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UPDATE ON THE RESEARCH OF SERUM BIOMARKERS TO ASSESS STROKE

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CONTENTS

SUMMARY

Serum biomarkers are becoming increasingly important in the assessment of patients with ischemic stroke. In this paper we perform an updated review of serum biomarkers in the assessment of ischemic stroke, with special interest on molecular markers associated with vascular risk, both in primary and secondary prevention, stroke diagnosis and the presence of neurological deterioration, infarct volume, hemorrhagic transformation, arterial recanalization and other prognostic variables. We also review the role of molecular markers during the acute phase of stroke. The knowledge of these serum biomarkers is important to improve functional outcome and to develop new therapeutic strategies in patients with ischemic stroke.

Key words: Ischemic stroke – Biomarkers – Outcome – Diagnosis

INTRODUCTION

Ischemic stroke is one of the leading causes of death and the leading cause of disability in adults in industrialized countries. Despite all the advances made in recent years, the only effective treatment in reducing the disability in stroke patients is thrombolytic therapy (1); however, it can only be administered in a small percentage of patients. This means that ischemic stroke represents a great problem in public health, which requires establishing and developing better strategies for its prevention and treatment in order to minimize the consequences and reduce its incidence.

In seeking factors that help to improve the stroke outcome during the last years, molecular markers are becoming increasingly important (2). On one hand, some molecular markers are useful to evaluate the risk of stroke and other vascular events, helping in primary and secondary stroke prevention. On the other hand, knowledge of the biochemical mechanisms of neurotoxicity and inflammation associated with cerebral ischemia helps in the identification of the best therapeutic options for each patient (3, 4).

In this article we review molecular markers in cerebral ischemia, both those related with the risk of stroke and other vascular events, and those that help in stroke diagnosis and treatment or to predict stroke evolution. The role of serum biomarkers to assess stroke is described in Table I.

SERUM BIOMARKERS AND RISK OF STROKE

Besides traditional risk factors for ischemic stroke, such as high levels of glucose or low-density lipoprotein (LDL) cholesterol, inflammatory markers are becoming increasingly of interest in assessing the risk of stroke and other vascular events. In fact, atherosclerosis is responsible for a large proportion of strokes (either directly by large artery disease or indirectly by cardioembolism, as a result of cardiac arrhythmias caused by heart disease or emboli after myocardial infarct), and is considered to be an inflammatory disease (5).

Epidemiological studies show that leukocyte count has been associated with the risk of first ischemic stroke (6), independent of other vascular factors, and has also been related to a higher risk of recurrent ischemic events in a high-risk population (7). High fibrinogen levels, another acute-phase reactant, have also been associated with a higher risk of stroke (8), and the high levels maintained following stroke increase the risk of recurrence (9). C-reactive protein (CRP) is an indicator of underlying systemic inflammation and may contribute to the development of atherosclerotic lesions and subsequent vascular events. Some studies show that high CRP levels predict a risk for stroke and myocardial infarct, with a twofold increased risk in men and an almost threefold increased risk in women (10, 11). Soluble CD40 ligand plays an important role in platelet activation

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Table I. *Role of serum biomarkers to assess stroke.*

Usefulness	Serum biomarkers
Risk of stroke	• Glucose • LDL cholesterol • Inflammatory markers (leukocytes, fibrinogen, CD40 ligand, metalloproteinases) • Lipoprotein-associated phospolipase A2
Diagnosis of ischemic stroke	• Fatty acid-binding protein • Neuronal specific enolase • Adhesion molecules (VCAM-1) • Protein S100-B • von Willebrand factor • B-type neurotrophic growth factor • Monocyte chemotactic protein
Diagnosis of cardioembolic stroke	• Brain natriuretic peptide • D-dimer
Diagnosis of athero-thrombotic stroke	• Haptoglobin • Serum amyloid A
Diagnosis of tissue at risk	• High levels of: IL-10 TNF-α Glutamate • Low levels of: Neuronal specific enolase IL-6 Matrix metalloproteinase-9
Infarct volume	• Glutamate • IL-6 • TNF-α • Toll-like receptors • Protein S100-B • Matrix metalloproteinase-9, -13 • Neurofilament
Neurological deterioration	• Glutamate • Ferritin • Nitric oxide • Inflammation (IL-6, TNF-α, adhesion molecules [VCAM-1, ICAM-1])
Cerebral edema	• Endothelin-1 • Matrix metalloproteinase-9 • Cellular fibronectin
Hemorrhagic transformation	• Matrix metalloproteinases • Cellular fibronectin • Plasminogen activator inhibitor 1 • Thombin-activable fibrinolysis inhibitor • C-reactive protein
Arterial recanalization	• Plasminogen activator inhibitor 1 • Alpha-2-antiplasmin • Thombin-activable fibrinolysis inhibitor
Stroke outcome	• Glutamate oxaloacetate transaminase • Toll-like receptors • Growth/differentiation factor 15 • Neuroserpin • Endothelial progenitor cells • Neurofilament
Stroke recurrence	• Inflammatory markers • Copeptin

and has inflammatory and prothrombotic properties. This molecule has proven to be a predictor of the risk of stroke and myocardial infarct in patients with non-valvular atrial fibrillation, with an increased relative risk of 20% (12).

Other inflammatory molecules have recently been associated with a higher risk of stroke, such as metalloproteinases (MMPs) and their tissue inhibitors (TIMPs). It has been shown that higher levels of MMP-9 and TIMP-1 increase the risk of vascular factors (13, 14) and the risk of death in patients with known cardiovascular disease (15, 16). In particular, higher levels of TIMP-1 are associated with an increased risk of stroke and vascular mortality (hazard ratio [HR] per standard deviation: 1.18 [1.04-1.35] and 1.22; 95% confidence interval [CI]: 1.09-1.37) (17).

Besides inflammatory markers, other molecules have been associated with stroke risk. High levels of lipoprotein-associated phospholipase A2 (Lp-PLA2), a serine lipase produced in macrophage-rich atherosclerotic lesions, have been associated with a higher risk of stroke and coronary heart disease (18). The risk of stroke is even higher when Lp-PLA2 and CRP levels are combined, with an 11-fold higher risk for ischemic stroke in patients with Lp-PLA2 levels ≥ 422 μg/L and CRP levels > 3 mg/L (19). Asymmetric dimethylarginine (ADMA) has also been shown to correlate with stroke risk. ADMA is a potent inhibitor of nitric oxide synthase (NOS), which mediates widespread endothelial dysfunction. It has been found that higher ADMA levels were associated with a sixfold risk of stroke in the elderly population (20).

SERUM BIOMARKERS IN ACUTE PHASE OF STROKE

During the acute phase of cerebral ischemia, an energetic failure occurs, leading to an intense depolarization of neuronal membranes, which release excitatory amino acids, such as glutamate (21). Glutamate stimulates several receptors, mainly AMPA and NMDA. The activation of AMPA receptors produces a greater depolarization by increasing intracellular sodium entry, being responsible for swelling and voltage-dependent channel opening. The activation of NMDA receptors increases the intracellular calcium concentration, which initiates the ischemic cascade, leading to cell death (Fig. 1). This glutamate excitotoxicity cascade is mediated by nitric oxide (NO) formation via neuronal nitric oxide synthase (nNOS), which produces immediate neuronal injury.

In addition to glutamate, other neurotransmitters are released in extracellular tissue after ischemia, such as glycine and γ-aminobutyric acid (GABA). Glycine is a co-activator of the NMDA receptor, and its release increases receptor activation, and therefore neuronal damage (22). GABA, however, exerts an inhibitory action. The inhibition of GABA release during and after ischemia may contribute to an overstimulation of glutamate vulnerable neurons, enhancing neuronal death.

Brain ischemia and subsequent reperfusion induces an inflammatory response. This response is initiated in microvasculature and contributes to cell destruction (23-25). The inflammatory response is initiated by the release of cytokines, mainly by endothelial cells, but also by neurons, astrocytes and microglia (26, 27). The cytokines responsible for initiating the inflammatory response are interleukin-1 beta (IL-1β) and TNF-α (27). This activation occurs very early,

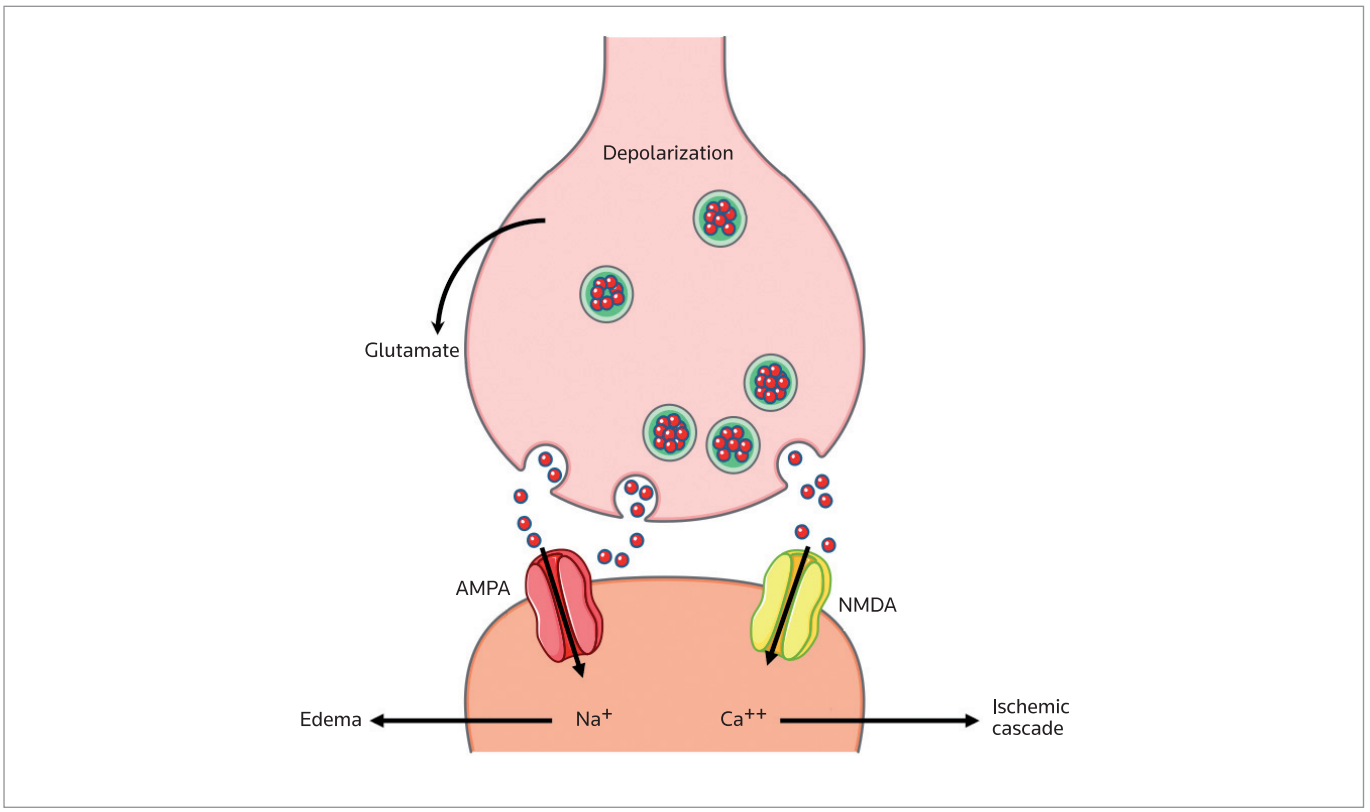

Figure 1. Excitotoxic effect of glutamate in brain ischemia. Glutamate is released as an effect of depolarization and activates AMPA receptors, being responsible for intracellular edema and NMDA receptors, initiating the ischemic cascade.

although it is transient. Subsequently, these cytokines induce a second inflammatory response, much more persistent, mediated by IL-6 and IL-8. These cytokines play an important role in the development of acute-phase reactants, including fever, CRP and fibrinogen (28), and the release of cell adhesion molecules, which cause the aggregation of leukocytes and subsequent adhesion to the vascular wall.

There are three groups of cell adhesion molecules: selectins, the immunoglobulin superfamily and integrins (29). Selectins contribute to the initial interaction between leukocytes and endothelial cells in the periphery of the infarct. The main molecules of the immunoglobulin superfamily are the intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion protein 1 (VCAM-1). Integrins are also involved in intracellular adhesion, as in the interaction of these cells with extracellular matrix elements, although the action takes place later (30). As a result of the activation of cell adhesion molecules, leukocyte recruitment and subsequent aggregation and adhesion to the vascular wall occur (Fig. 2).

The role of MMP-2 and MMP-9 in the tissue damage that occurs after ischemia has also been demonstrated (31). These molecules are responsible for the blood–brain barrier disruption, which leads to the development of vasogenic edema and facilitates hemorrhagic transformation (32). The presence of several cytokines, including IL-6 and TNF-α, stimulates the production of MMPs, especially MMP-9. Once leukocytes have been adhered to the vascular wall by a cytokine interaction, they use the production of MMPs to migrate through the endothelium and disrupt the blood–brain barrier, contributing to edema formation.

Although these molecular markers are increased during the acute phase of stroke, their expression may vary depending on certain situations. It has been found that during the acute phase of stroke, those patients without a previous history of hypertension who develop high blood pressure levels during the acute phase of stroke showed higher levels of IL-6, TNF-α, ICAM-1, VCAM-1 and MMP-9 compared to normotensive or chronic hypertensive patients, and this is associated with poor outcome (33).

SERUM BIOMARKERS WITH DIAGNOSTIC VALUE

Stroke diagnosis is based on clinical examination by experienced clinicians, supplemented by neuroimaging techniques, such as computed tomography (CT) and magnetic resonance imaging (MRI); however, there is increasing interest in molecular markers to improve stroke diagnosis. This is more evident in the etiological diagnosis of stroke subtypes, because in approximately one-third of cases stroke etiology remains undetermined, even with a complete complementary study. For all these reasons, molecular markers may be useful to clarify stroke origin and to establish a more appropriate secondary prevention.

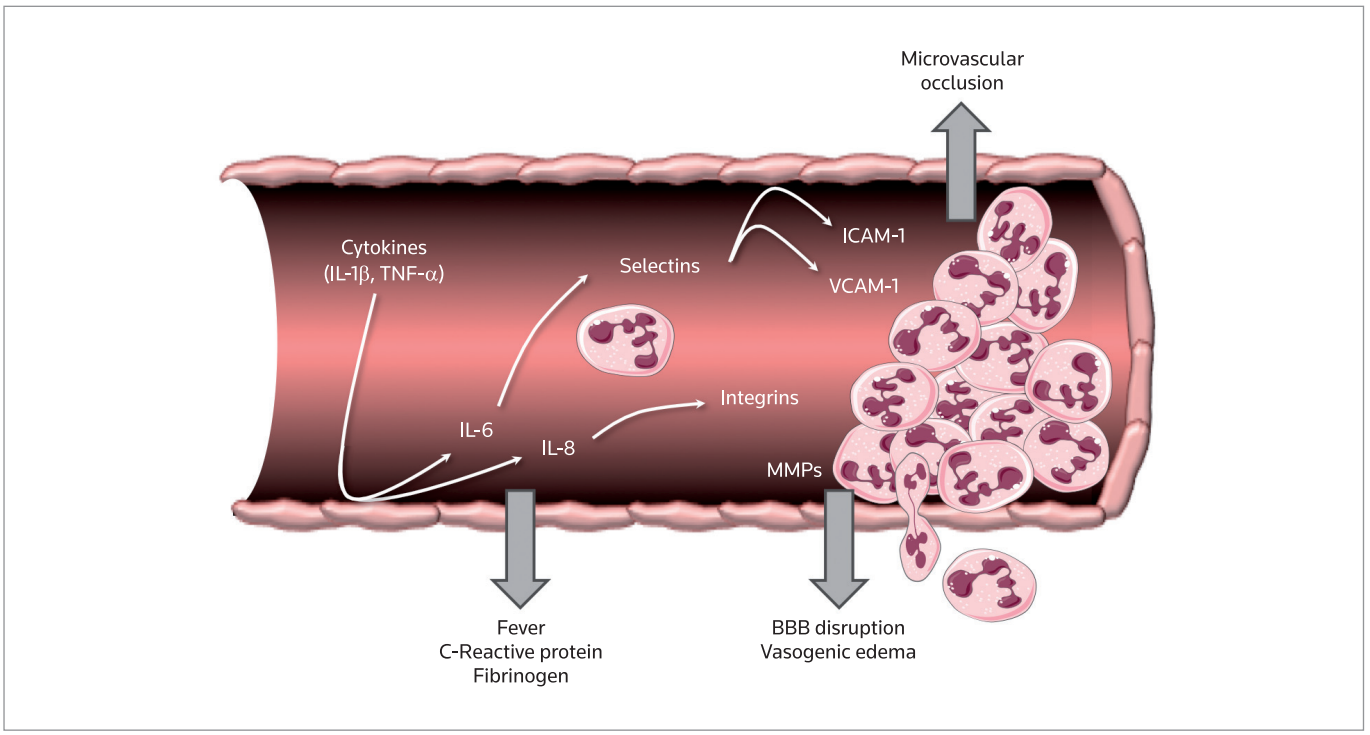

Figure 2. Inflammatory response in brain ischemia. The first response is initiated by interleukin-1 beta (IL-1β) and TNF-α. The second response is mediated by IL-6 and IL-8, responsible for acute-phase reactants and the activation of selectins and integrins, which promote cell adhesion and the release of intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1). Finally, matrix metalloproteinases (MMPs) disrupt the blood–brain barrier (BBB), causing vascular edema.

Serum biomarkers in the diagnosis of stroke

There have been many attempts to find a molecular marker to identify ischemic stroke; however, the development of serum biomarkers for ischemic stroke has several difficulties. The presence of blood–brain barriers makes the movement of brain molecules into the blood after ischemic stroke difficult, and many markers of brain ischemia are present in other conditions that mimic stroke.

Fatty acid-binding protein (FABP) (34) and neuronal-specific enolase (NSE) (34, 35) have been investigated as possible markers of stroke, and although theses biomarkers seem promising, the results should be taken with caution due to the small cohort of patients and methodological limitations of the studies.

Other studies have demonstrated the utility of the combination of several biomarkers in the diagnosis of ischemic stroke. In one study, five molecular markers (MMP-9, protein S100-B, von Willebrand factor, B-type neurotrophic growth factor [BNGF], and monocyte chemotactic protein [MCP]) were determined within the first 9 hours from stroke onset (36). The presence of three or more molecular marker levels above their respective thresholds was associated with the diagnosis of ischemic stroke, with 93% sensitivity and 93% sensibility. Another study evaluated 26 biomarkers involved in the acute phase of stroke determined within the first 24 hours from stroke onset (37). Among all of the biomarkers, MMP-9, VCAM-1, protein S100-B and von Willebrand factor were highly correlated with the diagnosis of ischemic stroke.

Serum biomarkers as tissue at-risk identifiers

Thrombolytic therapy with recombinant tissue-type plasminogen activator (rtPA) remains the only pharmacological treatment that has been demonstrated to be effective in acute ischemic stroke; however, its administration is limited only to a small percentage of patients, due moreover to the short therapeutic window. Neuroimaging has been considered the most appropriate technique to increase the therapeutic window for rtPA treatment. The perfusion–diffusion mismatch has been proposed as a tool to select patients with acute stroke who are more likely to benefit from reperfusion therapy, allowing a wider therapeutic window of 6 hours (38, 39). However, later studies suggest that caution is required for using these techniques, since perfusion–diffusion mismatch does not accurately predict lesion growth (40). Clinical–diffusion mismatch has been proposed as an easier alternative to perfusion–diffusion mismatch for selecting patients with salvageable ischemic tissue (41), based on the assumption that patients with severe clinical deficits, but with relatively small lesions on diffusion-weighted images, are likely to have ischemic penumbra.

A recent study has found a set of biomarkers that can accurately predict the presence of clinical–diffusion mismatch and consequently the presence of salvageable brain tissue (42). High levels of IL-10, TNF-α and glutamate, as well as low levels of NSE, IL-6 and MMP-9, have been associated with the presence of clinical–diffusion mismatch. The odds ratio for molecular markers to predict the presence

of clinical–diffusion mismatch are the following: IL-10 levels ≥ 23 ng/dL (odds ratio [OR]: 224; 95% CI: 20-1855), TNF-α ≥ 21 pg/mL (OR: 95; 95% CI: 11-754), glutamate ≥ 230 μmol/mL (OR: 55; 95% CI: 20-151), NSE ≥ 23 ng/mL (OR: 0.04; 95% CI: 0.02-0.11), IL-6 ≥ 10 pg/mL (OR: 0.05; 95% CI: 0.01-0.12) and MMP-9 ≥ 21 ng/mL (OR: 0.16; 95% CI: 0.03-0.42). This suggests that the tissue at risk, and potentially salvageable, can be identified by molecular markers of excitotoxic (higher glutamate levels) but antiinflammatory environment (higher IL-10 and lower IL-6 levels), with reduced neuronal damage (lower NSE levels) and blood–brain barrier disruption (lower MMP-9 levels).

Other molecules have been evaluated in animal models as markers of ischemic penumbra (43, 44), and even certain molecules such as glucose, heat shock proteins, hypoxia-inducible factor 1-alpha (HIF-1-alpha) or prostacyclin synthase are helpful in the differentiation between the penumbra and infarcted area in experimental models, although in clinical practice none of them has been useful to identify brain tissue at risk.

Serum biomarkers and etiological diagnosis of stroke

Ischemic stroke is an etiologically heterogeneous disease, and it is essential to make a correct etiological diagnosis of stroke, since prognosis, acute and long-term management to prevent recurrences varies considerably, depending on stroke subtype. Unfortunately, despite a complete diagnostic protocol, one-third of ischemic strokes remain undetermined because no potential etiological mechanism is detected. It is important, therefore, to find a marker that enhances the etiological diagnosis.

Some molecular markers have been evaluated to distinguish between cardioembolic and atherothrombotic stroke. In this regard, higher levels of brain natriuretic peptide (BNP; OR: 2.3; 95% CI: 1.4-3.7) and D-dimer (OR: 2.2; 95% CI: 1.4-3.7) were found in patients with cardioembolic stroke (45), and higher levels of haptoglobin and serum amyloid A in patients with atherothrombotic stroke (46). In addition, higher levels of BNP have been useful in the reclassification of undetermined stroke to possible cardioembolic stroke, finding that pro-BNP levels ≥ 360 mg/mL were independently associated with the possibility of a cardioembolic cause of stroke (OR: 35.8; 95% CI: 5.68-225.16) (47).

SERUM BIOMARKERS WITH PROGNOSTIC VALUE

Several studies have demonstrated the utility of serum biomarkers to predict the evolution of ischemic stroke. Many molecular markers have been implicated in the presence of neurological deterioration, infarct volume, risk of hemorrhagic transformation, arterial recanalization and other factors related with stroke outcome.

Serum biomarkers and neurological deterioration

In 25-40% of patients with ischemic stroke, neurological symptoms progress during the initial hours, which results in increased mortality and functional disability. One of the mechanisms responsible for this early neurological deterioration (END) is the transformation of the ischemic penumbra into necrotic tissue (23).

There are several biochemical mechanisms that have been implicated in the presence of END. Among these, excitotoxic amino acids, and in particular, high glutamate levels in plasma and cerebrospinal fluid (CSF), are the strongest biochemical predictors of progressing stroke. Glutamate plasma levels > 200 μmol/L predict with a 97% probability the risk of END in the acute phase of stroke (95% CI: 85-100) (48). In the subgroup of patients with lacunar infarct, plasma glutamate > 200 μmol/L and GABA < 240 nmol/L predicts neurological deterioration in 85% of cases (49).

Oxidative stress also plays an important role in the development of neurological deterioration. High plasma and CSF ferritin levels independently predict the presence of END in patients with acute hemispheric stroke (OR: 33.5; 95% CI: 4.7-235) (50). A possible explanation for the role of ferritin in the development of END may be the enhancement of excitotoxic and inflammatory mechanisms by ferritin, since a positive correlation between ferritin and glutamate and inflammatory levels has been found in clinical and experimental models (50, 51). NO has also been implicated in the development of END through the generation of free radicals. High levels of NO in CSF are independently associated with END in acute ischemic stroke, after adjustment of glutamate levels (52). Other molecules, such as L-arginine, also participate in the development of END mediated by NO. L-Arginine is the only known substrate for NO generation, and low levels of this molecule have been observed in those patients with END and poor outcome (53).

Inflammation has also demonstrated an important role in the presence of END. High levels of IL-6 both in CSF and plasma increase the risk of END and poor functional outcome in patients with acute ischemic stroke (OR: 37.7; 95% CI: 11.9-118.8) (24). In patients with lacunar infarcts, high levels of other inflammatory molecules, such as TNF-α and VCAM-1, have been associated with END, even after adjustment for glutamate and GABA levels (25). Cell adhesion molecules have also been implicated in stroke outcome. It has been found that higher levels of soluble ICAM-1 determined at admission predict the presence of neurological deterioration in patients with ischemic stroke (54). Moreover, the inhibition of E-selectin, ICAM-1 and VCAM-1 during the first days of ischemic stroke was associated with clinical improvement of patients, whereas those who did not improve showed no change in cell adhesion molecule levels (55).

Serum biomarkers and infarct volume

Infarct volume is one of the most important factors implicated in functional outcome in patients with ischemic stroke. As we have seen before, glutamate levels are the most powerful biomarker associated with clinical deterioration in stroke patients. The association of glutamate with stroke progression appears to be related with the increase in lesion volume. In fact, glutamate levels have been found to be the only independent predictor of ischemic lesion growth during the first 72 hours, after adjusting for other molecular markers (β = 0.21; standard deviation [SD] = 0.07; P = 0.004) (56).

Other molecules associated with infarct volume are related with inflammation. High levels of inflammatory molecules, such as IL-6 and TNF-α, and adhesion molecules, such as ICAM-1, are associated with a greater infarct volume (24, 57). In relation to inflammation, other immune markers predict the development of poor outcome and increased infarct volumes. Toll-like receptors (TLRs) are innate immunity receptors that activate inflammation and adaptive immunity. It has been shown that high levels of TLR-8 at admission

increase the risk of poor outcome in relation with higher infarct volume (58). Levels of this marker correlate with levels of other inflammatory markers, such as IL-6, TNF-α and IL-1β, suggesting a role in the pathophysiology of stroke through activation of inflammatory response.

Molecular markers related with neuronal and glial damage have recently been associated with final infarct volume. High levels of NSE and protein S100-B correlated with larger infarct volume in patients with acute ischemic stroke (59), which determines worse functional outcome (60). This association between protein S100-B and infarct volume is larger when this molecular marker is determined after 24 hours rather than at admission.

Finally, endothelial damage may also play an important role in the increase of infarct lesions. MMP-9 and MMP-13 have been related to infarct volume, finding a correlation between levels of these molecules and infarct growth determined by diffusion-weighted MRI (61).

Serum biomarkers and hemorrhagic transformation

Hemorrhagic transformation (HT) is a complication with significant clinical relevance in ischemic stroke, especially in those who receive thrombolytic therapy. Infarct volume, high blood pressure, anticoagulant treatment, old age, the presence of early ischemic signs on neuroimaging and high glucose levels increase the risk of HT (62).

One of the most important factors associated with HT is the loss of integrity of basal lamina, to which MMPs contribute (63). Clinical studies have demonstrated the association of high levels of MMP-9 and the risk of HT (OR: 12; 95% CI: 3-51), observing concentrations three times higher in those patients who presented HT (32). Higher levels of MMP-9 have also been found in those patients with HT who received thrombolytic therapy (64, 65). Fibronectins are adhesive glycoproteins synthesized by endothelial cells that contribute to the integrity of basal lamina. High plasma levels of cellular fibronectin (c-Fn) might be indicative of endothelial damage and predict hemorrhagic transformation in patients who receive thrombolytic therapy (OR: 2.1; 95% CI: 1.3-3.4) (65, 66). Albuminuria is a marker of chronic endothelial damage and is a good marker of hemorrhagic transformation in patients with acute ischemic stroke, especially to predict most severe bleedings (67).

Endogenous fibrinolytic inhibitors have also been reported to increase the risk of HT in patients treated with rtPA. Plasma plasminogen activator inhibitor 1 (PAI-1) levels are lower and thrombin-activable fibrinolysis inhibitor (TAFI) levels higher in patients with symptomatic HT after thrombolytic therapy. The combination of PAI-1 levels > 180% and TAFI levels < 21.4 ng/mL predicts the development of HT after rtPA with a sensitivity of 75% and a specificity of 97.6% (68).

Other molecular markers show a higher risk of HT after thrombolytic therapy. High levels of protein S100-B before thrombolytic therapy (69) and endogenous activated protein C (70) after rtPA administration have been associated with a higher risk of HT.

Serum biomarkers and brain edema

The development of brain edema is the major cause of death in patients with large ischemic strokes. As in the case of HT, the loss of integrity in basal lamina is one of the most important factors implicated in brain edema. In this regard, high levels MMP-9 and c-Fn have demonstrated utility in the prediction of malignant infarct, especially c-Fn (71). c-Fn levels > 16.6 µg/mL at admission are associated with the development of malignant infarction with high sensitivity (90%) and specificity (100%).

Endothelin-1 (ET-1) has been associated with water accumulation and brain edema in experimental models, suggesting that it may be a biomarker of blood–brain barrier disruption (72). In clinical studies, the utility of ET-1 to predict brain edema has also been demonstrated. ET-1 levels > 5.5 fmol/mL determined before rtPA administration are associated with severe brain edema in acute stroke patients who receive thrombolytic therapy (OR: 139.7; 95% CI: 19.3-1012.2) (73).

Serum biomarkers and arterial recanalization

The rates of recanalization after thrombolytic therapy are low, and recanalization is only achieved in less than half of patients. The activity of the fibrinolytic and coagulation system at the time of the arterial occlusion appears to play an important role in the dissolution of the clot when rtPA is administered, but there are other molecular markers that have been associated with arterial recanalization after thrombolytic therapy.

Levels of PAI-1, a marker of fibrinolysis, are significantly lower in patients who had recanalization after rtPA administration, and PAI-1 levels > 34 ng/mL were present in those with resistance to thrombolysis (74). Other markers of coagulation have been associated with arterial recanalization after thrombolytic therapy. Reduced levels of alpha-2-antiplasmin and TAFI at admission predict arterial recanalization with 25% sensitivity and 85% specificity (75).

Besides makers of coagulation, a recent study found that growth factors also predict recanalization after rtPA treatment (76). Patients with early recanalization showed higher levels of vascular endothelial growth factor (VEGF), granulocyte colony-stimulating factor (G-CSF) and angiopoietin-1 before rtPA treatment.

Other serum biomarkers associated with stroke outcome

There are other molecular markers that have been associated with functional outcome in patients with ischemic stroke, which may develop approaches to new therapeutic targets in acute ischemic stroke.

As we have seen before, higher glutamate levels are the most powerful biomarker of neurological deterioration and predict poor outcome. Glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) are two enzymes that are able to metabolize blood glutamate, facilitating the lowering of extracellular levels of brain glutamate. Recently, an association between high blood GOT and GPT levels and good outcome in patients with ischemic stroke has been found (77, 78). High levels of GOT and GPT were associated with lower levels of glutamate and smaller infarct volume.

Molecular markers that modulate the inflammatory response play an important role in functional outcome after stroke. Growth/differentiation factor 15 (GDF-15) is a stress-responsive cytokine that is induced after brain injury. In patients with ischemic stroke, higher levels of GDF-15 have been associated with poor functional outcome

in relation to higher inflammatory response (79). Neuroserpin is a brain-derived natural inhibitor of tPA. In patients with acute ischemic stroke, the decrease of neuroserpin levels during the first 24 hours has been associated with better functional outcome (80). The mechanism that explains this action is a decrease in the excitotoxic and inflammatory response during brain ischemia, because the decrease in neuroserpin levels is associated with a decrease in glutamate, IL-6, ICAM-1 and MMP-9 levels (81).

Novel molecules have also been involved in stroke outcome. High levels of copeptin, a fragment of pro-vasopressin, on admission are associated with poor functional outcome (combined areas under the curve, 0.79; 95% CI: 0.74-0.84; $P < 0.01$) and mortality (combined areas under the curve, 0.89; 95% CI: 0.84-0.94; $P < 0.01$) in patients with acute ischemic stroke (82). Phosphorylated neurofilament heavy protein (pNfH) is a molecule that reflects axonal damage. Serum pNfH levels are increased during the acute phase of stroke, and higher levels are significantly associated with stroke severity, infarct volume and outcome (83).

Finally, progenitor cells and growth factors have also been associated with functional outcome after stroke. The increase in circulating endothelial progenitor cells (EPCs) during the first week after ischemic stroke has been reported as a marker of good outcome and lower infarct volume (84), suggesting that EPCs might participate in neurorepair processes after ischemic stroke. In this regard, some growth factors, such as erythropoietin, are able to increase EPCs after ischemic stroke and improve outcome in those patients (85), which may constitute a new therapeutic target in the treatment of ischemic stroke.

SERUM BIOMARKERS AND RISK OF STROKE RECURRENCE

Recurrence of vascular disease events occurs more often during the first months post-stroke. The frequency of vascular recurrence ranges between 1.2% and 25%, depending on the stroke subtype, the population studied and the length of follow-up. The identification of factors that are associated with an increased risk of vascular disease recurrence would enable patients to be stratified to identify the high-risk subgroups that would most benefit from more active diagnostic and therapeutic interventions.

Inflammation biomarkers have demonstrated utility in the prediction of vascular recurrence after ischemic stroke. A study was specifically designed to evaluate the prognostic value of molecular markers of inflammation in relation to the risk of recurrence of vascular events in non-anticoagulated patients with ischemic stroke (86). Several inflammatory markers (CRP, IL-6, IL-10, ICAM-1, VCAM-1, MMP-9 and fibronectin) were obtained at baseline to predict vascular recurrence during 1 year of follow-up. IL-6 levels > 5 pg/dL and VCAM-1 levels > 1350 ng/dL increased by 20-fold and 3-fold, respectively, the risk of new vascular events or death of vascular origin.

Other molecular markers have been previously analyzed, but a modest association with vascular recurrence has been found. Levels of adiponectin (OR: 0.84: 95% CI: 0.7-1.01) (87), D-dimer (OR: 1.7; 95% CI: 1.3-2.2) (88), CRP (OR 1.7; 95% CI: 1.6-2.0) (89) and fibrinogen (OR: 1.8; 95% CI: 1.6-2.0) (89) have been slightly associated with vascular recurrence.

Stress hormones have recently been implicated in stroke recurrence. High copeptin levels increase the risk of cerebrovascular recurrence in patients with transient ischemic attack (90).

CURRENT LIMITATIONS OF SERUM BIOMARKERS

Molecular markers attempt to enhance functional outcome for stroke patients, improving the diagnosis and helping to select patients for specific treatments. However, their use in clinical practice has some limitations.

Although some pathophysiological mechanisms of cerebral ischemia are known, the use of these markers in ischemic stroke diagnosis is limited. On the one hand, the presence of the blood–brain barrier limits the transit of molecules from the brain to the peripheral blood, which can yield decreased serum concentrations of these markers. In addition, stroke is a heterogeneous disease, and there are different etiological subtypes that determine the release of various molecules. Another limitation is that many molecular markers of cerebral ischemia are present in other conditions that mimic stroke.

Several studies have attempted to find a molecular marker to diagnose ischemic stroke; however, they have several methodological problems in design and have been conducted in a small cohort of patients, which makes these results inconclusive. Although the combination of several molecular markers to diagnose stroke is promising, until now, any molecular marker has shown superiority to neuroimaging in the diagnosis of ischemic stroke.

FUTURE CHALLENGES IN SERUM BIOMARKERS

In seeking molecular markers for stroke there are still many unresolved issues. It would be useful that molecular markers could answer the question of whether a patient has suffered a stroke and could distinguish it from other stroke mimics, such as migraine or seizures, especially in the case of transient ischemic attack, in which the diagnosis may be more complicated. It would also be useful to find markers that could differentiate between ischemic stroke and intracerebral hemorrhage, and particularly that distinguish between different etiological subtypes to do an appropriate secondary prevention. Although many markers are promising for answering these questions, we still need more studies to confirm these findings and allow us to use these markers in clinical practice.

Another important area in the development of molecular markers is stroke outcome. Although several molecules are known that can predict the development of edema, hemorrhagic transformation or neurological deterioration, there are no therapeutic tools that can be used to limit these complications so far, which limits the usefulness of these molecules. It would also be important to develop neuroprotective drugs that limit the brain damage caused by ischemia, but none are yet available.

Finally, a novel field in the development of molecular markers is neurorepair after stroke. The knowledge of the mechanisms implicated in neuronal repair after brain ischemia would lead to the development of new therapeutic tools to improve the functional outcome of patients with ischemic stroke.

CONCLUSIONS

Serum biomarkers are becoming increasingly important in the assessment of patients with ischemic stroke. Some molecular markers are useful for evaluating vascular risk in both primary and secondary prevention. Other biomarkers have proven useful during the acute phase of stroke by helping to better diagnose or to assess the risk of complications in order to prevent them and improve functional outcome. Finally, the use of molecular markers is useful for finding and developing new therapeutic strategies to improve the care of patients with ischemic stroke.

DISCLOSURES

The authors state no conflicts of interest.

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