Invited Article

Acute ischemic stroke biomarkers: a new era with diagnostic promise?

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Stroke is considered as the first cause of neurological dysfunction and second cause of death worldwide. Recombinant tissue plasminogen activator is the only chemical treatment for ischemic stroke approved by the US Food and Drug Administration. It was the only standard of care for a long time with a very narrow therapeutic window, which usually ranges from 3 to 4.5 h of stroke onset; until 2015, when multiple trials demonstrated the benefit of mechanical thrombectomy during the first 6 h. In addition, recent trials showed that mechanical thrombectomy can be beneficial up to 24 h if the patients meet certain criteria including the presence of magnetic resonance imaging/computed tomography perfusion mismatch, which allows better selectivity and higher recruitment of eligible stroke patients. However, magnetic resonance imaging/computed tomography perfusion is not available in all stroke centers. Hence, physicians need other easy and available diagnostic tools to select stroke patients eligible for mechanical thrombectomy. Moreover, stroke management is still challenging for physicians, particularly those dealing with patients with "wake-up" stroke. The resulting brain tissue damage of ischemic stroke and the subsequent pathological processes are mediated by multiple molecular pathways that are modulated by inflammatory markers and post-transcriptional activity. A considerable number of published works suggest the role of inflammatory and cardiac brain-derived biomarkers (serum matrix metalloproteinase, thioredoxin, neuronal and glial markers, and troponin proteins) as well as different biomarkers including the emerging roles of microRNAs. In this review, we assess the accumulating evidence regarding the current status of acute ischemic stroke diagnostic biomarkers that could guide physicians for better management of stroke patients. Our review could give an insight into the roles of the different emerging markers and microRNAs that can be of high diagnostic value in patients with stroke. In fact, the field of stroke research, similar to the field of traumatic brain injury, is in immense need for novel biomarkers that can stratify diagnosis, prognosis, and therapy.

Key words: Biomarker, diagnostics, mRNA, rtPA, stroke, thrombectomy

INTRODUCTION

O F ALL HUMAN organs, the brain has the lowest tolerance to hypoxia. Sudden interruptions of cerebral blood circulation lead to brain tissue deprivation from oxygen, glucose, and other nutrients, causing brain dysfunction as well as other body functions that are under the regulation of the cerebral ischemic regions, known as ischemic stroke or cerebral ischemia.[1] Ischemic stroke is mainly caused by an embolus or thrombus in a major cerebral artery, which impairs the blood flow to the brain.[2] This will lead to brain tissue death and necrosis, which is known as "infarction". A hypoperfused area surrounding the infarct core is called "penumbra". Brain cells in the penumbra receive blood flow from collaterals that is too low to maintain electric activity but sufficient to keep it viable. If blood supply is not restored within a few hours, these cells will die and amplify the original damage. Penumbra is the target to salvage by chemical and mechanical reperfusion of blood supply. However, restoration of blood flow to these anoxic cells could result in further damage, known as reperfusion injury.[3] Studies have estimated by serial brain magnetic resonance imaging that approximately 1.9 million neuronal cells die per minute of ischemia, leading to the destruction of 14 billion synapses in human brains that were subjected to middle cerebral artery ischemic strokes.[4] Such damage could lead to extremely high risk of mortality and morbidity, depending on the location and extent of brain tissue damage.[5,6] In fact, stroke is one of the major causes of mortality and dysfunction that result in financial and social burden at the

individual and community levels.[7,8] Approximately 15 million people worldwide are affected by stroke annually.

Although ischemic stroke accounts for 80% of reported cases, hemorrhagic stroke has also been recognized.[9] Hence, due to the devastating effects of stroke on humans' functionality and mortality, sensitive and specific diagnostic tools are necessary to allow clinicians to provide fast and effective care. The goal of this paper is to review published work in order to identify potential biomarkers that could help in the early diagnosis and prediction of the onset of acute ischemic stroke (AIS).

Clinical treatment of ischemic stroke

An effective treatment approach for ischemic stroke is to restore blood flow to the hypoperfused brain areas. This approach is known as reperfusion therapy and it must be initiated as soon as possible.[10,11] Recanalization of blocked vessels could be achieved by chemical or mechanical means.[12] Recombinant tissue plasminogen activator (rtPA) is the only chemical treatment for ischemic stroke approved by the US Food and Drug Administration (FDA). However, the narrow therapeutic window for the initiation of the intravenous thrombolysis by rtPA, which usually ranges from 3 to 4.5 h of the stroke onset, limits this clinical approach.[13–15] In contrast, the main interventional treatment approach for ischemic stroke is mechanical thrombectomy. It is usually carried out using a microcatheter that goes into the blocked vessel in the cranial circulation from femoral/radial arteries and removes the thrombus in order to restore blood flow.[16] The management of patients with "wake-up" stroke, those who wake up with stroke symptoms that were not present prior to falling asleep and were known to be well for 6–24 h, has been quite challenging to physicians. Hence, many studies were carried out to evaluate the efficacy of different management approaches. For instance, in a multicenter prospective randomized study, open blinded end-point design was used to randomize wake-up stroke patients into a mechanical thrombectomy group and standard medical care group. It was found that patients who underwent mechanical thrombectomy had 2-fold neurological improvement compared to those who received standard medical care, and were more likely to achieve independence by 90 days. The results indicated that a significant proportion of patients with wake-up stroke might benefit from mechanical thrombectomy with a time range of 6–24 h once they had proper advanced imaging.[17] In addition, the DAWN trial showed those who were known to be well for 6–24 h and had a mismatch between clinical deficit and infarct, had a better outcome at 90 days with mechanical thrombectomy compared to standard care.[18] Although chemical recanalization by rtPA is

effective, there is a risk of hemorrhage that might lead to serious consequences.[4] Thrombectomy could be undertaken to a vessel with a diameter greater than 2 mm.[19,20]

Although immediate restoration of blood flow can minimize extensive brain neuronal tissue injury,[21,22] recanalization could cause further tissue necrosis and neuronal cell death. Clinically, that translates into cerebral edema or intracranial hemorrhage post-thrombolytic treatment, which is called ischemia/reperfusion injury.[23] The underlying mechanisms of ischemia/reperfusion injury have been attributed to different causes, such as free radical formation, inflammation, calcium overload, and cellular apoptosis.[24–27]

Diagnostic biomarkers for AIS

The usual work-up for patients with suspected stroke is history-taking, focusing on the time at which neurological symptoms or deterioration appeared. In addition to physical and neurological examinations, including the National Institute of Health Stroke Scale, radiological imaging, prothrombin time/international normalized ratio, complete blood count, electrocardiogram, and measurement of troponin, computed tomographic angiography/perfusion or magnetic resonance angiography/perfusion must be carried out when available to determine the extent of salvageable tissues and select good candidates for mechanical thrombectomy. This assessment must be done immediately for better patient outcome. The main objectives of the initial stroke assessment are to rule out intracranial hemorrhage, assess thrombolysis therapy contraindications, and characterize the type of stroke.[28] The fast and objective diagnosis of stroke type in acute settings enables treatment with rtPA within the narrow therapeutic window and undertaking mechanical thrombectomy when applicable, thereby improving overall patient outcome and neurological status.

Wake-up strokes, which usually occur during sleep, represent a diagnostic and therapeutic dilemma. Wake-up stroke accounts for approximately 25% of stroke cases.[29,30] In such cases, rtPA therapy cannot be applied due to the risk of hemorrhage as its therapeutic window might have been surpassed. Moreover, for a variety of reasons, computed tomographic angiography/perfusion or magnetic resonance angiography/perfusion are not readily available; additional diagnostic tools that are simple and available yet specific can help mitigate the diagnosis of patients with new ischemic strokes and give insight into selecting those who would be good candidates for reperfusion therapy without causing further damage.

In the history of medicine, diagnostic biomarkers have been widely used in different acute and chronic medical conditions. For instance, troponin is used for the diagnosis of

possible acute myocardial infarction, which is a sensitive test that has significantly improved patient care at the emergency department. Similarly, different diagnostic biomarkers could be used in the diagnosis of stroke that would help clinicians select the appropriate treatment, optimizing each patient's overall outcome. Nevertheless, monitoring such biomarkers during the patients' hospital stay would equip physicians with an insight regarding stroke progression.[29,31] This review gathers all inflammatory, neuroglial, and myocardial markers cited in published reports that could be potential biomarkers for the early diagnosis of AIS and prediction of its onset.

MATERIALS AND METHODS

Data sources and search strategy

IN ORDER TO identify the most relevant studies reporting on the use of biomarkers as diagnostic tools for AIS, a search of published works was carried out using electronic databases PubMed, MEDLINE, and Google Scholar. The search included reports published before December 2019. The search strategy involved the following MeSH terms: stroke, AIS, diagnosis, diagnostic*, biomarker*, miRNA, wake-up stroke, inflammatory biomarker, cerebral hemorrhage. The search was limited to English language with no restriction on the time of the publications. The snowballing method, finding further articles through the references of these articles, was applied.

Inclusion and exclusion criteria

In order to avoid heterogeneity in the selected articles, we applied good inclusion and exclusion criteria. We focused on articles reporting inflammatory, neuroglial, mRNA, and cardiac markers in AIS, whether measured in blood or cerebrospinal fluid (CSF); no previous systematic review combined all of these biomarkers. The sample must have been drawn less than 24 h from the onset of symptoms. Both human and animal studies using biomarkers for the diagnosis of AIS were included with no minimum sample size required. Studies reporting biomarkers in stroke mimics, such as space-occupying lesion, infection, and subdural hematoma, were excluded. In addition, studies reporting biomarkers in patients with transient ischemic stroke were excluded. Figure 1 shows a PRISMA flowchart of the study.

STROKE DIAGNOSTIC BIOMARKERS

IN FLAMMATORY BIOMARKERS PLAYS an important role as mediators in the acute phase of ischemic stroke and their levels are elevated in the peripheral blood.[32] Many studies evaluated the ability of these biomarkers to help in the diagnosis of ischemic stroke but with controversial results. Whiteley *et al.*, in a systematic review, reported that no inflammatory biomarker is recommended yet for the diagnosis of AIS. This is because of the limited reported data about the methods and the accuracy of the diagnostic tests used in the included studies.[33] An *et al.* showed that interleukin-6 (IL-6) expression was upregulated following brain ischemia when measured in the first 24 h in the peripheral blood. The IL-6 serum level was higher in patients with AIS (median 4.0 pg/ml) in comparison to patients with stroke mimics (median 1.2 pg/ml) (Table 1).[32] After correlating the levels of inflammatory biomarkers with the ischemic lesion volume and clinical outcome, Sotgiu *et al.* reported that IL-6 rise in peripheral blood had a neuroprotective effect and was associated with better outcome, but showed no value as a diagnostic tool.[34] In a Chinese population, procalcitonin level was significantly increased in ischemic stroke patients when compared to a normal control group. Patients with procalcitonin level above 1.20 ng/ml were at a higher risk for AIS in comparison to healthy individuals (median 0.25 ng/ml) (Table 1). It seemed to be an independent diagnostic marker in Chinese populations.[35] Future studies are required to confirm the diagnostic value of procalcitonin in AIS. Sotgiu *et al.* showed that tumor necrosis factor-α (TNF-α) is strongly correlated with the clinical severity and extent of the brain infarct. In his study, he included 66 patients with AIS and blood samples were withdrawn in the first 20 h. Patients with good outcome had a median TNF-α level of 37.9 pg/ml compared to 90 pg/ml in those with worse outcome (Table 1). Tumor necrosis factor-α reaches a high concentration in the first 6 h after AIS, but its similar behavior in other inflammatory and infectious processes limits its capacity to be used as a valuable diagnostic tool.[34]

Serum matrix metalloproteinase

Matrix metalloproteinases (MMP) are enzymes expressed in the central nervous system by infiltrating inflammatory cells such as macrophages and neutrophils in response to acute brain ischemia. They play an important role in the cascade leading to disruption of the blood–brain barrier and development of vasogenic edema.[36] In a case–control study, concentration of serum MMP-8 taken within 24 h of symptom onset was significantly higher in patients with AIS (58.3 ng/ml) when compared to a healthy group of patients (24.2 ng/ml). The author showed that serum levels varied between etiologic stroke subgroups as well, with higher levels measured in cardioembolic stroke and large vessel occlusion.[37] Ramos-Fernandez *et al.*, in a literature review, showed that
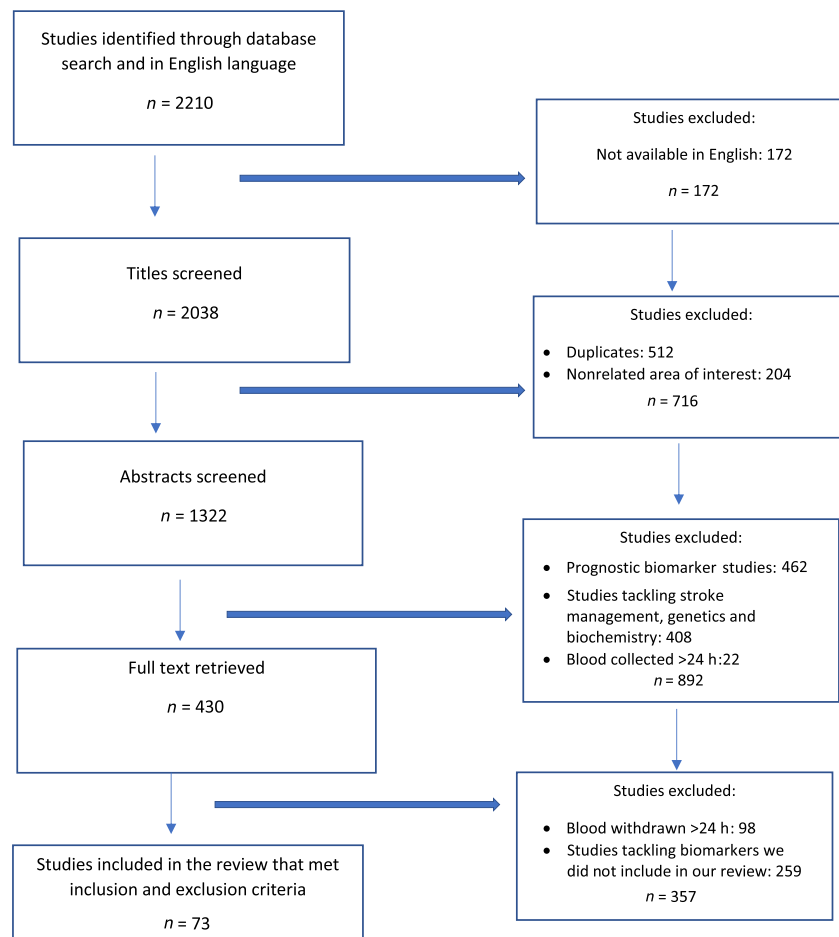
Fig. 1. PRISMA flow diagram of the search of published works to determine the current status of acute ischemic stroke diagnostic biomarkers.

MMP-9 levels were significantly overexpressed in the hyperacute phase of ischemic stroke, with a peak level after 24 h. The researchers also reported a strong correlation between MMP-9 serum level and the severity of stroke. Patients with progressive motor deficit had higher MMP-9 serum levels than those without clinical worsening (6.53 ± 2.16 ng/ml vs. 4.40 ± 1.78 ng/ml) (Table 1).[36] Serial measurement of MMP-9 was shown to be a useful marker for ongoing brain damage, and further studies are required to confirm its valuable diagnostic ability in AIS.

Thioredoxin

Oxygen free radicals are expressed in significant amounts during cerebral ischemia and they play a major role in cell death signal pathways that lead to brain damage in AIS.[38] Thioredoxin is a redox-regulating protein with antioxidant activity that can be a potential indicator of oxidative stress in strokes. It is released from cells in response to oxidative stress and plays a defensive role through its disulfide reductase activity.[39] Wu *et al.* undertook a prospective study on 312 patients admitted with AIS and fasting blood samples were withdrawn in the first 24 h from the event. Serum thioredoxin level was higher in patients with AIS when compared to healthy patients (15.03 ng/ml vs. 8.95 ng/ml) (Table 1). The author reported that serum thioredoxin levels of 11.0 ng/ml or higher corresponded to a 6.99-fold increase in risk of AIS compared to age and gender-matched normal cases.[40] Qi *et al.* reported similar findings and showed a strong correlation between increased thioredoxin serum levels and risk of AIS. However, major limitations were described in these studies that might have affected the results. These include the presence of immunoglobulins in the human serum that could bind to reagent antibodies used in the enzyme-linked immunosorbent assay (ELISA) method while detecting thioredoxin levels and lead to false positive

Table 1. Biomarkers that correlate with acute ischemic stroke and its clinical outcome

Biomarker	Study author/year	Level of biomarker/cut-off	Controls	Time of sampling
IL-6	An *et al*. (2013)	4 pg/ml (median)	1.2 pg/ml (median in stroke mimics)	6–24 h from onset
	Sotgiu *et al*. (2006)	9.2 pg/ml (median)	8.2 pg/ml (median in stroke mimics)	6–20 h from onset
TNF-α	Sotgiu *et al*. (2006)	90 pg/ml (worse outcome)	37.9 pg/ml (good outcome)	6–20 h from onset
Procalcitonin	Tian *et al*. (2015)	>1.2 ng/ml	0.25 ng/ml	Within 24 h from onset
MMP-9	Ramos-Fernandez *et al*. (2011)	6.53 ± 2.16 ng/ml	4.40 ± 1.78 ng/ml	Within 24 h from onset
MMP-8	Palm *et al*. (2018)	58.3 ng/ml	24.2 ng/ml	Within 24 h from admission
Thioredoxin	Wu *et al*. (2016)	15.03 ng/ml	8.95 ng/ml	Within 24 h from onset
GFAP	Ren *et al*. (2016)	0.02 ng/ml	0.004 ng/ml	Within 24 h from onset
UCH-L1	Ren *et al*. (2016)	0.13 ng/ml	0.05 ng/ml	Within 24 h from onset
sNfl	Onatsu *et al*. (2019)	89.5 pg/ml	25.2 pg/ml	Within 12 days from onset

GFAP, glial fibrillary acidic protein; IL-6, interleukin-6; MMP, matrix metalloproteinase; sNfl, serum neurofilament light chain; TNF-α, tumor necrosis factor-α; UCH-L1, ubiquitin C-terminal hydrolase.

results.[39] Thioredoxin could be a potential marker that can be used in future works to study diagnostic biomarkers in AIS.

Neuronal and glial markers

S100 is a glial protein that belongs to the calcium-binding protein family. It is released in blood in response to infarction of glial and Schwann cells. Nash *et al*., in a systematic review, found that S100 serum concentration was significantly higher in stroke patients compared to healthy controls. He also reported a strong correlation between S100 levels and stroke severity. However, its concentration does not reach its peak until 1–5 days after the event, which limits the diagnostic importance of S100 for early intervention in AIS.[41]

Glial fibrillary acidic protein (GFAP) is a brain-specific intermediate filament protein that is involved in astroglial cell structure. Glial fibrillary acidic protein is not detectable in the plasma until occurrence of necrosis and cytolysis, such as in ischemic stroke or intracranial hemorrhage. Foerch *et al*. showed that GFAP plasma level increases significantly in intracranial hemorrhage and in a more delayed fashion in ischemic stroke. This resembles the behavior of the previously discussed S100 marker, where plasma levels reach their peak after 48–78 h due to the gradual neuronal damage in AIS. As another limitation of GFAP as a potential diagnostic marker, the influence of other clinical variables on plasma levels such as renal function and infections were not taken into consideration.[42] Ren *et al*., in a controlled prospective study, showed that GFAP concentrations were

significantly elevated in patients admitted with AIS when compared to healthy volunteers (0.02 ng/ml vs. 0.004 mg/ml) (Table 1). This elevation was more significant in patients with associated intracranial hemorrhage.[43]

Ubiquitin C-terminal hydrolase (UCH-L1), a cytoplasmic enzyme of neurons that is associated with the brain's self-repair mechanism after injury, was shown in this study to be elevated significantly in patients with AIS (0.13 ng/ml) in comparison to healthy ones (0.05 ng/ml) (Table 1).[43]

Neurofilament light chain (NfL), is a major component of the neuronal cytoskeleton and plays an important role in axonal and dendritic branching, and growth. Onatsu *et al*. were able to detect NfL in blood using the novel ultrasensitive single molecule array (Simoa) assay. Neurofilament light chain serum levels were shown to be 3.5-fold higher in patients with AIS compared to those with transient ischemic attack (89.5 pg/ml vs. 25.2 pg/ml) (Table 1). The author also reported that serum NfL levels were the highest with AIS due to large vessel occlusion or cardioembolism.[44] Finally, neuronal and glial markers seem to be more specific in identification of cerebral ischemia and could potentially be utilized in the future for diagnostic purposes in AIS.

MicroRNAs

MicroRNA roles in brain development and stroke implications

MicroRNAs (miRNAs) are formed by a group of nonprotein coding genes that are present in all living organisms. MicroRNAs are involved in multiple biological reactions such as

cell growth, cell death, tissue differentiation, and embryonic development.[45] Variations in miRNA sequences affect miRNA regulation and have been associated with many human disorders.[46] It has also been found that miRNAs work as gene regulators in cerebrovascular diseases.[47] Due to their unique structures and functions, it has been proposed that miRNAs might be used as biomarkers in certain human diseases.[48] For instance, miRNAs play a key role in regulatory processes of leukocyte gene expression in AIS.[49] It has been shown that miRNA alterations following central nervous system injury stimulate neuronal cell death mediated by inflammation and oxidative stress.[50] Specific miRNAs have been shown in experimental studies to correlate with specific findings. For example, miRNA-17-92 was found to have a role in regulating the genes for T-box protein 2, phosphatase homolog and tensin homolog. These genes are known to play a role in radial glial cells.[51] It was found that miRNA-17-92 was upregulated in mice after stroke, which boosted the proliferation of neuronal progenitor cells.[52] In addition, the miRNA-124 cluster has been shown to have a role in the differentiation of neuronal progenitor cells. For instance, knockout mice of miRNA-124 caused reduction in brain size and anatomical abnormalities.[53] Hence, following an AIS, high levels of miRNA-124 were attributed to different cellular processes, such as inflammation, edema, cell death, and neurogenesis.[52,54,55] Lalwani *et al.* found out that miRNA-142-3p represses vascular endothelial cahedrins in zebrafish and as a result mediates vascular integrity. Increased levels of miRNA-142-3p were correlated with vascular hemorrhage, whereas low levels caused abnormal vascular remodeling.[56] Furthermore, Liu *et al.* found that miRNA-142-3p was elevated in rodents following ischemic stroke, which indicates its role in secular remodeling.[52] Similarly, miRNA-126 targets vascular endothelial growth factor (VEGF). Thus, it mediates vascular development.[57–59] In rodents, miRNA-126 inhibits VEGF and affects retinal neovascularization post-ischemic stroke.[60,61] Moreover, in humans, elevated levels of miRNA-126 were established as a biomarker for AIS.[62]

MicroRNA involvement in stroke formation and miRNAs as diagnostic tools for AIS

Atherosclerosis, diabetes, and hypertension are comorbidities mostly associated with stroke.[63,64] It has been reported that hypertension is the number one risk factor for stroke formation due to its effects on vessel elasticity, making them easy to rupture, which leads to hemorrhagic stroke.[65] In one of the studies carried out on rats with hypertension, it was shown the levels of miRNA-155 were reduced in these rats.[66] In addition, it was observed that miRNA-155 plays a

role in vessel relaxation as it targets nitric oxide synthase and angiotensin II receptors.[54,67] Hence, miRNA-155 has a crucial role in stroke formation as it modulates blood pressure. Moreover, miRNA-22 has been found to target chromogranin A, leading to a boost in catestatin, which regulates blood pressure.[68–70] In animal studies, rats that were treated with miRNA-22 antagonist had a decrease in blood pressure.[71] In a comparison of hypertensive and normotensive rats, a sequence of 24 miRNAs has been shown to be expressed in the brainstem of hypertensive rats.[72] Similarly, a sequence of 30 miRNAs were found to be upregulated in human endothelium of vasculature that was thought to play a role in hypertension.[73]

Alterations in miRNAs were found to occur in atherosclerotic vessel walls and serum, implying their involvement in atherosclerosis formation and progression.[74] For instance, miRNA-155 was found to play a role in the inflammatory processes that accompany atherogenesis by targeting pro-inflammatory transcription factors such as Ets1 and AT1R, thus being atheroprotective.[75] In mouse studies, knockdown of miRNA-155 led to Fas apoptosis protein downregulation, along with TNF-α downregulation.[76,77] In addition, antagonism of miRNA-155 enhanced lipid uptake and inflammation.[78] Similarly, downregulation of miRNA-320a, which is involved in VEGF signaling pathways, as well as miRNA-92a, which regulates shear stress genes, had atheroprotective effects.[79–81] Moreover, type II diabetic patients who had ischemic stroke were found to have low levels of miRNA-223 and high levels of miRNA-144. It was found that miRNA-144 levels were low in the serum of diabetic patients following stroke.[82] In diabetic mice, it was established that the levels of miRNA-200a and miRNA-466a were downregulated in neural stem cells.[83]

In 2009, the first study to compare the expression of miRNAs in healthy people and patients with acute ischemia, as per WHO clinical criteria, was published by Tan *et al.* They found out that 157 miRNAs were expressed in stroke patients. Among those, 138 miRNAs were highly expressed; among them, 17 were upregulated (miR-25, 181a, 513a5p, 550, 602, 665, 891a, 923, 933, 939, 1184, 1246, 1261, 1275, 1285, 1290, and let-7e). Hence, it was concluded that these miRNAs could be used in the diagnosis of AIS as well as in the differentiation of large and small artery stroke.[84] In 2013, the Tan *et al.* research team found that 21 miRNAs were similarly expressed in all AIS patients (hsa-miR-1258, 125a5p, 1260, 1273, 149, 220b, 23a, 25, 26b, 29b1, 302e, 34b, 4835p, 488, 4903p, 498, 506, 659, 890, 920, and 934). Among these 21 miRNAs, four were downregulated (miR-25, 34b, 4835p, and 498).[85]

Levels of miRNAs in blood were compared between patients who had strokes and controls, and it was found that

miR-122, 148a, 19a, 320, and 4429 were low and miR-363 and 487b were elevated in patients with AIS.[86] Similarly, comparison of miR-210 levels in blood in ischemic stroke patients and healthy controls showed that miR-122 levels were downregulated in stroke patients, especially during the 7–14-day period following stroke onset, which suggested that the miR-122 level could be useful in stroke diagnosis a few days after stroke onset.[87]

Researchers in one study found that miR-16 was higher in ischemic stroke patients compared to hemorrhagic stroke patients with an odds ratio of 9.75, which indicates that miRNA is not only a stroke diagnostic biomarker, but also could be used in distinguishing between ischemic and hemorrhagic stroke.[88] In contrast, miR-21 was reported previously to have cardioprotective effects in ischemia reperfusion-induced cardiocyte cell death. It was identified that miR-21 is significantly downregulated in AIS patients within the first 24 h, suggesting its potential as a diagnostic tool at the early stage of cerebral ischemia.[89] Furthermore, Let-7 is a type of miRNA, with a family of 12 members in humans, that plays an important role in central nervous system gene expression regulation.[90] It has been proposed that Let-7 promotes neurodegeneration by the activation of RNA-sensing Toll-like receptors.[91] Serum Let-7e was found to be elevated in ischemic stroke patients within the first 24 h of stroke onset with a specificity and sensitivity of 73.4% and 82.8%, respectively, for the diagnosis of ischemic stroke.[92] In contrast, another member of the Let family, Let-7c-5p, was reduced in the plasma of ischemic stroke patients within the first 24 h.[93]

Troponin

Cardiac troponin I (cTnI) and cardiac troponin T (cTnT) are part of the troponin complex of microfilaments that form the cardiac-specific contractile apparatus (sarcomere) of cardiomyocytes,[94] and are crucial biomarkers in cardiac emergencies. Cardiac troponin is released transiently in the blood, as a result of acute myocardial infarction or ischemia. Although the mechanism of troponin release is still poorly understood, the main reason is necrosis.[95] This release is mediated by a complex molecular mechanism involving Ca^{2+}, adenosine 5′-triphosphate, and O_2.[95] Elevated levels signify myocardial injury and allow for the diagnosis of myocardial infarction.[96] Conversely, the basic cause of myocardial infarction is not essentially attributed to elevated cTn levels.[96] In fact, it is vital to recognize that cTn elevations can be attributed to factors other than acute or chronic coronary heart disease.[97] Multiple different pathologies have been correlated with elevation in cTn, including AIS.[98] In a study undertaken by Schietz *et al*., cTn levels were measured in 715 ischemic stroke patients at admission. Interestingly, high levels of cTn were reported one in every seven patients with AIS, and these levels were reported to be independently associated with meagre short-term outcome and mortality.[96] In addition, Král *et al*. reported a study on 107 patients with AIS. These patients were admitted to the hospital less than 12 h post-stroke and their cTn levels were measured and compared to multiple baseline values.[99] They also concluded that elevated troponin levels can be regularly detected in patients with AIS.[99] Chang *et al*. also undertook a study on 1,234 patients with AIS, claiming an association exists between positive troponin levels and large vessel occlusion (LVO) in patients with AIS.[100] In contrast, a systematic review published in 2018 by VanHouten *et al*. claimed that troponin I levels might become elevated in patients with AIS, but seldom rise above 2 ng/ml.[101] Moreover, in a study led by Yildiz *et al*., serum troponin I levels of 41 patients with acute coronary syndrome and ischemic stroke were compared to patients with acute coronary syndrome only.[102] Shockingly, cTn levels were comparable between the two groups, raising the claim that elevated troponin levels were mainly due to cardiac abnormalities, rather than cerebral ones, resulting in troponin I not being a consistent acute phase key player in patients with AIS.[102] In summary, these findings suggest that troponin might not be a suitable biomarker for AIS diagnosis. Table 2 provides a comprehensive summary of serum biomarkers with potential diagnostic characteristics for AIS.

Accessibility and biokinetics of stroke biomarkers

The Brain Trauma Indicator was approved by FDA in 2018 as the first blood test to evaluate concussion and prevent unnecessary imaging. The Brain Trauma Indicator measures the UCH-L1 and GFAP, which are released by the brain cells within 12 h post-injury and the test can be available within 3 h, predicting the presence of intracranial lesions. Accordingly, physicians decide whether imaging is needed or not. In addition, another assay that measures the levels of GFAP, neuron-specific enolase, S100B, and TNF-α has been proposed by a research team in Arizona. The assay is able to detect the levels of these biomarkers within 90 s in the blood, predicting intracranial injury that could be caused by trauma or stroke depending on the clinical context and patient's presentation.[103,104]

Multiple studies have been published comparing the concentration of biomarkers in case of focal or diffuse injury to the brain. For instance, serum GFAP level was significantly

Table 2. Summary of serum biomarkers with potential diagnostic characteristics for acute ischemic stroke (AIS)

Study (year)	Study design	Sample size	Biomarker	Diagnosis/ significance	Results	References
Diagnostic accuracy of plasma glial fibrillary acidic protein for differentiating intracerebral hemorrhage and cerebral ischemia in patients with symptoms of acute stroke (2012)	Prospective study, Jun 2009–May 2010	205	GFAP	Plasma GFAP analysis performed within 4.5 h of symptom onset can differentiate ICH and ischemic stroke	GFAP concentrations were increased in patients with ICH compared with patients with ischemic stroke. Diagnostic accuracy of GFAP for differentiating ICH from ischemic stroke and stroke mimic was high	24
Assessment of serum UCH-L1 and GFAP in acute stroke patients (2016)	Prospective study	189	GFAP	Higher GFAP levels were associated with stroke severity and history of prior stroke. Confirmed the potential of GFAP as a tool for early rule-in of ICH	GFAP concentrations were significantly greater in ICH patients than in controls ($P < 0.0001$). GFAP yielded an AUC of 0.86 for differentiating between ICH and IS within 4.5 h of symptom onset with a sensitivity of 61% and a specificity of 96% using a cut-off of 0.34 ng/ml	25
Limited clinical value of multiple blood markers in the diagnosis of ischemic stroke (2013)	Prospective study, Sep 2008–Oct 2010	278	IL-6	Clinical usefulness of these biomarkers is limited due to low discriminating ability when compared to clinical parameters alone in diagnosis of ischemic stroke	Only IL-6, S100B, and MMP-9 were independently associated with ischemic stroke in multivariate analysis. The addition of biomarkers (IL-6, S100B, and MMP-9) did not improve the diagnostic performance of baseline clinical models with added biomarkers versus baseline clinical models alone	14
Inflammatory biomarkers in blood of patients with acute brain ischemia (2005)	Prospective study	66	Inflammatory biomarkers	IL-6, in the context of a complex pro-inflammatory network occurring during stroke, is associated with	Some markers showed a direct significant correlation with both initial and final NIH scale and with infarct size, particularly	16

Table 2. (Continued)

Study (year)	Study design	Sample size	Biomarker	Diagnosis/ significance	Results	References
				neuroprotection rather than neurotoxicity in patients with ischemic brain injury.	tumor necrosis factor alpha (TNF-a) (P 1⁄4 0.002), intercellular adhesion molecule-1 ($P < 0.01$) and matrix metalloproteinase 2/9 (P 1⁄4 0.001). interleukin-6 (IL-6) serum level showed a significant inverse correlation with both final neurological impairment and infarct size ($P < 0.001$).	
Serum matrix metalloproteinase-8, tissue inhibitor of metalloproteinase and myeloperoxidase in ischemic stroke (2018)	Cross-sectional case–control study	1,279	MMP-8	Concentrations of serum neutrophil markers are increased after ischemic stroke and associate with stroke severity and etiology.	Levels of MMP-8, MMP-8/TIMP-1 ratio, and MPO were independently associated with ischemic stroke. MMP-8 levels differed between etiologic stroke subgroups (p 1⁄4 0.019, ANOVA), with higher levels in cardioembolic stroke and stroke due to large vessel disease, and lower levels in microangiopathic stroke. MMP-8, MMP-8/TIMP-1 ratio and MPO ($P < 0.001$) concentrations showed positive associations with stroke severity independent of stroke etiology.	[19]
Matrix metalloproteinase-9 as a marker for acute ischemic stroke: a systematic review (2011)	Systematic review	–	MMP-9	MMP-9 is a possible marker for ongoing brain ischemia, as well as a predictor of hemorrhage in patients treated with t-PA	Higher MMP-9 values were significantly correlated with larger infarct volume, severity of stroke, and worse functional outcome. MMP-9 was a predictor of the development of	[18]

Table 2. (Continued)

Study (year)	Study design	Sample size	Biomarker	Diagnosis/ significance	Results	References
					intracerebral hemorrhage in patients treated with thrombolytic therapy	
Serum neurofilament light chain concentration correlates with infarct volume but not prognosis in acute ischemic stroke (2019)	Prospective single-center observational cohort study nested within Embodete CT Eastern Finland Study, March 2005–November 2009	136 + 3,096	sNfL	Cases with stroke were distinguishable from those with TIA following the determination of sNfL in the blood samples. The presence and amount of axonal damage estimated by sNfL correlated with the final cerebral infarction volume but was not predictive of degree of disability	sNfL was markedly higher in patients with AIS (89.5 pg/ml [IQR, 44.7–195.3]) than with TIA (25.2 pg/ml [IQR: 14.6-48.0], $p = $ <0.001). sNfL concentration ≥49 pg/ml proved to be the best cut-off value to differentiate between patients with stroke and those with TIA (sensitivity, 73%; specificity, 80%). sNfL concentration significantly correlated with cerebral infarction volume ($r = 0.413$, $P \leq 0.001$). Patients with AIS due to cardioembolism or large artery atherosclerosis had the highest sNfL concentrations	26
S100 as a marker of acute brain ischemia: a systematic review (2008)	Systematic review	–	S100	S100 was significantly increased after stroke onset, and correlates with infarct volume, stroke severity, and functional outcome, and was a possible marker for ongoing ischemia. Its serum concentration during acute stroke is a useful marker of infarct size and long-term clinical outcome	S100 peaks from symptom onset between 24 and 120 h with significantly raised values measured from 0 to 120 h. Higher S100 values indicated significantly larger infarction volumes, more severe strokes, and worse functional outcome. There was a significant difference in S100 levels between AIS patients and controls	23
Thioredoxin is a novel diagnostic and	Prospective study,	346	Thioredoxin	Elevated serum TRX level at admission	There was a significant positive association	21

Table 2. (Continued)

Study (year)	Study design	Sample size	Biomarker	Diagnosis/ significance	Results	References
prognostic marker in patients with ischemic stroke (2015)	Jan 2012–Dec 2013			was a novel diagnostic and prognostic marker in patients with acute ischemic stroke.	between serum TRX levels and NIHSS scores (r1⁄4 0.476, Po0.0001). Based on the ROC curve, the optimal cut-off value of serum TRX levels as an indicator for auxiliary diagnosis of AIS was projected to be 11.0 ng/ml, which yielded a sensitivity of 80.3% and a specificity of 73.7%, with the AUC at 0.807	
Cardiac biomarkers predict large vessel occlusion in patients with ischemic stroke (2019)	Prospective study, Jan 2016–Jun 2017	1,234	Troponin	Cardiac biomarkers, particularly serum troponin levels, are associated with acute LVO in patients with ischemic stroke	There was an association between positive troponin and LVO after adjusting for age, sex, and other risk factors (adjusted OR 1.69 [1.08-2.63], $p = 0.022$). There was an association between LAD and LVO after adjusting for age, sex, and risk factors (adjusted OR per mm 1.03 [1.01-1.05], $P = 0.013$) but this association was not present when AF was added to the model (adjusted OR 1.01 [0.99-1.04], $p = 0.346$)	[82]
Frequency, determinants, and outcome of elevated troponin in acute ischemic stroke patients (2012)	Prospective study, Oct 2009–Oct 2010	917	Troponin	Elevation of cTnT occurs in every seventh patient with AIS, is independently associated with poor short-term outcome and mortality. Patients with strokes affecting the insular cortex are particularly prone to myocardial	Factors independently associated with increased cTnT were higher stroke severity ($p = 0.04$), renal insufficiency ($p = 0.001$), pre-existing coronary artery disease ($p = 0.03$), hypercholesterolemia ($p = 0.02$), and insular cortex involvement ($p < 0.001$). Increased	[78]

Table 2. (Continued)

Study (year)	Study design	Sample size	Biomarker	Diagnosis/ significance	Results	References
				injury, justifying intensive cardiac monitoring	cTnT on admission was an independent predictor of unfavorable outcome (adjusted OR 2.65 [95% CI, 1.29–5.46]) and in-hospital mortality (4.51 [1.93–10.57]). There was a trend towards a negative association of cTnT elevation with major neurologic improvement (0.54 [0.27–1.07])	
Circulating troponin I level in patients with acute ischemic stroke (2018)	Systematic review	1,226	Troponin	Troponin I levels >2 ng/ml should not be attributed to an AIS, but should prompt a thorough evaluation for coronary artery disease	20.6% had a circulating troponin I level elevated over the reference range, but 99% were below 2.13 ng/ml. This is significantly lower than the distribution observed in a cohort of 89,423 unique cases of acute coronary syndrome ($p < 2.2 - 16$)	[21]
Is troponin really a reliable marker in patients with acute ischemic stroke? (2018)	Prospective study, Nov 2011–Jan 2014	183	Troponin	Abnormal troponin levels were more likely to be due to cardiac causes than cerebral ones in this first study evaluating the cTnI levels in patients with ACS concomitant with AIS. The severity of IS, lesion location in the anterior circulation and higher troponin levels were associated with mortality	cTnI levels were found to be similar in both groups. Presence of diabetes mellitus, coronary artery disease, and previous myocardial infarction were more frequent in patients with AIS. cTnI levels in patients with cranial lesion in the anterior circulation were higher ($P = 0.039$). Presence of AIS, cTnI levels higher than 20 ng/ml, and left ventricular ejection fraction <40% were found to be independent risk factors for mortality ($p < 0.05$)	[80]
		107	Troponin T			[77]

Table 2. (Continued)

Study (year)	Study design	Sample size	Biomarker	Diagnosis/ significance	Results	References
Troponin T in acute ischemic stroke (2013)	Prospective study, 2011			Elevated cTnT can be frequently detected in patients with AIS. To reliably identify patients with current acute myocardial impairment, more in-depth clinical investigation is needed	cTnT levels were correlated significantly with age (r [0.448) and the levels of N-terminal pro-brain natriuretic peptide (r [0.528), cystatin C (r [0.457), creatine kinase-MB mass (r [0.253), urea (r [0.281), and albumin (r [L0.219). Multiple logistic regression analysis found creatinine >90 mmol/L (OR 3.45; 95% CI, 1.09 −10.85), N-terminal pro-brain natriuretic peptide (OR 100 mg/L increase 1.09; 95% CI, 1.03–1.16), and creatine kinase-MB mass (OR per 1 mg/L increase 1.45; 95% CI, 1.04–2.04) were associated with cTnT elevation in patients with AIS	
Assessment of serum UCH-L1 and GFAP in acute stroke patients (2016)	Prospective study	189	UCH-L1	No clinical significance in AIS	UCH-L1 concentrations were significantly greater in ICH patients than in controls ($p < 0.0001$)	24

higher in focal brain injury compared to diffuse brain injury.[105] In addition, Czeiter *et al.* found that GFAP 99% of determining CT-positive patients post-stroke or TBI compared to clinical examinations used in contemporary decision rules was 99% sensitive in determining.[106]

Kinetic metrics of these biomarkers are quite important as each biomarker might have a different yield at different times post-injury. Very limited data is available concerning the kinetics of these biomarkers. Ubiquitin C-terminal hydrolase is one of the most studied biomarkers in terms of kinetic metrics. It was found the that levels were significantly high in serum in the first 24 h following brain injury compared to its level in CSF. Moreover, it was found that there is a strong between the median concentration between the levels of UCH-L1 in the serum and CSF over a 7-day period. This result is of significant clinical importance as peripheral blood sample would replace the need for invasive methods to obtain a CSF sample in order to determine the presence and the time elapsed following brain injury.[107] Table 1 summarizes the different levels of biomarkers and the time of sampling compared to controls.

CONCLUSION

STROKE IS THE second leading cause of death and the third leading cause of disability worldwide. Ischemic

stroke management is still challenging for physicians, particularly in predicting the elapsed time and selecting the appropriate candidates for treatment without causing reperfusion injury. Fast assessment and management are crucial in predicting patient outcome. Recombinant tissue plasminogen activator is the only FDA approved chemical treatment for ischemic stroke, with a narrow therapeutic window of 3.5 h, and mechanical thrombectomy proved to be useful up to 6–24 h. A wealth of research and studies have been undertaken to come up with an objective diagnostic method that would help physicians to apply the best management especially if the timeframe of the onset is unknown. Certain inflammatory, neuroglial, and miRNA markers in blood have been identified as potential and promising diagnostic tools for ischemic stroke. However, further studies are needed as some of this promising biomarker research is still at an early stage. It might be of benefit if future trials come up with a battery of these potential diagnostic markers, like a custom ELISA kit, capable of predicting the time of stroke onset and selecting the appropriate candidates for treatment.

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