Neurobiological Markers of Post-Stroke Rehabilitation

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Stroke is a devastating neurological condition derived from the permanent or transient interruption of the blood flow

Ischemic stroke is a complex sequence of events that occur in the brain and that evolve over time and space.
The neurovascular unit is a modular concept defined at an intercellular level

Moskowitz, M A. et al., Neuron, 2010
Ischemic stroke enhances the interactions of brain endothelium with extravascular CNS cells (astrocytes, microglia, neurons), as well as intravascular cells (platelets, leukocytes), and that these interactions contribute to the injury process.
The cerebral vasculature assumes the following phenotypes:

1) poor capillary perfusion of brain tissue
2) pro-adhesive for circulating cells
3) pro-inflammatory
4) pro-thrombogenic
5) diminished endothelial barrier function
6) leukocytes and neutrophils accumulation in post-ischemic tissues prior to the onset of tissue injury
Microglia responses in cerebral ischemia range from induced neurotoxicity to neuroprotection and depend on the severity of ischemic stress, the damage signals released, the duration/timing of the insult, the microenvironment, and the interaction with other brain cells.

Early on, production of cytokines upregulates adhesion molecule expression (e.g., ICAM-1, P and E-selectin) and, along with integrins, promote leukocyte rolling and sticking to the vessel surfaces.

Patel A.R et al., Int J Physiol Pathophysiol Pharmacol. 2013
Soluble factors in stroke in acute and recovery phase: a possible source for biomarkers in stroke

Microglial cells and astrocytes can produce both proinflammatory cytokines and neuroprotective factors

Simats et al., BBA Molecular basis of diseases. 2016
Ideally, a biomarker should be:
• Rapidly measured
• Reproducible,
• Reliable,
• Accurate
• Using a method that can be applied across a range of diverse clinical settings.
• Should be present in body fluids

To date, >250 markers have been evaluated for the diagnosis of stroke, and several of these have been combined into biomarker panels.
Early biomarkers associated with a cause of stroke

Given the heterogeneity of patients, a single biomarker application may not be able sufficient

Glen C. Jickling and Frank R. Sharp *Biomarker Panels in Ischemic Stroke* *Stroke*. 2015;46:915-920
### Early biomarkers associated with a cause of stroke

<table>
<thead>
<tr>
<th>Duration</th>
<th>Biomarker Panel</th>
<th>Method</th>
<th>Proteins/Markers</th>
<th>Sensitivity/Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>6h from stroke</td>
<td>6 protein panel</td>
<td>ELISA</td>
<td>Caspase-3, Chimerin, D-dimer, MMP-9, Secretogolin, sRAGE</td>
<td>17%/98%</td>
</tr>
<tr>
<td>24h from stroke</td>
<td>5 protein panel</td>
<td>ELISA</td>
<td>BNGF, MCP-1, MMP-9, S100B, vWF</td>
<td>92%/93%</td>
</tr>
<tr>
<td></td>
<td>4 protein panel</td>
<td>ELISA</td>
<td>BNP, D-dimer, MMP-9, S100B</td>
<td>86%/37%</td>
</tr>
<tr>
<td></td>
<td>4 protein panel</td>
<td>ELISA</td>
<td>MMP-9, S100B, VCAM1, vWF</td>
<td>90%/90%</td>
</tr>
<tr>
<td></td>
<td>4 protein panel</td>
<td>Immunoassay</td>
<td>Eotaxin, EGFR, S100A12, Metalloproteinase inhibitor-4, Prolactin</td>
<td>73%/72%</td>
</tr>
<tr>
<td></td>
<td>5 protein panel</td>
<td>Immunoassay</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


Gandolfi M. et al., Hindawi Neural Plasticity 2017, Article ID 1389475,
### Table 1: List of the main assessed and emerging circulating biomarkers in stroke.

<table>
<thead>
<tr>
<th>Biomarker group</th>
<th>Molecule</th>
<th>Diagnostic or prognostic value</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Irin</strong></td>
<td>Irisin</td>
<td>Good prognostic marker of stroke recovery with training</td>
<td>[21, 22]</td>
</tr>
<tr>
<td><strong>Myokines</strong></td>
<td>Myostatin (GDF-8)</td>
<td>Muscle wasting</td>
<td></td>
</tr>
<tr>
<td><strong>PDE5</strong></td>
<td>PDE5</td>
<td>Good prognostic marker of stroke (muscular level)</td>
<td>[23-26]</td>
</tr>
<tr>
<td><strong>DPP4</strong></td>
<td>DPP4</td>
<td>Good prognostic marker of stroke (angiogenic level)</td>
<td>[27-30]</td>
</tr>
<tr>
<td><strong>Osteonectin (SPARC)</strong></td>
<td>FGF-21</td>
<td>Ameliorating stroke recovery</td>
<td>[31, 32]</td>
</tr>
<tr>
<td><strong>Brain-derived neurotrophic factor (BDNF)</strong></td>
<td>Improvement in stroke recovery</td>
<td>Bad prognosis stroke recovery</td>
<td>[33, 34]</td>
</tr>
<tr>
<td><strong>Neurotrophic factors</strong></td>
<td>Neurotrophin-3</td>
<td>Biomarkers of stroke onset</td>
<td></td>
</tr>
<tr>
<td><strong>Neurotrophin-4</strong></td>
<td>CrT</td>
<td>Biomarkers of stroke onset</td>
<td></td>
</tr>
<tr>
<td><strong>Brain-derived neurotrophic factor (BDNF)</strong></td>
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<td>Biomarkers of stroke onset</td>
<td></td>
</tr>
<tr>
<td><strong>Neuropeptides</strong></td>
<td>Neuropeptide Y</td>
<td>Good prognostic biomarker in certain SNP patterns</td>
<td>[35]</td>
</tr>
<tr>
<td><strong>PACAP</strong></td>
<td>Substance P</td>
<td>Bad prognosis in stroke progression</td>
<td>[36]</td>
</tr>
<tr>
<td><strong>Growth factors and GF-like molecules</strong></td>
<td>VEGF</td>
<td>Good prognosis in ischemic stroke progression (remodeling)</td>
<td>[37, 38]</td>
</tr>
<tr>
<td><strong>Cytokines</strong></td>
<td>Interleukin-6 (IL-6)</td>
<td>Stroke onset and progression</td>
<td>[39]</td>
</tr>
<tr>
<td><strong>Interleukin-33 (IL-33)</strong></td>
<td>Interleukin-15 (IL-15)</td>
<td>Prognostic value to be reviewed</td>
<td>[40]</td>
</tr>
<tr>
<td><strong>Interleukin-2 (IL-2)</strong></td>
<td>Interleukin-2 (IL-2)</td>
<td>Brain injury</td>
<td>[41]</td>
</tr>
</tbody>
</table>

*Arrows show the plasma and/or serum level or the level in peripheral blood.*

Gandolfi M. et al., Hindawi Neural Plasticity 2017, Article ID 1389475,
Serum biomarkers to monitor the self repair process

<table>
<thead>
<tr>
<th>IL-6</th>
<th>MMP 9</th>
<th>Irisin</th>
<th>BDNF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fractalkine</td>
<td>VEGF</td>
<td>Myostatin</td>
<td>NT-3</td>
</tr>
<tr>
<td></td>
<td>V-CAM</td>
<td>Follistatin</td>
<td>GFAP</td>
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<tr>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CAF 22</td>
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<td></td>
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<tr>
<td></td>
<td></td>
<td>IGF-1</td>
<td></td>
</tr>
</tbody>
</table>

Chmielewska N et al., Neurol Neurochir Pol. 2018
Inflammatory factors induce rescue

Angiogenesis stimulates neurogenesis and vice versa

VEGF
MMP 9
V - CAM

T0: 72 H after stroke
T3: 5-12 wk from rehabilitation

Cosky EE, Ding Y. Brain Circ 2018
Muscle can induce neuroplasticity

Irisin
IGF-1
Myostatin
Follistatin
CAF 22

T0: 72 H after stroke
T1: after stroke
T3: 5-12 wk from rehabilitation
Peripheral biomarkers of neurons and glia

BDNF

NT-3

GFAP

S100 B

T0: 72 H after stroke
T1: 1-2 wk after stroke
T3: 5-12 wk from rehabilitation

Constans A et al., Front. Aging Neurosci., 2016

Neural survivor
Angiogenesis
Neurogenesis
Synaptic plasticity

Memory functions
Spatial learning
Learning speed
Conclusions

• Biomarkers in stroke represent a current challenge in the diagnostic and prognostic evaluation of stroke onset and pathogenesis.

• Identification of biomarkers of recovery of stroke (an other neurological diseases) is still in its infancy

• Many of the molecules described here are still under investigation and may become promising biomarkers.
Grazie per l’attenzione!
Hvala za pozornost!
The stroke-induced injuries include:

- **A fall in glucose-dependent ATP generation**, resulting in the flow of numerous ionic species into the cell

- **A reduction of oxygen supply** leading to the accumulation of lactate via anaerobic glycolysis and so to acidosis, that interferes with intracellular protein synthesis

- **Calcium overload**: Ca++ ions entry in the cell resulting in activation of a number of proteases, kinases, lipases, and endonucleases,

- **Excitotoxicity**: Glutamate accumulation in the extracellular space inducing alterations in the concentration of intracellular ions (mainly Ca++ and Na+) by the prolonged stimulation of AMPA and NMDA ionotropic receptor

- **Free radicals production**: act as additional triggers of cell death

Zhao, Li-Ru, and Alison Willing. Progress in neurobiology, 2018.
Plasma Proteome Database (PPD) was developed as a part of Human Proteome Organization's (HUPO) initial effort to characterize human plasma proteome. The HPPP was initiated in 2002.

Specimens of human serum and EDTA-, citrate-, and heparin-plasma to 55 participating laboratories worldwide. This is one of the largest resources on proteins reported in plasma and serum.

Relative Abundance of plasma proteins

12 Proteins Comprise ~96% of the Protein Mass in Plasma