthermore, the prominence of the imaging-defined penumbra in both research and clinical practice has increased following the completion of two pivotal EVT trials in 2018.^{1,2} In this context, we briefly outline the definitions and challenges associated with the imaging-defined penumbra, aiming to provide new insights into stroke prevention and treatment strategies (Figure 1).

2.1. Definition

The penumbra refers to a region of reversible hypoperfusion characterized by impaired electrical activity but preserved cellular metabolism and viability, surrounding the infarct core.^{20,21} However, this cellular activity does not necessarily indicate functional viability; without timely reperfusion, the penumbra may progress to infarction within 1–2 h.²² Imaging examinations define the tissue window by assessing the degree of injury within the ischemic penumbra. At present, two primary methods are employed to evaluate the ischemic penumbra: the “mismatch approach” and the “threshold-based approach.” CTP imaging and magnetic resonance imaging (MRI) are commonly used tools for detecting the imaging-defined penumbra.

CTP is a contrast bolus-tracking technique that can be utilized on virtually any multidetector computed tomography (CT) scanner.²³ The parameters obtained from CTP, including cerebral blood volume (CBV), cerebral blood flow (CBF), mean transit time (MTT), and time to peak (TTP), are essential for assessing the perfusion status of brain tissue. To evaluate the volume of ischemic penumbra, two randomized trials utilized CTP mismatch criteria to select patients who demonstrated improved clinical outcomes following intravenous treatment.^{24,25} The CTP “mismatch” analysis focuses on the disparity between CBF and CBV parameters. Specifically, in the ischemic penumbra, CBF is reduced, while CBV remains unchanged or increases relative to the contralateral hemisphere—this mismatch is a hallmark of the penumbra. In contrast, the infarct core exhibits concurrent reductions in both CBF and CBV, reflecting severe ischemia. In the DEFUSE-3 study, the threshold-based CTP approach defined the infarct core as regions with CBF <30% of normal, and the ischemic penumbra as regions with TTP >6 s, excluding the infarct core.² The EXTEND and HOPE studies employed less restrictive perfusion mismatch imaging criteria, defining the perfusion lesion-ischemic core mismatch as a ratio greater than 1.2 between the volume of hypoperfusion and the volume of the ischemic core, along with an absolute volume difference greater than 10 mL.^{26,27}

The EPITHET trial, along with two prospective studies, demonstrated that patients with a perfusion-diffusion

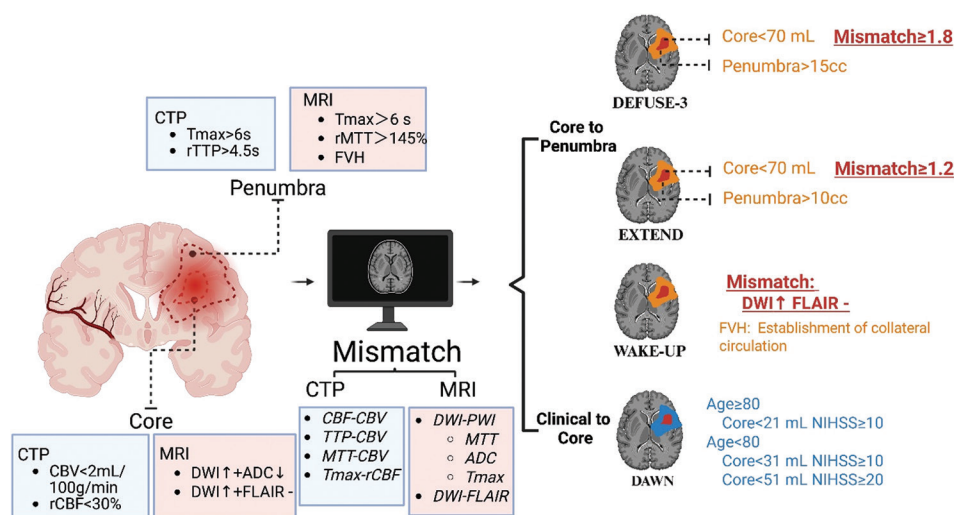


Figure 1. Imaging definitions of mismatch selection in acute ischemic stroke. Schematic created with BioRender.

Abbreviations: ADC: Apparent diffusion coefficient; CBF: Cerebral blood flow; CBV: Cerebral blood volume; CTP: Computed tomography perfusion; DWI: Diffusion-weighted imaging; FLAIR: Fluid-attenuated inversion recovery; FVH: FLAIR vascular hyperintensity; MRI: Magnetic resonance imaging; MTT: Mean transit time; PWI: Perfusion-weighted imaging; rCBF: Relative cerebral blood flow; rMTT: Relative mean transit time; rTTP: Relative time to peak; TTP: Time to peak.

magnetic resonance mismatch who achieved successful reperfusion following either intravenous recombinant tissue plasminogen activator or EVT exhibited better clinical outcomes compared to those without reperfusion.²⁸⁻³⁰ MRI employs two important mismatch approaches: the combination of diffusion-weighted imaging (DWI) with perfusion-weighted imaging (PWI), and the combination of DWI with fluid-attenuated inversion recovery (FLAIR). When PWI, which indicates perfusion deficits, exceeds DWI, which delineates the infarct core, the DWI/PWI mismatch serves as a critical marker for identifying regions with reduced perfusion but without significant cytotoxic edema, known as the salvageable penumbra.³¹ The DEFUSE-3 study utilized the DWI/PWI mismatch to establish eligibility criteria for EVT beyond the traditional time window, defining the target population as patients with a hypoperfusion volume/infarct core volume ratio > 1.8, an infarct core volume ≤ 70 mL, and a penumbra volume ≥ 15 mL. The DEFUSE-3 study demonstrated that the group receiving EVT combined with standard medical therapy had a higher proportion of reperfusion and better clinical outcomes at 90 days compared to the group receiving medical therapy alone.² Meanwhile, the WAKE-UP study utilized DWI/FLAIR mismatch to select an eligible population for intravenous treatment beyond 4.5 h.³² This study defined eligibility as high signal intensity on DWI without obvious lesions on FLAIR images, which typically indicates the presence of salvageable brain tissue, and the results showed that the proportion of 3-month excellent

functional outcomes (mRS score 0–1) was higher in the intravenous treatment group.

2.2. Challenges

The “mismatch” and threshold-based approaches of CTP and MRI for defining the tissue window have yielded significant clinical benefits in AIS, applicable to both intravenous thrombolysis and EVT.^{1,2,26,27,32-34} However, various imaging modalities for detecting the ischemic penumbra exhibit distinct advantages and limitations. First, in delayed time windows (e.g., 6–24 h post-AIS), CTP may underestimate the infarct core volume due to limited scan coverage.⁹ Conversely, in ultra-early time windows, CTP may overestimate the baseline infarct core in patients achieving rapid reperfusion, a phenomenon attributed to the “phantom infarct core” effect.³⁵ Second, while MRI offers superior spatial resolution compared to CT, its practical application is limited by instrumental and operational constraints, including prolonged inspection times, expensive equipment, and complex image processing.³⁶ Moreover, the DWI/PWI mismatch criterion does not fully align with the heterogeneity of ischemic tissue viability, as it may include reversible diffusion-restricted lesions. Third, studies indicate that perfusion thresholds for ischemic injury vary based on gender, age, and tissue type,³⁷ underscoring the inadequacy of relying solely on a single imaging feature for individualized and precise penumbra assessment.

In recent years, advancements in EVT technology have reshaped clinicians’ understanding of the imaging-

defined penumbra. Since 2022, six randomized controlled trials have demonstrated that patients with large anterior circulation infarct cores can derive significant benefits from EVT.³⁻⁸ For example, in the SELECT-2 study, the therapeutic benefit of EVT was sustained across all prespecified imaging subgroups, encompassing patients with all the Alberta Stroke Program Early CT Score (ASPECTS, a criteria to assess the extent of early ischemic changes on non-contrast CT scans in patients with ischemic stroke), patients with core volumes of ≥ 150 mL, and notably cases lacking a target mismatch profile.⁵ Furthermore, an exploratory analysis of the SELECT-2 trial indicated that EVT improved clinical outcomes across a diverse range of infarct volumes, with findings suggesting that the penumbra volume had no significant effect on treatment efficacy.⁹ A recent secondary *post hoc* analysis of the TENSION trial also demonstrated that follow-up infarct volume had limited explanatory power for functional outcomes compared to early neurological status in patients with low ASPECTS.³⁸ These findings suggest that the benefits of EVT may extend up to 24 h from symptom onset in patient populations exclusively composed of those with low ASPECTS, individuals with low ASPECTS and large perfusion core volumes, and even in patients with very small areas of reversible ischemia as defined by CT perfusion (penumbra). This challenges the role of both penumbra and infarct volume as predictive and therapeutic imaging markers.

While both MRI and CTP can yield quantitative measurements to identify areas of inadequate blood flow in the brain, the thresholds or criteria used to define these regions, commonly referred to as mismatch zones, may not accurately reflect the health status of brain tissue. In addition, the lack of advanced tools for visualizing these critical regions has resulted in a limited understanding of the extent of salvageable cells that could provide protection during the ischemia and reperfusion cascade. These observations underscore the limitations of conventional imaging in capturing the full spectrum of biological information. As such, the assessment of ischemic penumbra cannot depend solely on imaging techniques. Instead, it must integrate the dynamic pathophysiological processes of cerebral ischemia to more accurately identify and evaluate the penumbra, thereby informing clinical decision-making.

3. Imaging-defined ischemic core: Definition and challenges

Aside from the volume of the penumbra, the infarct core is another parameter that requires attention. A prespecified substudy of RESCUE-Japan LIMIT trial emphasized the

impact of ischemic core volumes on both prognosis and the effectiveness of EVT.³⁸ MRI and CTP are commonly used in clinical routine for estimation of ischemic core, outcome prediction, and treatment decision-making in the acute stroke setting.¹⁷ However, due to constraints such as the limited therapeutic time window and considerable regional disparities in healthcare resources, the evaluation of ischemic core is predominantly performed using a more straightforward and expedited non-contrast computed tomography (NCCT) approach. This involves the utilization of ASPECTS to quantify the extent of the infarcted tissue. Traditional clinical guidelines for EVT typically excluded patients with large ischemic cores; nonetheless, recent investigations have indicated that even those with substantial infarction in imaging may experience certain benefits from EVT.³⁻⁸ Therefore, we undertook a retrospective review of the prevailing definitions of ischemic core to elucidate their limitations and to propose avenues for future research.

3.1. Definition

When blood flow to a specific area of the brain is interrupted, the ischemic core represents the region experiencing the most severe form of hypoperfusion, which can rapidly progress to irreversible damage.³⁹ In the late window (6 h after onset), ischemic tissue becomes infarcted when the CBF is reduced by more than 70% of its initial value (and not 30%) and, if compared with the contralateral hemisphere, is indicated as relative CBF (rCBF) $< 30\%$.⁴⁰ Studies have consistently shown that the ischemic core volumes predicted by CTP often exaggerate the irreversible infarct volume after EVT, particularly in a very early time window, known as the “ghost core phenomenon.”⁴¹

In the initial stages of ischemic stroke, cellular cytotoxic edema manifests as restricted diffusion on MRI. This high signal intensity can be detected as early as 11 min post-onset in DWI, revealing information pertinent to the initial infarct core volume. In DWI, the infarct core volume is defined by an apparent diffusion coefficient (ADC) of $< 620 \times 10^{-6} \text{ mm}^2/\text{s}$.⁴² Research indicates that the sensitivity of DWI for detecting infarct core volume is 73–92% at 3 h and 95–100% at 6 h post-stroke onset.⁴³ However, heterogeneity in imaging protocol leads to significant changes in the ADC distribution, ischemic core location, size, and association with clinical scores. Work is needed in standardizing imaging protocols to improve the reliability of ADC as an imaging biomarker in stroke management protocols.⁴⁴

When cerebral tissue experiences ischemia, it presents as low density on NCCT. These low-density regions, previously considered as core areas, may be

reversible within a specific time window through EVT. The ASPECTS, based on CT scans, is a semi-quantitative method for evaluating anterior circulation blood supply, distinguishing between viable brain tissue and infarcted tissue.⁴⁵ It effectively predicts the initial infarct volume; higher ASPECTS correlate with smaller infarct volumes. In 2015, the American Heart Association/American Stroke Association recommended EVT for patients with AIS who have an ASPECT score ≥ 6 .⁴⁶ Infarcted tissue is affected by mean partial volume and vasogenic edema, causing the density values on CT to decline with prolonged ischemic time.⁴⁷ The assessment of ASPECTS on NCCT shows time-dependent characteristics, with a reduced detection rate for ultra-early infarction. Moreover, the CT-derived initial infarct core volume does not always equate to the final infarct core volume, indicating certain limitations. However, multimodal imaging approaches can enhance the identification of infarct core volumes.

3.2. Challenges

The ischemic core refers to the early phase of AIS, during which the fate of the tissue has not yet been definitively determined based on baseline imaging. In contrast, infarction designates tissue that exhibits histologically confirmed necrosis, as evidenced by follow-up imaging (NCCT or DWI-MRI). Meanwhile, the determination of whether and to what extent the baseline ischemic core progresses to infarction can only be made retrospectively. In the context of ischemic stroke, two distinct patterns of cell death are recognized: pan-necrosis, which involves the necrosis of all cell types—including neurons, microglia, astrocytes, and endothelial cells—and incomplete infarction. The former is commonly referred to by clinicians as the ischemic core on baseline imaging, and as infarction on follow-up imaging. In contrast, the term “incomplete infarction” is utilized to describe scattered regions of necrosis interspersed with areas of preserved tissue. While this interpretation of imaging findings is widely accepted, it remains uncertain whether it fully captures the underlying pathology.¹⁷

Simultaneously, the measurement of the imaging-defined infarct core does not always align completely with histologically confirmed infarction, similarly to the concept of the penumbra. Previous studies have shown that infarct volumes increase steadily for about 3 days after symptom onset and then decrease slowly due to resolution of vasogenic edema; therefore, it is likely that the volumetric differences between early ischemic core and final infarct were overestimated due to early edema in some patients.⁴⁸ Follow-up NCCT may underestimate infarct size due to the fogging effect.⁴⁹ This phenomenon occurs when initially hypodense ischemic areas become

transiently isodense to normal brain tissue, typically around 2–3 weeks after the onset of ischemia. In addition, infarction can be extremely patchy, making both human and automated infarct volume estimation difficult. Even if imaging could precisely define the volume of infarction on follow-up imaging, the validation of core volumes would require immediate and complete recanalization.⁵⁰ Both the degree and timing of reperfusion are particularly relevant to final infarct volume.⁵¹ Moreover, the ischemic core of certain patients can still be salvaged due to early and complete reperfusion, complicating core measurements.

Defining a definite tissue state (e.g., ischemic core or penumbra) is to quantify ischemic brain to ensure more precise treatment decision-making.^{52–54} However, this dogmatic approach is undermined by an oversimplification that results in a loss of critical data. For example, the transition from ischemic core at baseline imaging (*i.e.*, areas of severe ischemia) to actual infarction is non-linear and complex.^{55,56} The progression to infarction varies across cell types—for example, neurons may be already severely damaged in regions where astrocytes are only minimally injured⁵⁷—and this progression is also variable in both time and space. The classification of tissue state using binary thresholds of 100% and 0% infarction probability results in a precision that is not attainable within the available data.¹⁵ This approach inadequately represents the complexities of histopathological realities and neglects the influence of multivariate confounders that affect tissue outcomes at specific brain regions over time.⁵⁵ Additional factors, including ischemic preconditioning (e.g., neuroprotective agents or hypothermia therapy), the location of the infarct (e.g., white matter infarcts often contribute to increased leukoaraiosis), the level of vessel occlusion, and the processes of reperfusion and recanalization, also influence the dynamic nature of the ischemic core.^{54,58}

4. Evolution of the ischemic regions from imaging and biological perspectives

As research on large infarct cores progresses, there is growing consensus that assessment of the imaging-defined penumbra must incorporate molecular biology approaches. Enhancing our understanding of the ischemic penumbra requires identifying characteristic imaging and biological biomarkers of neural injury and repair. Integrating biological metabolism analysis with imaging markers will enable the development of a unified imaging-biological framework for diagnostic and therapeutic decision-making. Such an approach has the potential to markedly enhance functional outcomes in AIS.

4.1. Imaging ischemic regions: Dynamic, multiparametric model at multiple level

The current definitions of the infarct core and penumbra may be limited due to their oversimplification.⁵⁹ Macro and static neuroimaging methods are insufficient to meet the needs of expanding treatment populations and enhancing therapeutic efficacy (Table 1). Therefore, it is essential to consider the infarct core and penumbra from a dynamic, multimodal and multilevel perspective, viewing them as continuously evolving entities. The underlying cellular and molecular mechanisms of these structures are in a state of constant flux regarding their metabolism and function. In practical applications, understanding these dynamic and multilevel characteristics will provide valuable predictive information about tissue fate following reperfusion, thereby offering a theoretical basis for optimizing treatment strategies and improving clinical outcomes.

4.1.1. CBF, collateral status: Individual difference

Due to physiological variability among individuals, factors such as age, metabolic status, and collateral circulation significantly influence cerebral perfusion. Consequently, assessing the biological penumbra—particularly through the analysis of residual CBF (rCBF) and collateral circulation—is a viable approach. Blood oxygen content, a critical component of rCBF, can be assessed by measuring blood oxygenation level-dependent (BOLD) changes to estimate the oxygen extraction fraction, thereby aiding penumbral evaluation.⁶⁰ Near-infrared light is a form of electromagnetic radiation characterized by wavelengths that lie between those of visible light and mid-infrared light.⁶¹ This technology is non-invasive, high-resolution, and capable of real-time targeting, thereby facilitating continuous observation of changes in collateral vessels and perfusion.⁶²⁻⁶⁴

The MR CLEAN-LATE, DAWN, and DEFUSE 3 trials support the hypothesis that both collateral flow-based

and CTP-based selection identify patients with relatively small infarct cores and preserved penumbras.⁶⁵ Collateral circulation, closely linked to penumbral survival time, is crucial for predicting favorable functional outcomes in AIS patients following reperfusion therapy. The hypoperfusion intensity ratio (HIR), calculated as the ratio of Tmax >10 seconds to Tmax >6 seconds, serves as an indirect measure of collateral circulation status, with values ranging from 0 to 1. Poor collateral status is defined as a HIR >0.5, whereas a HIR ≤0.5 indicates good collateral status.⁶⁶ Collateral blood flow status is commonly utilized as a radiological surrogate marker for assessing infarct growth, a critical concept that reflects patterns of ischemic injury. This marker provides insights into the peripheral CBF and serves as an ideal neuroimaging surrogate for the selection of neuroprotective agents aimed at mitigating cellular damage in the brain.⁶⁷ In addition, sensitive and reliable biomarkers—such as genes, proteins, plasma metabolites, or inflammatory mediators—are essential for assessing collateral status.

4.1.2. Brain frailty: Tissue vulnerability

“Brain reserve” denotes the redundancy in brain networks that affects the presence and severity of symptoms following brain injury, including AIS.⁶⁸ Recently, brain frailty garnered increasing attention, as a concept encompassing the decline of “brain reserve.”⁶⁹ Clinical surrogates include cardiovascular comorbidities (e.g., diabetes, smoking, hypertension, and hypercholesterolemia), prior stroke, or traumatic brain injury. Imaging surrogates for brain frailty, easily detectable on NCCT, include brain atrophy (cortical and subcortical) and features of cerebral small vessel disease (SVD, for example, lacunes, white matter disease, and chronic infarctions). A retrospective study found that large vessel occlusive AIS patients with concurrent cerebral SVD have increased odds of uncompensated collaterals and unfavorable clinical outcomes at 90 days.⁷⁰

Table 1. Summary of the current and future imaging modalities

Imaging modality	Advantages	Limitations
NCCT	Directly and quickly visualize the ischemic core; hypodensity can reflect brain frailty	A pseudonormalization of infarct density on NCCT can be observed, a phenomenon known as the fogging effect; not directly assess penumbra
CTP	Fast and easily accessible in the acute setting of ischemic stroke; practical for pre-reperfusion	Pre-treatment imaging can be inaccurate because of the ‘ghost’ large infarcts; several other variables are crucial to ischemic infarct growth, such as blood glucose and age
MRI	High-resolution, multimodal imaging provides an in-depth analysis of the cellular metabolic characteristics of the infarct core	DWI reflects bioenergetic failure, not histopathology; diffusion imaging protocol heterogeneity

Abbreviations: CTP: Computed tomography perfusion; DWI: Diffusion-weighted imaging; MRI: Magnetic resonance imaging; NCCT: Non-contrast computed tomography.

As markers of SVD, atrophy may mediate stroke outcomes through collaterals, while lacunes directly influence clinical outcomes in the retrospective study.⁷⁰ Therefore, SVD markers are also essential in assessing stroke patients, along with collaterals. A *post hoc* analysis of the Safety and Efficacy of Nerinetide (NA-1) in Subjects Undergoing Endovascular Thrombectomy for Stroke (ESCAPE-NA1) randomized clinical trial, markers of brain frailty on routine CT images (brain atrophy, chronic infarcts, and SVD) mediated 85.1% of the total association of age with 90-day functional outcome after thrombectomy.⁶⁸ Another *post hoc* analysis of the ESCAPE-NA1 trial established an association between brain frailty, particularly as indicated by chronic infarctions and brain atrophy on basic non-contrast CT scans, and the initial clinical presentation of stroke, as measured using the NIHSS, in patients with large vessel occlusive stroke undergoing mechanical thrombectomy.⁷¹ Moreover, neurologic recovery within 90 days after stroke was less favorable in those with a greater overall brain frailty burden.⁷¹ However, different imaging markers contribute variably to overall brain frailty, with global cortical atrophy identified as a key driving factor. Future research should explore how frailty markers can be effectively combined with other omics markers to maximize their clinical significance.

4.1.3. Net water uptake (NWU): Degree of infarction

A recent study reported that the benefits of successful vessel recanalization are significantly mediated by infarct growth and the reduction of edema.⁷² In patients with a low ASPECTS, which inversely correlates with infarct volume, several factors—including the extent of tissue damage, infarct topography, disruption of brain network connectivity, and selective neuronal loss—may significantly influence clinical outcomes beyond the volume of the infarct itself.³⁸ Consistent with these findings, NWU in the parenchyma, serving as a surrogate for the extent of tissue damage, has recently been shown to be associated with clinical outcomes in patients with large established infarcts. Automated quantification of NWU on non-contrast CT in the emergency department (or even directly in the neuro-angiography suite) could quantify pre-reperfusion infarction without significantly delaying treatment and predict the age of a lesion.⁷³⁻⁷⁵ Moreover, studies have demonstrated that NWU is a superior predictive marker for hemorrhagic transformation compared to both the ASPECTS and collateral blood flow status.^{76,77} Another potential advantage of NWU is its capability to monitor the therapeutic effects of agents specifically targeting edema inhibition, such as glibenclamide.⁷³

4.1.4. Gray matter and white matter: Anatomic heterogeneity

The mammalian neocortex is a substantial structure characterized by its laminar architecture and experiences rapid enlargement during early development. In humans, the combined volume of neocortical gray matter and adjacent white matter constitutes approximately 80% of total brain volume.⁷⁸

Metabolic demand with the gray matter is more susceptible to ischemia than the white matter.⁷⁹ It was reported that patients with cognitive impairment often experienced gray matter reduction, and the vast majority of reduction occurred in the thalamus in ischemic stroke (reflecting group differences in multiple cognitive domains).⁸⁰ Gray matter volume was also applied in other diseases to explain the severity of the clinical symptoms, such as Parkinson's disease and schizophrenia.⁸¹⁻⁸³

Similar to gray matter, white matter is critically dependent on a continuous supply of oxygen and glucose. However, white matter receives less collateral circulation than gray matter and has a smaller blood supply, leading to extreme susceptibility to ischemia. Therefore, ischemic stroke rapidly and profoundly damages white matter. White matter hyperintensities are well-established structural imaging biomarkers of cerebral SVD.⁸⁴ The Framingham Heart Study demonstrated that the presence of severe white matter ischemia at baseline is associated with a two-fold increase in the risk of stroke-related mortality and a four-fold increase in the risk of subsequent dementia.⁸⁵ A retrospective single-center study provides evidence supporting the prognostic value of baseline white matter hyperintensities concerning functional outcomes following post-stroke intervention.⁸⁶ The observed poorer response to ischemic stroke events in patients with a greater burden of white matter disease is consistent with the associations between white matter disease burden and indicators of brain and cerebrovascular health. Notably, the study revealed associations between functional outcomes and periventricular white matter disease volume, but not with deep white matter involvement.⁸⁷

4.2. Biological ischemic regions: Biomarkers based on pathological changes

Biological penumbra is a region surrounding the infarct core where physiological function is lost but tissue viability is preserved. With timely reperfusion, this region can fully recover its function. Conversely, if reperfusion is delayed, collateral circulation deteriorates over time, leading to progressive enlargement of the infarct core

and contraction of the penumbra, ultimately resulting in irreversible infarction. Following stroke onset, the biological penumbra undergoes a series of intricate pathophysiological processes, including impaired mitochondrial ATP production, blood-brain barrier (BBB) breakdown, aberrant cross-talk between brain innate and peripheral adaptive immunities, upregulation of proinflammatory mediators, tissue remodeling, and angiogenesis-related to matrix metalloproteinases, among others.⁷³ Driven by the ischemic cascade, the penumbra gradually progresses toward irreversible infarction. We will mainly introduce some of the dynamic and interwoven metabolic, molecular, and cellular processes, as well as biomarkers with diagnostic and therapeutic potential.

4.2.1. Impaired ATP production

Molecular definitions of the ischemic core and penumbra have focused on protein synthesis, adenosine triphosphate (ATP) production, and heat-shock protein (HSP) induction. A decrease in protein synthesis is observed when the rCBF value declines to 30% to 50% of baseline levels. This condition may be reversible with the preservation of ATP within the penumbra. However, if ATP levels are completely depleted, irreversible translation blockade and subsequent infarction occur in the core region of the affected tissue.⁷³ Therefore, some researchers propose that defining the penumbra based on metabolic alterations—specifically, suppressed protein synthesis but preserved ATP production in ischemic neural tissue—could provide more precise guidance for clinical assessment and treatment of acute cerebral infarction.⁸⁸ Only regions with sufficient energy reserves can translate heat shock protein 70 (HSP70) into functional proteins, indicating potential for recovery. Due to varying ischemic vulnerabilities among cell types, tissue samples may show heterogeneous infarction patterns related to differences in energy reserves and protein synthesis capacity.⁷³

4.2.2. Excitotoxicity

Since 2010, glutamate-mediated toxicity has been a focal point of neuroprotection research, with magnesium sulfate and NA-1 (Tat-NR2B9c) recognized as notable neuroprotective agents in clinical trials.⁸⁹⁻⁹¹ The activity of glutamatergic N-methyl-D-aspartate receptors (NMDARs) and NMDAR-mediated Ca^{2+} influx is critical for neuronal function and cell survival. Memantine preferentially inhibits extrasynaptic N-methyl-D-aspartate receptors (NMDARs) and has received approval from the Food and Drug Administration (FDA) for the symptomatic treatment of moderate-to-severe

Alzheimer's disease, exhibiting variable efficacy; this makes it a promising therapeutic agent for stroke patients.⁹² SHPL-49, a structurally modified derivative of salidroside, was proved its efficacy of reducing early glutamate excitotoxicity by promoting the NR2A-CAMKII α -Akt/CREB pathway.⁹³ Calpain processing impairs the function of postsynaptic density protein-95 (PSD-95) following the overactivation of excitatory glutamate receptors (excitotoxicity) during stroke. Enhancing PSD-95 stability presents a viable approach for potentially recoverable ischemic areas.⁹⁴

4.2.3. Oxidative stress

Release of reactive oxygen species (ROS) begins within minutes after middle cerebral artery occlusion in rodents and gradually increases to reach a plateau around 100 min after the start of occlusion although further transiently increases following reperfusion.⁹⁵ During the acute phase, glial cell activation in the ischemic penumbra is a critical determinant of stroke prognosis, releasing pro-inflammatory cytokines that directly correlate with neuronal death and BBB disruption.^{96,97} Positron emission tomography studies in rodents, as well as research on patients and non-human primates with stroke, found that the subjects subsequently develop neurotoxic microglial activation over time both within and around the infarct, irrespective of the occurrence of recanalization.⁹⁵ For instance, studies suggest that distinct microenvironmental signals drive the emergence of ischemic core-associated microglia (ICAM) and ischemic penumbra-associated microglia (IPAM) subpopulations. In the ischemic core, damage-associated molecular patterns (DAMPs) released from injured cells, such as high-mobility group box 1 (HMGB1, an endogenous ligand for Toll-like receptor 4), induce ICAM formation. In contrast, in the penumbra, where cellular damage is relatively mild, corticosterone may influence the emergence of IPAM.⁹⁸ Moreover, enhancing microglial mitochondrial energy metabolism suppresses ROS production, alleviates neuronal injury, and mitigates post-ischemic cognitive dysfunction.^{99,100}

4.2.4. Endothelial dysfunction

Endothelial dysfunction is a critical factor influencing the entire spectrum of AIS, such as early-onset cryptogenic ischemic stroke in men and age-related stroke.¹⁰¹ Inflammatory markers such as matrix metalloproteinases are released by injured endothelium early in the course of ischemia, leading, among other events, to neutrophil activation, marginalization, and tissue infiltration.⁹⁵ The endothelial glycocalyx, composed of a complex mixture of carbohydrates attached to proteins on the surface of

endothelial cells in blood vessels, plays a crucial role in maintaining vascular integrity; thus, preserving its functionality is a vital therapeutic strategy in the management of AIS.¹⁰² In addition, capillary pericytes are particularly sensible to ischemia and may exacerbate the phenomenon of no-reflow; therefore, they represent viable and promising therapeutic targets for mitigating no-reflow following ischemic stroke.¹⁰³ Endothelial dysfunction is featured with suppressed endothelial nitric oxide synthase (eNOS) with concomitant nitric oxide deficiency, restoring endothelial nitric oxide represents a promising approach to treating stroke injury.¹⁰⁴ Pretreatment with a 1064-nm laser (near-infrared II light) at an irradiance of 50 mW/cm² improved CBF, eNOS phosphorylation, and stroke outcomes in a mouse model of ischemic stroke.¹⁰⁴ The progression of the ischemic penumbra to infarction is closely linked to microcirculatory function.¹⁰⁵ Studies demonstrate that fingolimod (FTY720), by modulating G-protein-coupled receptors (specifically sphingosine-1-phosphate receptor 1, S1P₁), inhibits lymphocyte-driven thrombo-inflammatory responses, enhances vascular endothelial cell function, and improves microvascular perfusion. This results in reduced expansion of the infarct core, enhanced reperfusion efficacy, and improved safety profiles.¹⁰⁶

4.2.5. Inflammation

The inflammatory cascade represents a key therapeutic target for future stroke interventions. By elucidating the specific roles of inflammatory signaling mechanisms in various forms of vascular brain injury, we can uncover the underlying mechanisms of disease phenotypes.¹⁰⁷ Following AIS, damaged tissues are enriched with dying or injured cells and debris. Studies suggest that Forkhead box P3 (FoxP3), a transcription factor, may serve as a feasible molecular target for enhancing macrophage phagocytic efficiency and promoting inflammatory resolution at brain injury sites.¹⁰⁸ Circulating blood biomarkers are invaluable tools for predicting the biological risks of stroke and vascular cognitive impairment (VCI). Research indicates that an interleukin-18 (IL-18)-centered inflammatory network correlates with radiographic features of cerebral SVD and may serve as a biomarker for future stroke susceptibility.¹⁰⁹ Moreover, a study reported that CircOGDH (circular RNA derived from oxoglutarate dehydrogenase) is a novel biomarker for detection of penumbra.¹¹⁰ Their findings demonstrate that circulating CircOGDH levels are positively correlated with cerebral penumbra volume in patients with AIS. In addition, crosstalk between ischemic penumbral neurons and

endothelial cells is mediated by circular RNA originating from CircOGDH.¹¹¹ Meanwhile, the inflammatory marker IL-6 not only correlates with specific stroke subtypes but also demonstrates strong associations with brain imaging biomarkers in Alzheimer's disease.^{112,113} Furthermore, experiments in mice utilizing the transient middle cerebral artery occlusion model revealed that B cells begin to infiltrate from day 7 onward.¹¹⁴ Research has indicated that B cells may exhibit distinct characteristics compared to other immune cells and that their presence confers a protective effect on brain tissue—a conclusion supported by findings from studies involving B-cell-deficient mice and mice administered with a large quantity of B cells.¹¹⁴ While these potential biomarkers significantly influence stroke prognosis, their explicit relationship with ischemic regions remains unclear. This ambiguity necessitates further investigation to enhance their clinical utility and broaden their application potential.

4.2.6. Other possible biomarkers

At the cellular level, following ischemia, the significant reduction in CBF within the biological penumbra compels neurons to shift their energy metabolism from oxidative phosphorylation to glycolysis under hypoxic conditions, resulting in excessive lactate production.¹¹⁵ Consequently, specific biomolecules that are dynamically altered in the post-stroke penumbra may serve as critical biomarkers for predicting penumbral status. For instance, brain lactate accumulates significantly after the onset of ischemia, a process that is clinically relevant for identifying the ischemic penumbra, as it reflects early changes in glucose metabolism.¹¹⁶ Moreover, during an energy crisis, lactate can be transported from astrocytes to neurons, serving as an energy substrate that helps alleviate the energy deficiency in neurons. Accumulating evidence suggests that lactate acts as a promising neuroprotective agent.

Cardiac disease is often associated with the onset of AIS and is frequently a contributing factor to its occurrence. Consequently, cardiac biomarkers may serve a crucial role in indicating the presence of significant cerebral imaging markers. A recent study has found that elevated soluble suppression of tumorigenicity-2 receptor, growth differentiation factor-15, and N-terminal pro-B-type natriuretic peptide (NT-proBNP) is significantly associated with an increased risk of post-stroke cognitive impairment.¹¹⁷ This relationship highlights the importance of recognizing that structural brain lesions, such as white matter hyperintensities and cerebral microbleeds, frequently co-occur with cardiac diseases; notably, higher

levels of NT-proBNP have been linked to reduced total brain, gray matter, and white matter volumes, independent of cardiovascular risk factors.¹¹⁸

5. Conclusion

The treatment of AIS is currently evolving from a traditional time-based window concept to a more precise tissue-based approach, underscoring the essential role of imaging techniques in identifying salvageable brain tissue. However, significant challenges persist with current imaging methods, which often fail to capture the intricate pathological and physiological changes occurring in brain cells. These methods do not adequately reflect the heterogeneous responses of brain cells to treatment, leading to ambiguous definitions and inconsistent standards when defining the ischemic penumbra and core.

This paper critically examines these challenges and innovatively proposes a multidimensional evaluation system that integrates quantitative imaging features with molecular and pathological markers. By enhancing the accuracy of stroke lesion diagnosis, this approach aims to overcome the limitations of existing imaging techniques. Future research should prioritize an in-depth exploration of multimodal imaging technologies, as well as the integration and validation of biological marker data. By establishing a unified framework that fuses imaging with biological markers, we can pave the way for more precise and personalized treatments for AIS. This integrated strategy promises to optimize clinical decision-making and ultimately improve patient outcomes, addressing the current challenges faced in the management of this condition.

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Conflict of interest

The authors declare that they have no competing interests.

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