



# Blood-Based Biomarkers: A Forgotten Friend of Hyperacute Ischemic Stroke

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Ischemic stroke (IS) is the second leading cause of death worldwide. Multimodal neuroimaging techniques that have significantly facilitated the diagnosis of hyperacute IS are not widely used in underdeveloped areas and community hospitals owing to drawbacks such as high cost and lack of trained operators. Moreover, these methods do not have sufficient resolution to detect changes in the brain at the cellular and molecular levels after IS onset. In contrast, blood-based biomarkers can reflect molecular and biochemical alterations in both normal and pathophysiologic processes including angiogenesis, metabolism, inflammation, oxidative stress, coagulation, thrombosis, glial activation, and neuronal and vascular injury, and can thus provide information complementary to findings from routine examinations and neuroimaging that is useful for diagnosis. In this review, we summarize the current state of knowledge on blood-based biomarkers of hyperacute IS including those associated with neuronal injury, glial activation, inflammation and oxidative stress, vascular injury and angiogenesis, coagulation and thrombosis, and metabolism as well as genetic and genomic biomarkers. Meanwhile, the blood sampling time of the biomarkers which are cited and summarized in the review is within 6 h after the onset of IS. Additionally, we also discuss the diagnostic and prognostic value of blood-based biomarkers in stroke patients, and future directions for their clinical application and development.

**Keywords:** hyperacute ischemic stroke, blood-based biomarker, genetic and genomic, glial activation, neuronal injury, oxidative stress, inflammation, angiogenesis

## INTRODUCTION

As a disease with high incidence, disability, mortality, and recurrence rates, stroke is a significant economic burden globally because of costs associated with treatment and post-stroke care. Stroke is now the second leading cause of death worldwide (1), with Asia accounting for nearly two-thirds of the total mortality due to stroke. In China, the age-standardized prevalence, incidence, and mortality rates of stroke from 2012 to 2013 were 1.115, 0.247, and 0.115%, respectively, with  $\approx$ 2.4 million new stroke cases, 1.1 million stroke-related deaths, and 11.1 million stroke survivors annually (2).

The two main subtypes of stroke are ischemic stroke (IS) and hemorrhagic stroke (HS). Management strategies for hyperacute IS include intravenous thrombolysis and intravascular treatment, for which the therapeutic time windows are <4.5 and <6 h, respectively, after symptom onset (3). Early detection and intervention are critical to ensure a good clinical outcome in hyperacute IS. A number of studies have focused on how to shorten the door-to-needle time (DNT) and door-to-puncture time (DPT). However, because laboratory and imaging examinations cannot be performed outside of the hospital setting, IS diagnosis and treatment are not possible during the pre-hospital stage, i.e., from symptom onset to on-site first aid and until the patient reaches the emergency room.

Additionally, the time nodes division standard of hyperacute IS has not been unified so far. In clinical research, the mainstream time nodes mainly include 6 or 8 h after symptom onset (4, 5). However, at present, almost all the major early diagnosis and treatment guidelines of IS recommend that intravenous thrombolysis should be carried out within 4.5 h after symptom onset, while mechanical thrombectomy should be carried out within 6 h after symptom onset. Although mechanical thrombectomy within 6–24 h after symptom onset can be carried out based on the strict inclusion criteria including endovascular therapy following imaging evaluation for ischemic stroke 3 (DEFUSE) and diffusion-weighted imaging or computed tomography perfusion assessment with clinical mismatch in the triage of wake-up and late presenting strokes undergoing neurointervention with trevo (DAWN), both of which are complex and need special imaging technique such as the rapid processing of perfusion and diffusion (RAPID) software, which significantly limits the use of mechanical thrombectomy beyond 6 h after symptom onset in the vast underdeveloped areas and community hospitals of China (6, 7).

Moreover, neuroimaging is the main technique for the diagnosis of IS in the hyperacute phase. Non-contrast computed tomography (NCCT) is the most common method used to detect stroke in the emergency room because of its simplicity, reasonable cost, and the rapidity with which results can be obtained. However, NCCT has low specificity and sensitivity for hyperacute IS (8). The development of multimodal neuroimaging techniques such as T1-, T2-, and diffusion-weighted or fluid-attenuated inversion recovery magnetic resonance imaging (MRI) has significantly facilitated diagnosis, but they are not widely used in underdeveloped areas and community hospitals in China because of their inherent shortcomings such as high cost and lack of trained operators. Additionally, current multimodal neuroimaging techniques do not have sufficient resolution to detect changes in the brain at the cellular and molecular levels after IS onset, including in cell structure, the neurotransmitter level, oxidative stress, neuroinflammation, angiogenesis, and metabolism, although these can provide important information on the prognosis of IS.

We hope that blood-based biomarkers can attract the attention of clinicians and researchers in the diagnosis of IS in the hyperacute phase, better guide clinical diagnosis, treatment, and prognosis evaluation, and supplement the shortcomings of current multimodal imaging techniques by the publication of this review manuscript. Therefore, in this review, we

summarize the current state of knowledge on blood-based biomarkers of hyperacute IS including those associated with glial activation, neuronal injury, inflammation and oxidative stress, vascular injury and angiogenesis, coagulation and thrombosis, and metabolism as well as genetic and genomic biomarkers. Meanwhile, the blood sampling time of the biomarkers which are cited and summarized in the review is within 6 h after the onset of IS. Additionally, we also discuss the diagnostic and prognostic value of these biomarkers in stroke patients, and future directions for their clinical application and development.

## BIOMARKERS ASSOCIATED WITH NEURONAL INJURY

### Ubiquitin C-Terminal Hydrolase L1 (UCH-L1)

UCH-L1, a neuronal protein that is associated with neurodegeneration, is highly expressed in the central nervous system (CNS) and is associated with synaptic plasticity, synaptic homeostasis, and self-repair of the brain after injury (9–11). Serum UCH-L1 level was elevated after 3 and 6 h of ischemia following middle cerebral artery occlusion (MCAO) in rats, while the level in intracerebral hemorrhage (ICH) rats was unchanged (12). Consistent with this finding, in rats subjected to MCAO for 30 min or 2 h, serum UCH-L1 level was significantly elevated 6 h later (13). However, a clinical study reported that serum UCH-L1 within 4 and 6 h after symptom onset was higher in HS patients than in IS patients and stroke mimics (SM), respectively (14); and in a single-center study of hyperacute IS, the area under the receiver operating characteristic curves (AUCs) of serum UCH-L1 for distinguishing IS patients from controls and IS from HS patients within 4.5 h after symptom onset were 0.64 and 0.62, respectively (15).

To sum up, the different expression of UCH-L1 in IS and HS suggests that it may be a potential biomarker in the diagnosis of hyperacute IS.

### N-Methyl-d-Aspartate Receptor (NMDAR)

Both embolic and thrombotic vascular occlusions induce neurotoxicity mediated by the excitatory NMDAR, leading to biochemical changes in brain tissue, the blood-brain barrier (BBB), and cerebral vasculature (16). Within 3 h after symptom onset, patients with IS showed elevated serum NMDAR autoantibody levels compared to healthy control subjects (HCs), which had an AUC of 0.99 for diagnosing IS with a sensitivity of 97.0% and specificity of 98.0% using the best cutoff point of 2.0 µg/l (16).

In a word, NMDAR is an independent and sensitive serologic marker capable of detecting IS with a high post-test probability, and may potentially be useful in assisting the diagnosis of hyperacute IS in the emergency setting.

### Neurofilament Light Chain (NfL)

When brain damage occurs, neuronal injury and disruption of axonal membranes lead to the release of cytoskeleton proteins, such as neurofilaments, into the interstitial fluid and eventually into the cerebrospinal fluid (CSF) and blood (17). Neurofilaments are highly specific structural, neuronal cytoskeletal proteins that

consist of four neurofilament subunits: neurofilament light chain (NfL), neurofilament medium chain (NfM), neurofilament heavy chain (NfH), and  $\alpha$ -internexin (17). In a cohort study, within 3 h after symptom onset, patients with IS showed elevated serum NfL levels compared to transient ischemic attack (TIA) (18). In summary, NfL was associated with clinical severity on admission and hyperacute IS diagnosis.

## BIOMARKERS ASSOCIATED WITH GLIAL ACTIVATION

### Glial Fibrillary Acidic Protein (GFAP)

As an intermediate filament protein that is almost exclusively expressed in the CNS, GFAP maintains the structure and facilitates the migration of astrocytes (19). The time window between 2 and 6 h after symptom onset is critical for differentiating HS from IS based on serum GFAP level, which has a diagnostic accuracy of >80.0% (20). The AUC of plasma GFAP for differentiating HS from IS and SM within 4.5 h after symptom onset was found to be 0.915, with a sensitivity of 84.2% and specificity of 96.3% using a cutoff value of 0.29  $\mu\text{g/l}$  (21); another study reported an AUC of 0.86 with a sensitivity of 61.0% and specificity of 96.0% using a cutoff of 0.34  $\mu\text{g/l}$  (15). Additionally, within 4 and 6 h after symptom onset, serum GFAP had an AUC of 0.866 for differentiating HS from IS and SM, with a sensitivity of 75.0% and specificity of 84.0% using an optimal cutoff point of 72 ng/l (14); another study reported an AUC of 0.872 with a sensitivity of 77.8% and specificity of 94.2% using a cutoff of 0.03  $\mu\text{g/l}$  within 6 h after symptom onset (22). A recent clinical study reported that plasma GFAP within 4.5 h after symptom onset was higher in HS patients than in IS patients (23).

In conclusion, different expression of GFAP in IS, HS, and SM suggests it may be a potential biomarker in the diagnosis of hyperacute IS; and GFAP levels were also associated with stroke severity.

### S100 Calcium-Binding Protein B (S100 $\beta$ )

S100 $\beta$  is an acidic calcium-binding protein that is predominantly expressed in astrocytes in the mammalian brain and is involved in cell cycle progression and differentiation, astrocyte–neuron communication, and CNS development and maintenance (24, 25). In a mouse model of focal cerebral infarction, plasma S100 $\beta$  level increased after 4 h of ischemia, which was correlated with infarction volume and the degree of neurologic deficit (26). A clinical study found that a plasma S100 $\beta$  concentration of 67 ng/l within the first 6 h after symptom onset could differentiate HS from IS with a sensitivity of 95.7% and a specificity of 70.4% (27). Additionally, a high serum concentration of S100 $\beta$  at 6 h after stroke onset was associated with increased risk of post-stroke infections (28). Another study reported that a plasma S100 $\beta$  concentration was lower than 1.364 pg/ml within 6 h after symptom onset could predict the development of epilepsy after a hyperacute stroke event (include IS and HS) (29).

As mentioned above, S100 $\beta$  may be a potential biomarker in the diagnosis of hyperacute IS; and a high serum concentration of S100 $\beta$  was related to severity and poor functional outcome.

## BIOMARKERS ASSOCIATED WITH INFLAMMATION AND OXIDATIVE STRESS

### Cytokines

Interleukin 10 (IL-10) is an anti-inflammatory cytokine that is secreted mainly by lymphocytes and monocytes/macrophages (30). The IL-4 receptor (IL-4R) is expressed by hematopoietic, endothelial, epithelial, and muscle cells; in fibroblasts and hepatocytes; and in brain tissue, consistent with the broad target range of IL-4 (31, 32); and IL-4R binds to IL-4 to exert anti-inflammatory properties (33). Clinical-diffusion mismatch (CDM) and perfusion-diffusion mismatch (PDM) have been proposed as surrogates for the ischemic brain that is at risk of infarction. A clinical study found that along with CDM and PDM, the serum level of IL-10 at admission could identify IS patients who were candidates for thrombolytic therapy with the tissue plasminogen activator (tPA) (34). Moreover, a serum IL-10 level  $\geq 30$  pg/ml predicted a favorable functional outcome at 3 months with a sensitivity of 86.0% and specificity of 88.0% (34). Another study showed that plasma IL-4R level within 4.5 h after stroke onset was an independent predictor of poor neurologic prognosis with a sensitivity of 53.0% and specificity of 72.0% at 24 h and a sensitivity of 52.0% and specificity of 73.0% at 48 h after stroke onset using the same cutoff point of 503.40 ng/l (33).

In short, cytokines (including IL-10 and IL-4R) are independent predictors of poor neurologic prognosis and functional outcome.

### Neutrophils, Neutrophil to Lymphocyte Ratio (NLR), and Platelet to Lymphocyte Ratio (PLR)

NLR and PLR have recently been reported as potential novel biomarkers of the baseline inflammatory process and could serve as outstanding predictors in patients with IS (35, 36). Before intravenous thrombolysis treatment, the AUCs of NLR and PLR for predicting post-thrombolysis early neurological deterioration and NLR for predicting post-thrombolysis early neurological improvement were 0.763, 0.703, and 0.695, respectively (37). Additionally, within 4.5 h after symptom onset, serum neutrophil counts and NLR were positively correlated with IS severity, and higher neutrophil counts and NLR were independently associated with worse outcomes and higher mortality rates at month 3 (38).

Anyway, neutrophils, NLR, and PLR may be potential biomarkers in assessing the severity and prognosis of hyperacute IS.

### Serum Amyloid Protein (SAP)

SAP is a member of the pentraxin family and plays a key role in innate immunity and inflammation, and serum amyloid A (SAA) is an acute-phase protein, which is upregulated by a variety of inflammatory stimuli (39). In IS patients who were treated with thrombolysis, the baseline (before thrombolysis treatment) SAP remained significantly and independently associated with 3-month death (40). Additionally, within the first 6 h after symptom onset, SAA had an AUC of 0.76 for predicting stroke-associated infections (41).

To sum up, SAP remained significantly and independently associated with 3-month death and can predict stroke-associated infections in hyperacute IS.

## Others Potential Biomarkers Associated With Inflammation and Oxidative Stress

Human  $\beta$ -defensin 2 (HBD-2) and chitotriosidase (ChT) are involved in the immune response and inflammation, and both are related to neurologic outcomes. Specifically, ChT is a sensitive parameter of macrophage activation and its elevated level in plasma reflects an inflammatory response (42). The baseline (pre tPA treatment) plasma ChT activity in IS patients was shown to be a short-term (i.e., at 48 h after stroke onset) predictor of tPA treatment outcome (43). HBD-2 mainly acts as an antimicrobial peptide and chemoattractant (44); baseline plasma HBD-2 level (within 4.5 h after symptom onset) was linked to neurologic decline at 24 and 48 h after stroke onset (33).

Retinol-binding protein 4 (RBP4), an adipokine primarily secreted by adipocytes and hepatocytes, has been implicated in oxidative stress, inflammation, and coronary artery calcification (45). Plasma RBP4 is a promising biomarker for distinguishing IS from HS patients during the hyperacute phase (within 6 h after symptom onset) as it was detected at a higher level in the former group (46). Moreover, the combination of RBP4 and GFAP improved the detection of IS (46). A recent clinical study reported that plasma RBP4 within 4.5 h after symptom onset was higher in IS patients than in HS patients (23).

Fluorescent molecular peroxidation products (FMPPs) are potential biomarkers of molecular oxidative damage that can increase the permeability of the BBB (47). Plasma FMPP level in IS patients before thrombolytic therapy predicted early neurologic deterioration at 48 h after symptom onset and was related to the occurrence of symptomatic HS after thrombolytic treatment of IS (47).

Adenosine acts as a vasodilator, inhibits inflammation, and might be neuroprotective (48). Within 3.3 h after symptom onset, serum adenosine showed a high value in separating IS from SM (49). In conclusion, RBP4 is a promising biomarker for distinguishing IS from HS patients during the hyperacute phase; and HBD-2 is a potential biomarker in assess prognosis of hyperacute IS. Additionally, ChT and FMPPs were predictors of tPA treatment outcome. And adenosine is a promising biomarker for distinguishing IS from SM patients during the hyperacute phase.

## POTENTIAL BIOMARKERS ASSOCIATED WITH VASCULAR INJURY AND ANGIOGENESIS

### Matrix Metalloproteinase-9 (MMP-9)

Matrix metalloproteinase-9 (MMP-9) is responsible for degradation of type IV collagen, laminin, and fibronectin, which are the major components of the basal lamina; and the loss of integrity of the basal lamina is considered to be the primary cause of edema after focal cerebral ischemia and hemorrhagic transformation (50). In IS patients within 6 h after

symptom onset, plasma MMP-9 level substantially increased and correlated with the severity of the disease and infarct volume (51). Additionally, the baseline (pre tPA treatment) plasma MMP-9 level in IS patients predicted parenchymal hematoma (PH) appearance after tPA treatment (52). Another study showed that serum MMP-9 level  $\geq 140 \mu\text{g/l}$  within 3 h after stroke onset and before tPA therapy predicted the occurrence of PH after therapy with a sensitivity of 92.0% and specificity of 74.0% (53).

In short, MMP-9 is a potential biomarker to evaluate the severity of hyperacute IS and predict the complications after tPA treatment.

### Metabolites of the L-Arginine Pathway

Nitric oxide (NO) is critical for the maintenance of vascular integrity (54). Asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) are protein degradation products of L-arginine that can reduce NO production through direct or indirect pathways (55, 56). The levels of serum metabolites of the L-arginine pathway including L-arginine, ADMA, and SDMA were elevated in IS within 6 h after symptom onset compared to asymptomatic carotid stenosis, indicating a greater degree of endothelial dysfunction (57). Meanwhile, the serum concentration of L-arginine and ADMA/SDMA ratio were correlated with thrombo-inflammation within 6 h after IS onset (58); these correlations were in turn independently associated with risk of post-stroke infection but not other outcomes (58). Additionally, within 3.3 h after symptom onset, serum ADMA and SDMA showed a high value in separating IS from SM (49).

In conclusion, the different expression of metabolites of the L-arginine pathway in IS control and asymptomatic carotid stenosis patients prompts it as a potential biomarker in the diagnosis and assessment of the prognosis of hyperacute IS.

### Cellular Fibronectin (c-Fn)

Fibronectin exists in two forms: plasma fibronectin (p-Fn), which is primarily produced by hepatocytes; and c-Fn, which is mainly synthesized by endothelial cells. A high plasma level of c-Fn is indicative of endothelial damage (59). Plasma c-Fn level before tPA therapy was independently associated with tPA-induced hemorrhagic transformation (HT) (59), whereas serum c-Fn level  $\geq 3.6 \text{ mg/l}$  within 3 h after stroke onset and before tPA therapy predicted PH after tPA therapy with a sensitivity of 100.0%, specificity of 60.0%, and negative predictive value of 100.0% (53). Therefore, c-Fn is a potential biomarker to predict the complications after tPA treatment.

### Endostatin

Endostatin, an inhibitor of endothelial cell proliferation and migration, is derived from collagen XVIII, which is a major constituent of both endothelial and epithelial basement membranes (60). High plasma endostatin level (within 3 h after symptom onset) was found to reflect an acute antiangiogenic status and predicted worse long-term functional outcome in IS patients (61). Another study reported that a plasma endostatin concentration higher than  $1.203 \text{ ng/ml}$  within 6 h after symptom onset could predict the development of epilepsy after a hyperacute stroke event (include IS and HS) (29). A recent



clinical study reported that plasma endostatin within 4.5 h after symptom onset was higher in IS patients than in HS patients (23). As mentioned above, endostatin may be a potential biomarker in assessing the prognosis and complications of hyperacute IS.

### Other Potential Biomarkers Associated With Vascular Injury and Angiogenesis

Caveolin-1, which is highly expressed in endothelial cells, is an important regulator of endothelial permeability after cerebral ischemia (62). A low serum level of caveolin-1 (within 4.5 h after stroke onset) was associated with symptomatic HT after recombinant tPA therapy (63).

B-type natriuretic peptide (BNP), a neurohormone produced by the heart ventricles and the brain, promotes natriuresis and diuresis in the body, acting as a vasodilator with countering vasoconstrictor effects (64). Two fragments after the cleavage of a propeptide include N-terminal proBNP (NT-proBNP) which does not have biological activity, and formed-BNP which is biologically active (64). A recent clinical study reported that plasma NT-proBNP within 4.5 h after symptom onset was higher in IS patients than in HS patients (23).

Therefore, caveolin-1 is a potential biomarker to predict complications after tPA treatment. And NT-proBNP is a promising biomarker for distinguishing IS from HS patients during the hyperacute phase.

## POTENTIAL GENETIC AND GENOMIC BIOMARKERS

Epigenetic analysis and gene profiling have been used as diagnostic tools in cardiovascular diseases and cancer, and are increasingly being applied to stroke.

### RNAs

MicroRNAs (miRNAs) are a group of small, non-coding, endogenous single-stranded RNA molecules that are the main regulators of homeostasis in neurons; their dysregulation has been linked to specific pathologic processes in the brain (65, 66). In rats, miR-223-3p was upregulated whereas let-7b-3p was downregulated in the blood at 4 h after permanent MCAO-induced cerebral ischemia (67); and in patients with IS, circulating serum miRNA-221-3p and miRNA-382-5p levels were lower than in HCs within 6 h after symptom onset (68). Additionally, within 6 h after symptom onset, plasma miR-16 had an AUC of 0.775 for differentiating IS, with a sensitivity of 69.7% and specificity of 87.0%; and miR-16 AUC, sensitivity, and specificity in IS patients reached 0.95, 100.0, and 91.3% in stroke derived from large artery atherosclerosis (69). Moreover, plasma miR-16 level was higher in the poor prognosis (mRS 3–6) group than in the good prognosis (mRS 0–2) group (69). Another study showed that the levels of plasma miR-125a-5p, miR-125b-5p, and miR-143-3p were upregulated in IS within 6 h after symptom onset compared to HCs; and the set of plasma miRNAs (miR-125a-5p, miR-125b-5p, miR-143-3p) had an AUC of 0.90 for differentiating IS from HCs, with a sensitivity of 85.6% and specificity of 76.3%, which was superior to multimodal

cranial computed tomography obtained for routine diagnostics (sensitivity: 72.5%) (70).

Circular RNAs (circRNAs) are a class of RNA molecules that may hold the key to understanding and properly manipulating tightly regulated gene expression patterns, as they are more highly expressed in brain tissues than in other tissues (71, 72). The levels of blood circPHKA2 (hsa\_circ\_0090002) and circBBS2 (hsa\_circ\_0039457) were downregulated in IS within 6 h after symptom onset compared to control subjects (73).

Transfer RNAs (tRNAs) are best known for their role in protein synthesis, tRNAs and tRNA-derived fragments (tRFs) also play a role in regulatory processes such as gene expression and translational control (74). Within 6 h after symptom onset, plasma tRFs had an AUC of 0.986 for differentiating HS from IS and SM (75).

Long non-coding RNAs (lncRNAs) represent an extensive, largely unexplored functional component of the genome, with multiple studies demonstrating that the brain expresses the highest amounts of lncRNAs among all tissue types, H19 is one of the best characterized lncRNA genes (76). A study showed that the levels of plasma H19 were significantly higher in IS patients within 3 h after symptom onset compared to HCs; and the plasma H19 had an AUC of 0.910 for differentiating IS from HCs, with a sensitivity of 80.6% and specificity of 92.0% (77).

As mentioned above, the different expression of RNAs in IS, HS, and SM suggests that it may be a potential biomarker in the diagnosis of hyperacute IS.

### Genetic Analysis and Gene Profiling

In a case–case prospective study, CD40-1C>T polymorphism (rs1883832) in peripheral blood was found to be associated with brain vessel re-occlusion after fibrinolysis in the early phase (within 3 h) after stroke onset (78). It was also reported that a large number of genes showed altered expression in the peripheral blood of humans as early as 3 h after IS onset, which was mainly attributable to neutrophils and was thought to contribute to tissue damage after stroke (79). Gene expression profiles in the peripheral blood (within 3 and 5 h after stroke onset) differ after cardioembolic compared with large-vessel atherosclerotic stroke (80). More specifically, genes showing altered expression in large-vessel atherosclerotic stroke were associated with platelets and monocytes and are known to be involved in the modulation of hemostasis, whereas those that were altered in cardioembolic stroke were expressed in neutrophils and related to the immune response to infection (80).

In short, CD40-1C>T polymorphism (rs1883832) can predict brain vessel re-occlusion after fibrinolysis after stroke onset; and genes may be potential biomarkers in assessing the complications of hyperacute IS.

## POTENTIAL BIOMARKERS ASSOCIATED WITH COAGULATION, THROMBOSIS, AND METABOLISM

Mean platelet volume (MPV), an index of platelet size, is related to thrombus formation and propagation and may contribute

to acute thrombotic events (81). Disabling or fatal IS in thrombolytic patients was associated with high blood MPV level before initiation of recombinant tPA treatment (82).

Apolipoprotein CI and CIII (ApoC-I and ApoC-III, respectively) are involved in triglyceride metabolism. Plasma concentrations of ApoC-I and ApoC-III were lower in HS patients than in IS patients within 6 h after symptom onset (83).

Branched-chain amino acids (BCAAs) including valine, leucine, and isoleucine were reduced in rat plasma within 2 h after IS onset (84). A similar decrease in BCAAs was observed in human plasma within  $6 \pm 2$  h after IS onset, and the degree of reduction was correlated with worse neurologic outcome (84).

A mass spectrometry-based proteomic analysis revealed that within 4.5 h after symptom onset, gelsolin, dihydropyrimidinase-related protein 2 (DPYSL2), and cystatin A in peripheral blood were independent predictors of poor outcome in IS (85).

Pregnenolone sulfate is one of the circulating metabolites. Within 3.3 h after symptom onset, serum pregnenolone sulfate had a high value in differentiating IS from SM (49).

As described above, MPV is a potential biomarker to predict the complications after tPA treatment. ApoC-I and ApoC-III, valine, leucine, isoleucine, gelsolin, DPYSL2, cystatin A, and pregnenolone sulfate are potential biomarkers to diagnose and assess the prognosis of hyperacute IS.

## DISCUSSION

Stroke is a serious global public health problem. Multimodal neuroimaging is the standard method used for the diagnosis of hyperacute IS. However, it cannot detect changes in specific molecules and cell types in the brain, which is valuable for assessing etiology and prognosis. The pathophysiological mechanism of cerebral ischemia plays an important role in specifying the prevention and treatment of ischemic stroke, so the blood-based biomarkers in this manuscript are classified according to the pathophysiological mechanism of IS. Specifically, IS initiates a wide range of events called ischemic cascades at the beginning of cerebral ischemia. Ischemic events begin with gradual or sudden cerebral hypoperfusion, including cellular bioenergy failure, excitotoxicity, oxidative stress, blood-brain barrier dysfunction, microvascular injury, hemostatic activation, inflammation, and eventual neuronal, glial, and endothelial cell necrosis (86). Blood-based biomarkers can provide important information on clinical status beyond that obtained by standard tests and diagnostic procedures. Further, we list the main research results of the relevant cited literature in **Supplementary Table 1**, which includes biomarkers and classification, participants, sample type, onset to measurement time, major outcomes, association with stroke, study design, and references.

Delays in treatment greatly increase the disability and mortality rates of IS. Although a standardized stroke management protocol can shorten DNT and DPT, there is an inevitable delay between the time from symptom onset to arrival at the emergency room during which medical interventions cannot be administered. There are no methods for

rapid detection of IS, which is mainly based on the observation of symptoms. Moreover, CT and MRI equipment are not widely installed in ambulances in China. Therefore, new tools for detecting IS outside of the hospital setting that are easy to operate without specialized knowledge and yield rapid results are needed. Blood-based biomarkers can serve as a foundation for the development of such diagnostic tests.

As an important part of the cytoskeleton of neurons, NfL has been proven to be associated with a variety of nervous system diseases, including Alzheimer's disease (AD), multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD), and frontotemporal dementia (FTD), etc. It is conducive to predict, diagnose, monitor the progress, and evaluate the efficacy and prognosis of these diseases. Of note, NfL and cerebrovascular-related diseases (e.g., stroke, subarachnoid hemorrhage, traumatic brain injury, etc.) have also become research hotspots in recent years. Three important clinical studies also showed the associations of NfL with stroke. Specifically, one of these important studies showed that higher plasma NfL levels was independently associated with 3- or 6-month functional disability and higher all-cause mortality (87). Meanwhile, another study showed that serum NfL levels increased with the grade of age-related white matter changes and were able to predict unfavorable clinical outcome 90 days after stroke (88). Additionally, it is shown that serum NfL levels 7 days post-stroke independently predicted mRS 3 months post-stroke (89). In this clinical research, NfL was expected to objectively be one of the most promising surrogate markers for evaluating the clinical outcome of stroke. However, in the three studies, the authors did not state clearly that the blood sampling time was within 6 h after ischemic stroke onset (20 d, 24 h, 24 h, respectively), which was why we did not cite and summarize them before. Thus, we eagerly anticipate that more NfL studies will emerge in the future to better confirm the clinical value of blood-borne biomarkers in stroke.

Biomarkers are physiologic features or biological substances that can be objectively and reproducibly measured. The ideal biomarker has the following characteristics (25, 90): (1) accurate and reproducible; (2) measured in a standardized fashion; (3) acceptable to the patient and easy to interpret by clinicians; (4) non-invasive and inexpensive; (5) has sensitivity and specificity of at least 80%; (6) explains a reasonable proportion of the outcome independent of established predictors, with consistency across multiple studies; and (7) there is evidence that clinical management strategies can be determined based on biomarker level.

Some blood-based biomarkers have been identified for IS diagnosis, differentiation, and evaluation of severity and prognosis. However, there are several limitations associated with their use in clinical practice. Firstly, alterations in the brain may not be reflected by blood-based biomarkers because of the presence of the BBB. Secondly, IS is a heterogeneous clinical syndrome with various etiologies and complex clinical manifestations that cannot be represented by a single biomarker. Thirdly, blood-based biomarkers can be influenced by comorbidities such as hypertension and diabetes. Fourthly, most data on potential biomarkers of

hyperacute IS have come from single-center studies with small samples in which candidate biomarker levels varied. Finally, a standard set of criteria for biomarker cutoff values is currently lacking.

In summary, blood-based biomarker testing can serve as a valuable adjunct to routine clinical examinations and neuroimaging in the diagnosis of hyperacute IS. Further exploration of the relationship between these biomarkers and the pathophysiology of hyperacute IS can guide treatment selection and facilitate prognostic assessment of hyperacute IS patients, thereby improving clinical outcomes.

## AUTHOR CONTRIBUTIONS

YX and XW contributed to the conception and organization of the article. ZL and CY contributed to the literature search and

manuscript writing. All authors contributed to the article and approved the submitted version.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fneur.2021.634717/full#supplementary-material>

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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