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General review

Blood biomarkers in the early stage of cerebral ischemia

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ABSTRACT

In ischemic stroke patients, blood-based biomarkers may be applied for the diagnosis of ischemic origin and subtype, prediction of outcomes and targeted treatment in selected patients. Knowledge of the pathophysiology of cerebral ischemia has led to the evaluation of proteins, neurotransmitters, nucleic acids and lipids as potential biomarkers. The present report focuses on the role of blood-based biomarkers in the early stage of ischemic stroke—within 72 h of its onset—as gleaned from studies published in English in such patients. Despite growing interest in their potential role in clinical practice, the application of biomarkers for the management of cerebral ischemia is not currently recommended by guidelines. However, there are some promising clinical biomarkers, as well as the N-methyl-D-aspartate (NMDA) peptide and NMDA-receptor (R) autoantibodies that appear to identify the ischemic nature of stroke, and the glial fibrillary acidic protein (GFAP) that might be able to discriminate between acute ischemic and hemorrhagic strokes. Moreover, genomics and proteomics allow the characterization of differences in gene expression, and protein and metabolite production, in ischemic stroke patients compared with controls and, thus, may help to identify novel markers with sufficient sensitivity and specificity. Additional studies to validate promising biomarkers and to identify novel biomarkers are needed.

1. Introduction

The US National Institutes of Health Biomarkers Definitions Working Group defined a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" [1]. Biomarkers may have many valuable applications as:

- diagnostic tools for identification of patients with abnormal conditions;
- tools for staging diseases or classifying their extent;
- indicators of prognosis;
- and predictors of response to an intervention [1].

According to the above definition, a biomarker can be an indicator measured by clinical or imaging evaluation, or by biological tests using body fluids [2]. Knowledge of the pathophysiology of cerebral ischemia has led to the evaluation of proteins, neurotransmitters, nucleic acids and lipids as potential biomarkers [2,3]. In stroke patients, blood-based biomarkers could be of interest for the diagnosis of ischemic origin and subtype, prediction of

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outcomes and targeting of treatments for selected patients [2–5].

The present review focuses on the role of blood-based biomarkers in patients in the early stage of ischemic stroke (IS), as defined by samples collected within 72 h of stroke onset.

2. Pathophysiology of ischemic stroke

When vascular occlusion occurs, the central region of the ischemic brain territory, the 'ischemic core', is characterized by total flow interruption. Tissue is irreversibly damaged because of adenosine triphosphate (ATP) depletion and aerobic glycolysis failure, which evolves into tissue death within a few minutes. Surrounding the core, the region called the 'ischemic penumbra' is characterized by a cerebral blood flow drop to below functional thresholds. The penumbra is functionally impaired, but still viable [6,7], as neurons and other brain cells in this area can survive longer due to a metabolic change to anaerobic status. However, if cerebral blood flow is not restored, the tissue will die. Ionic, biochemical and cellular events overlap in a tissue already compromised [7] and occur in a time-dependent manner [8].

The first response after cerebral ischemia is neuronal excitotoxicity and calcium overload within minutes of stroke onset [7]. ATP depletion induces ion pump failure, which causes a release of glutamate into the extracellular compartment. The consequential overactivation of glutamate receptors increases the intracellular influx of calcium, sodium and water, and activates cellular catabolic processes. Moreover, calcium overload activates calcium-dependent enzymes and leads to production of nitric oxide, arachidonic acid metabolites and superoxide. Oxidative and nitrosative stress is also a mediator of ischemic injury.

The secondary or delayed response to ischemia is characterized by an inflammatory response within hours [7]. Cerebral blood vessels, the first to experience the ischemic insult, trigger the inflammatory response. Vascular cells and perivascular microglia macrophages release cytokines: interleukin (IL)-1β and tumor necrosis factor (TNF)-α start the inflammatory response and induce activation of IL-6 and IL-8, responsible for the more long-lasting inflammatory response. These latter cytokines mediate the development of acute-phase reactions [fever, C-reactive protein (CRP) and fibrinogen] and upregulate three types of cell adhesion molecules:

- selectins (such as P- and E-selectin), which aid the interaction between leukocytes and endothelial cells in the periphery of the infarct;
- the immunoglobulin superfamily [such as intercellular adhesion molecule type 1 (ICAM-1) and vascular adhesion molecule type 1 (VCAM-1)];
- and integrins, which promote leukocyte migration to the vascular surface [9].

The first blood-borne cells to be recruited in the brain are neutrophils, followed by monocytes and, within the first 48 h, lymphocytes. Proinflammatory cytokines, mainly IL-6 and TNF-α, stimulate matrix metalloproteinase (MMP) production, especially MMP-9. MMPs induce blood–brain barrier (BBB) disruption, and mediate the migration of leukocytes, which adhere to vessel walls, through endothelium. Edema and hemorrhagic transformation (HT) may arise as a consequence of BBB disruption [9]. Blood-borne and microglia-derived macrophages accumulate in the border zone to clear away debris and dead cells, and produce proinflammatory mediators and toxic molecules [7]. Calcium overload and oxidative stress lead to cell death mediated by apoptosis or necrosis, which can be maintained for hours or even days after stroke onset [7].

According to the pathophysiology of ischemia, serum biomarkers are classified into markers related to excitotoxicity, astrocyte activation, endothelial dysfunction, inflammation, neuroplasticity or neurotrophicity, fibrinolysis, metabolism, oxidative stress and other mechanisms. The characteristics of blood biomarkers, their pathophysiological classifications and relation to brain ischemia are detailed in Table 1.

3. Blood biomarkers for ischemic origin of stroke

In the presence of a sudden focal neurological deficit, biomarkers could contribute by helping to distinguish brain ischemia from hemorrhagic stroke and other neurological events that mimic stroke. In an in-hospital setting, IS can easily be differentiated from intracerebral hemorrhage (ICH) by computed tomography (CT) and magnetic resonance imaging (MRI) scans. In a prehospital setting in the absence of ambulances with mobile CT scanners [10], there is no possibility of differentiating ischemia and hemorrhage [11], leading to delay that can add to the emergency situation.

Few individual biomarkers show enough sensitivity and specificity to identify IS on their own. Nevertheless, the most promising are autoantibodies (aAbs) to the N-methyl-D-aspartate receptor (NMDA-R), particularly the NR2A/2B subunits, which may be able on their own to distinguish IS from controls in the early acute phase and rule out ICH within the first 72 h [12]. Not only aAbs directed against the receptor could be a useful biomarker, but the NMDA-NR2 antigen might be as well. Produced by proteolytic degradation of the NMDA-R, this antigen might also be able to distinguish IS from stroke mimics and people with vascular risk factors from healthy controls (HC) with good accuracy [13]. Serum glial fibrillary acidic protein (GFAP) has also shown high diagnostic accuracy for differentiating ICH and IS, with no significant heterogeneity between two pooled meta-analyses [11,14] and a review [15] based on just a few studies of this topic [16–19]. The temporal pattern of GFAP release, measured at regular intervals during the first 24 h after stroke, appears to differ between patients with IS and ICH: in ICH, median serum GFAP levels are significantly higher at 2 h, peak at 6 h and 12 h, and decline after 24 h; in IS, GFAP levels are barely measurable before 24 h and only start to increase after 48 h [18]. The time windows of 3–4 h [4] and 1–6 h [11] after onset are best for using GFAP to differentiate between ICH and IS [18], respectively, although these are not compatible with clinical needs. In a small sample of IS patients, serum apolipoprotein C-III (ApoC-III) levels and, to a small extent, ApoC-I levels, were found to be relatively higher in IS compared with ICH

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