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Network per la riabilitazione mentale e motoria dell'ictus
Združenje za kognitivno in gibalno rehabilitacijo po možganski kapi

Neurobiological Markers of Post-Stroke Rehabilitation

E. Tongiorgi (Università di Trieste)

Bibione, 25 ottobre 2018

UNIVERSITÀ DEGLI STUDI DI TRIESTE
REGIONE DEL VENETO ULSS4 VENETO ORIENTALE
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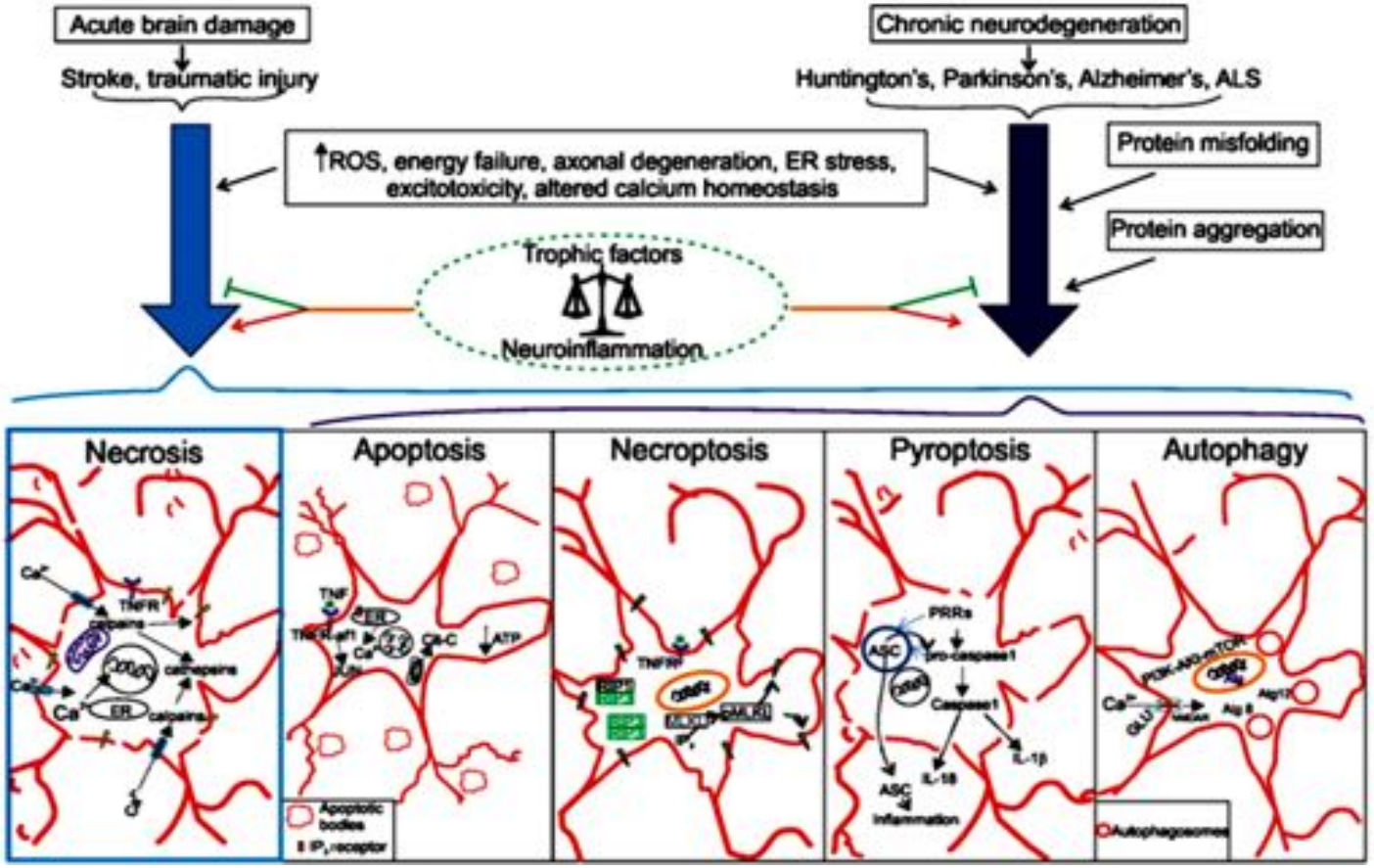
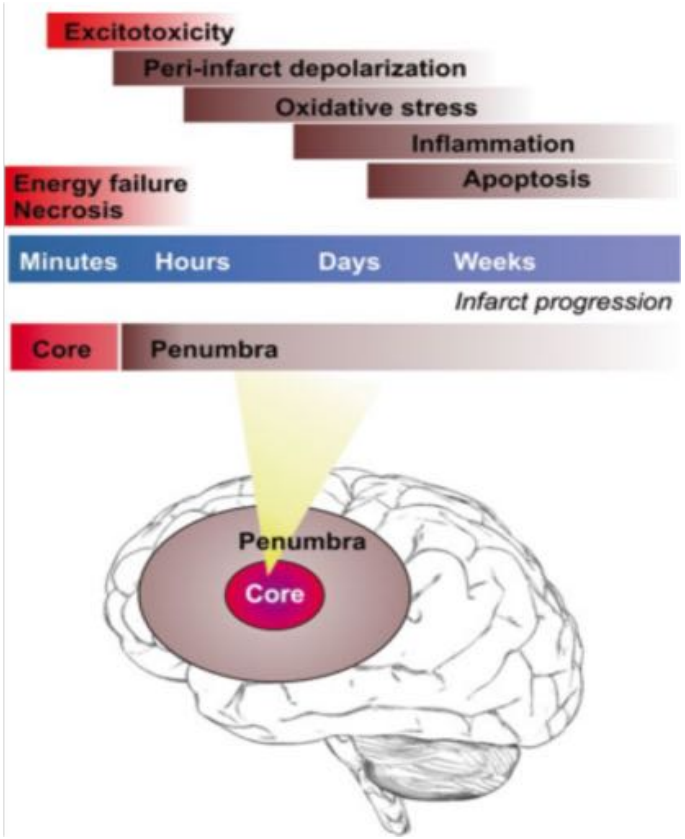
Stroke is a devastating neurological condition derived from the permanent or transient interruption of the blood flow



Tovar-y-Romo, et al., *Neurochem.* 2016

Ischemic stroke is a complex sequence of events that occur in the brain and that evolve over time and space



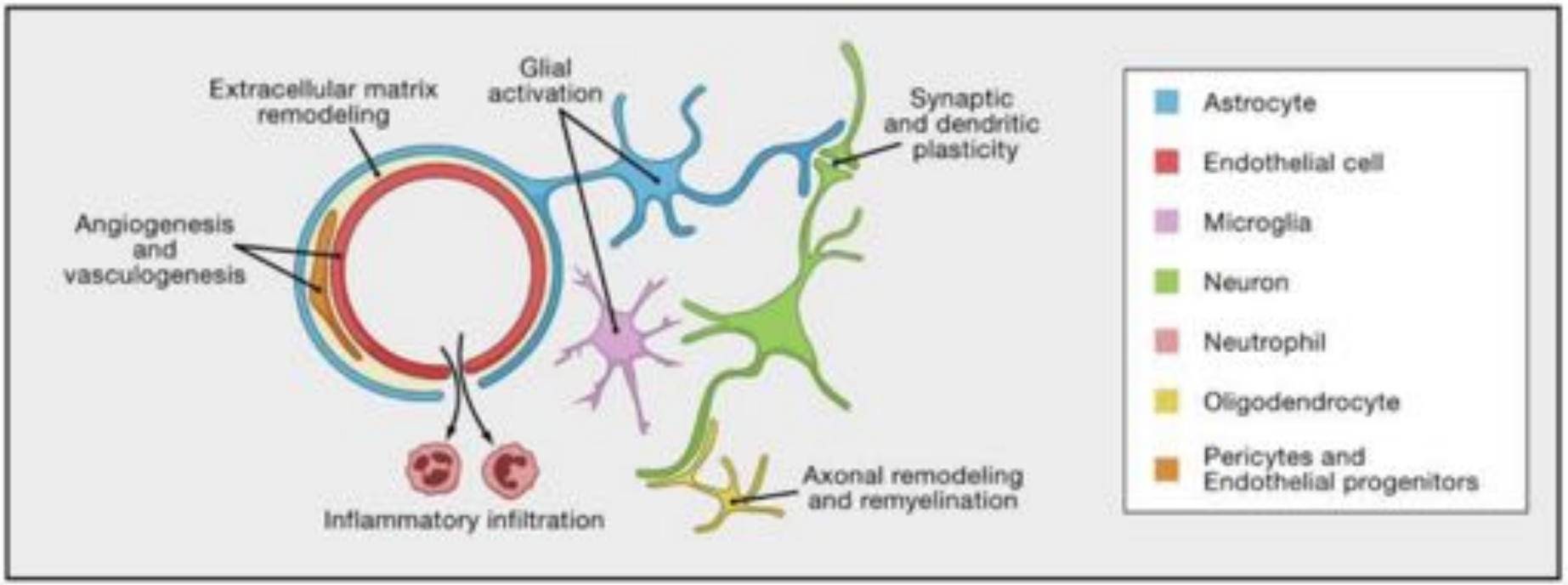
Cipriani R. et al. *Ischemia and stroke, 2014*

Between the core and the normal brain, the cerebral blood flow deficit is lower, residual perfusion persists due to collateral blood vessels, and partial energy metabolism is maintained.

This area, called **penumbra**, is on the edge between life and death: it could survive for a certain time, but, without treatment, the tissue in the penumbra also becomes progressively damaged.

(*Lo, 2008*)

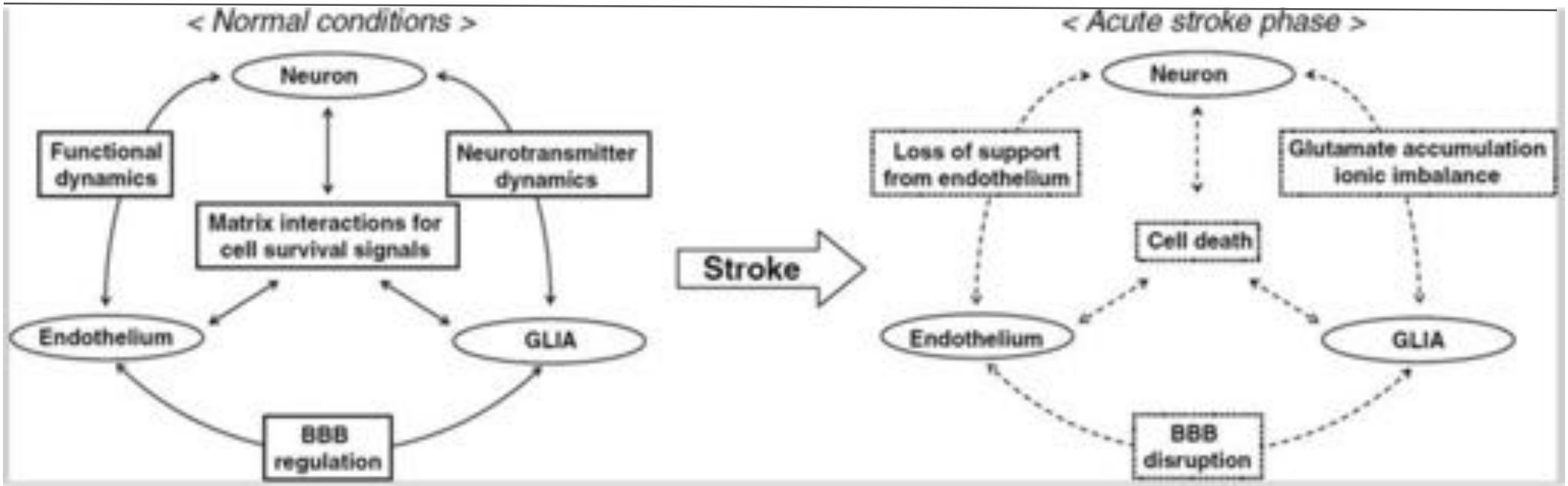
The neurovascular unit is a modular concept defined at an intercellular level



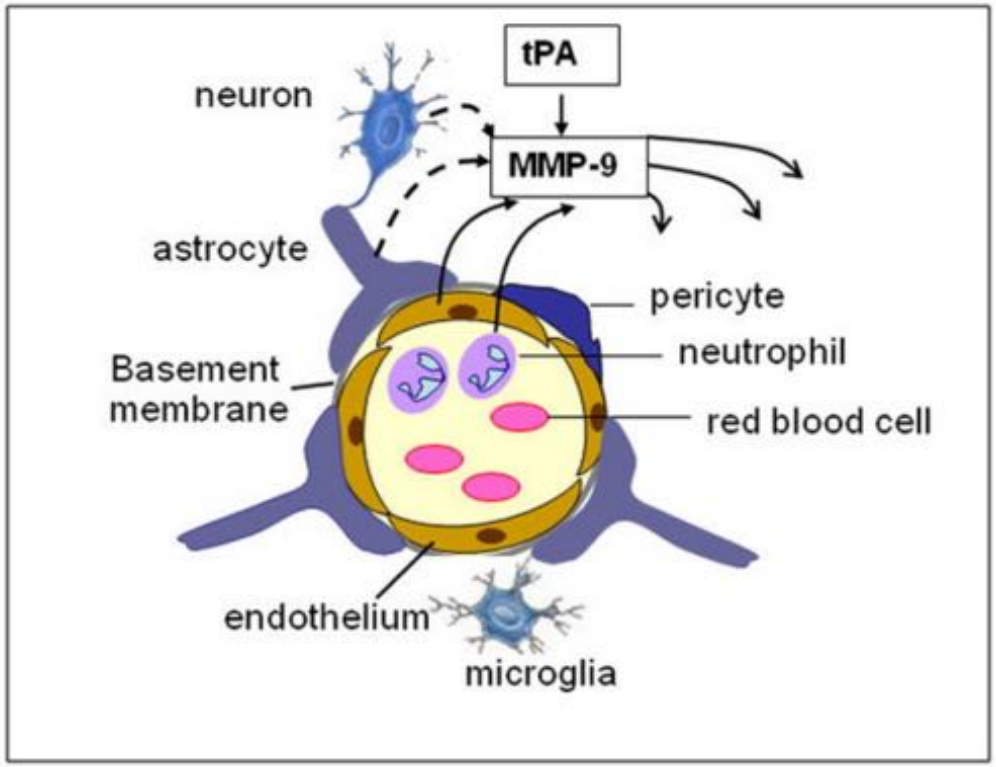
Moskowitz, M A. et al., Neuron, 2010

Effects of stroke at the level of the neurovascular unit

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



Dysfunctional signaling in the neurovascular unit after stroke



The cerebral vasculature assumes the following phenotypes:

1) poor capillary perfusion of brain tissue

2) pro-adhesive for circulating cells

3) pro-inflammatory

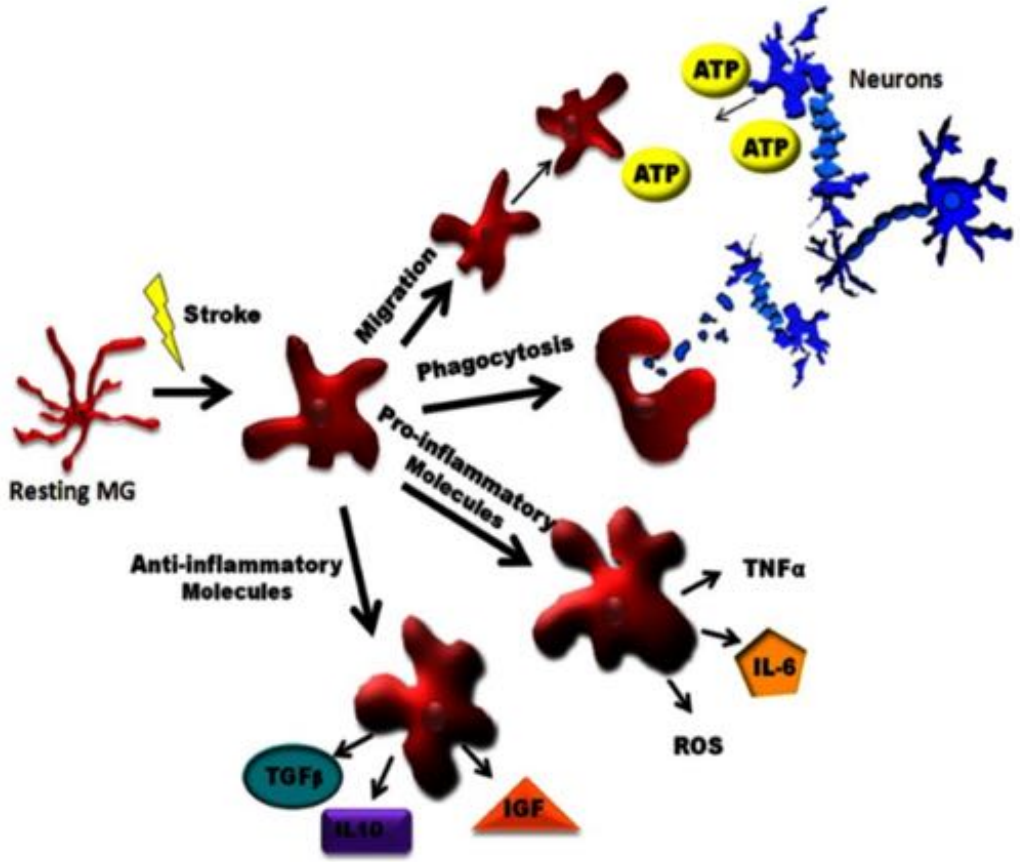
4) pro-thrombogenican

5) diminished endothelial barrier function

6) leukocytes and neutrophils accumulation in post-ischemic tissues prior to the onset of tissue injury

Rong Jin, et al. Neurobiol Dis. ;38(3):376-385, 2010

Microglia: the double-edge sword



Patel A.R et al., Int J Physiol Pathophysiol Pharmacol. 2013

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

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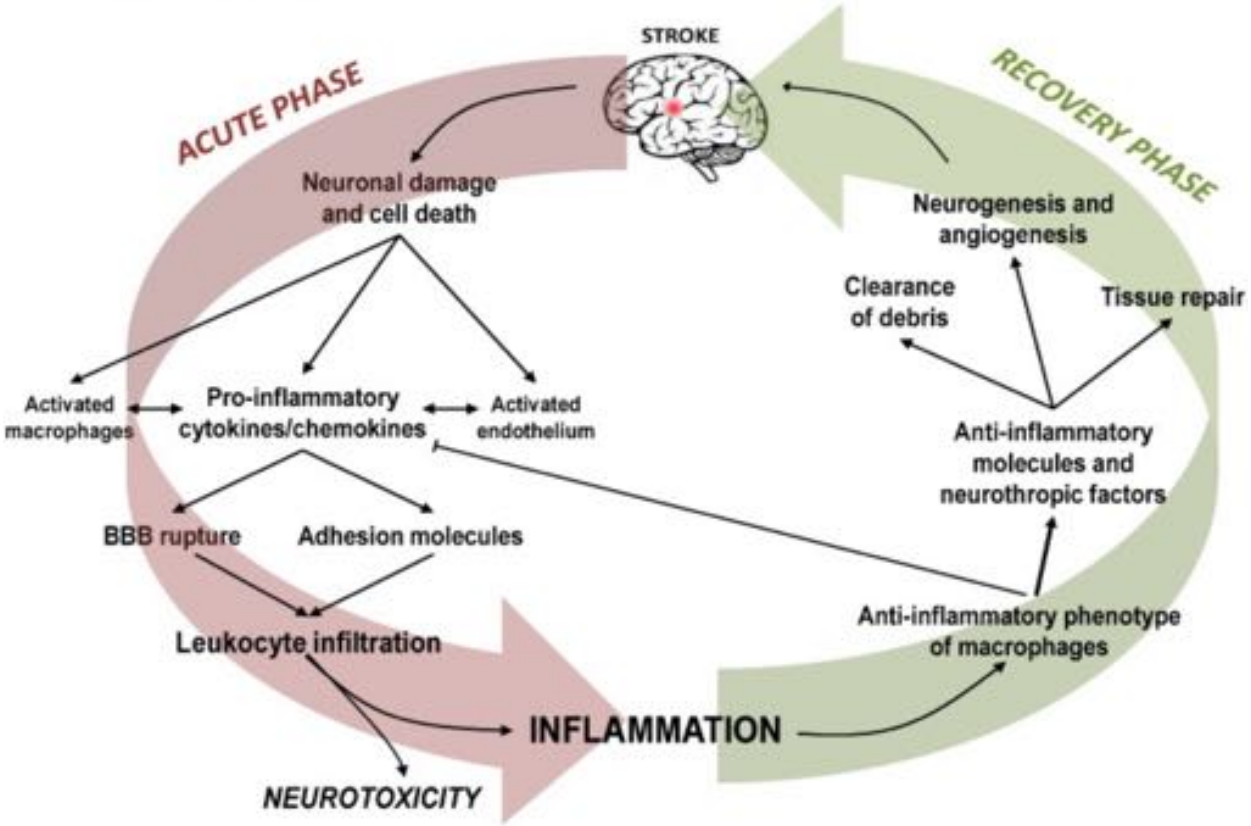
Soluble factors in stroke in acute and recovery phase: a possible source for biomarkers in stroke



Simats et al., BBA Molecular basis of diseases. 2016

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

Requirements for biomarkers in stroke

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

Biomarker	Cause of Stroke	Description of Biomarker
BNP[21,22]	Cardioembolic	Vasoactive peptide hormone
von Willebrand factor[23,24]	Cardioembolic	Glycoprotein
Interleukin-6[25,26]	Cardioembolic, lacunar	Inflammatory cytokine
TNF-α[25]	Cardioembolic, lacunar	Inflammatory cytokine
D-dimer[18,27–29]	Cardioembolic, large vessel	Fibrin degradation product
C-reactive protein[30,31]	Cardioembolic, large vessel, lacunar	Acute phase protein
ICAM-1[32–34]	Lacunar, large vessel	Adhesion molecule
sRAGE[18]	Lacunar, large vessel	Transmembrane receptor
Fibrinogen[31,35]	Large vessel	Glycoprotein
P-selectin[36]	Large vessel	Cell adhesion molecule
Adiponectin[37]	Large vessel	Adipose tissue hormone
Thrombomodulin[34]	Lacunar	Thrombin cofactor
RNA panel[19,20,38]	Cardioembolic, large vessel, lacunar	Nucleic acid

BNP indicates brain natriuretic peptide; ICAM-1, intracellular adhesion molecule-1; sRAGE, soluble receptor for advanced glycation end products; and TNF-α, tumor necrosis factor-α.

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

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Early biomarkers associated with a cause of stroke

	6 protein panel[17]	Ischemic+hemorrhagic stroke vs control	Protein, plasma	ELISA	1005	Caspase-3, Chimerin, D-dimer, MMP-9, Secretagogin, sRAGE	17%/98%	
50/	5 protein panel[13]	Ischemic+hemorrhagic stroke vs control	Protein, plasma	ELISA	437	BNGF, MCP-1, MMP-9, S100B, vWF	92%/93%	6h from stroke
	4 protein panel[14]	Ischemic+hemorrhagic stroke vs control	Protein, plasma	ELISA	946+343	BNP, D-dimer, MMP-9, S100B	86%/37%	
	4 protein panel[15]	Ischemic stroke vs control	Protein, plasma	ELISA	222	MMP-9, S100B, VCAM1, vWF	90%/90%	
	4 protein panel[16]	Ischemic stroke vs control	Protein, whole blood	Immunoassay	155	BNP, D-dimer, MMP-9, S100B	73%/72%	
224/	5 protein panel[17]	Ischemic+hemorrhagic stroke vs control	Protein, plasma	Immunoassay	130	Eotaxin, EGFR, S100A12, Metalloproteinase inhibitor-4, Prolactin	90%/84%	24h from stroke

13. Reynolds MA, Kirchick HJ, Dahlen JR, Anderberg JM, McPherson PH, Nakamura KK, et al. Early biomarkers of stroke. *Clin Chem*. 2003;49:1733–1739.

17. Sharma R, Macy S, Richardson K, Lokhnygina Y, Laskowitz DT. A blood-based biomarker panel to detect acute stroke. J Stroke Cerebrovasc Dis. 2014;23:910–918.

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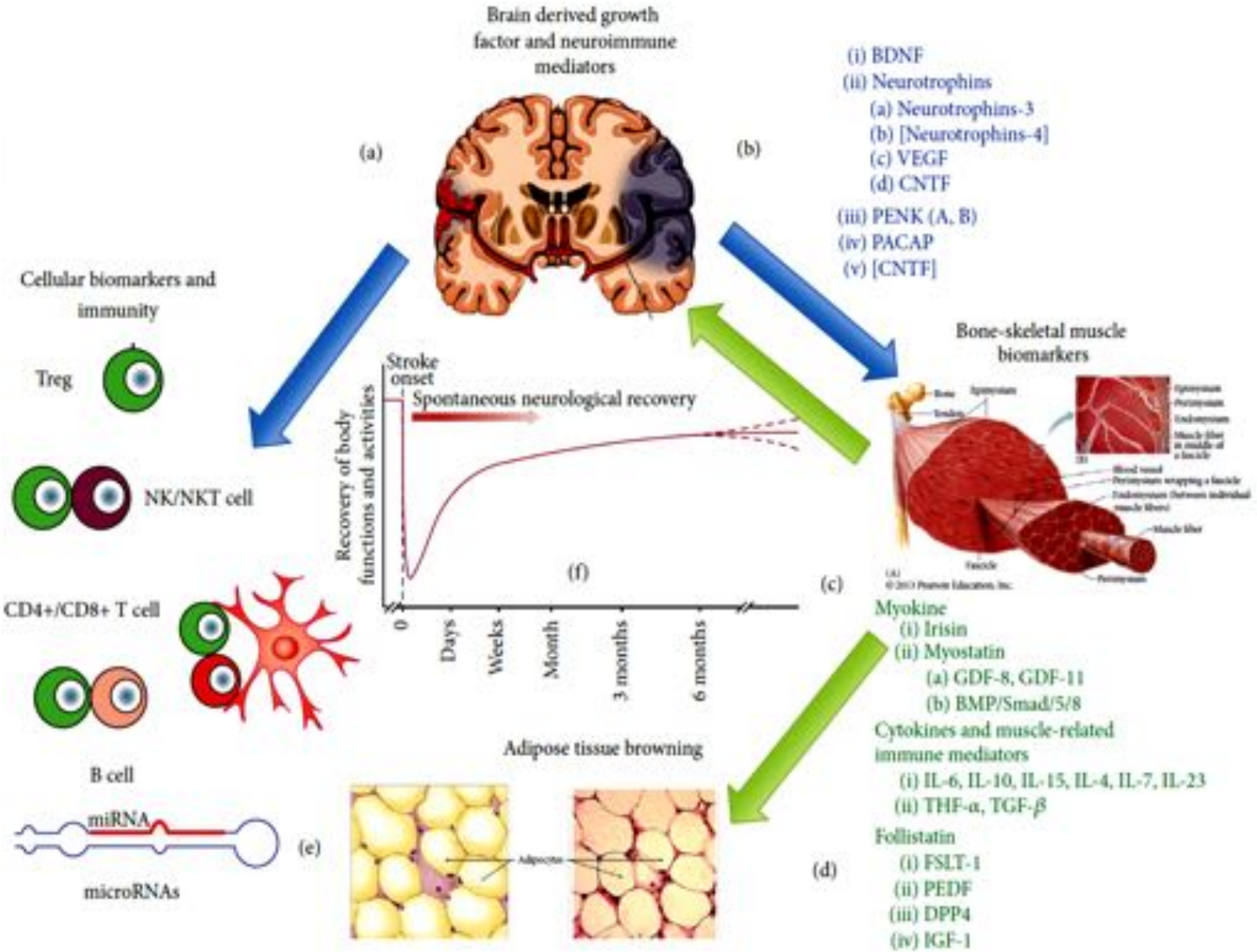

Gandolfi M. et al.,
Hindawi Neural Plasticity
2017, Article ID 1389475,

Biomarkers associated with prognosis/recovery of stroke

Table 1: List of the main assessed and emerging circulating biomarkers in stroke.

Biomarker group	Molecule		Diagnostic or prognostic value[31]			References
Myokines	Irisin			↑	Good prognostic marker of stroke recovery with training	[21, 22]
	Myostatin (GDF-8)	↑	Muscular biomarker of stroke Muscle wasting			[23–26]
	Follistatin			↑	Good prognostic marker of stroke (muscular level)	[27–30]
	PEDF			↑	Good prognostic marker of stroke (angiogenic level)	[31, 32]
	DPP4			↓	Ameliorating stroke recovery	[33, 34]
	Osteonectin (SPARC)			↑	Neural repair following stroke	
	FGF-21	↑	Negatively associated with stroke			[35]
Neurotropic factors	Brain derived neurotropic factor (BDNF)	↑	Improvement in stroke recovery Biomarker of stroke onset	↓	Bad prognosis stroke recovery	[17, 19, 20, 36]
	Neurotrophin-3	↑	Biomarkers of stroke onset	↑	Stroke recovery	[37, 38]
	Neurotrophin-4	↑	Biomarkers of stroke onset			[39]
	CNTF	↑	Biomarkers of stroke onset			[40]
Neuropeptides	Neuropeptide Y			↑	Good prognostic biomarker in certain SNP patterns	[41]
	Proenkephalin A			↑	Bad prognosis in stroke progression	[42–44]
	PACAP			↑	Bad prognosis in hemorrhagic stroke progression	[45]
	Substance P			↑	Very bad prognosis in ischemic stroke progression	[46]
Growth factors and GF-like molecules	VEGF	↑	Biomarkers of stroke onset			[47–49]
	IGF-I, IGF-II			↑	Good prognosis in ischemic stroke progression (remodelling)	[50–52]
Cytokines	Interleukin 6 (IL-6)	↑	Stroke onset and progression	↑	Prognostic value to be reviewed	[1, 15, 16]
	Interleukin-33 (IL-33)	↑	Biomarkers of stroke onset	↓	Bad prognosis in ischemic stroke progression	[53]
	Interleukin 15 (IL-15)	↑	Biomarkers of stroke onset	↑	Brain injury	[54]
	Interleukin-II (IL-II)	↑	Biomarkers of stroke onset			[55, 56]

[31] 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,

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Serum biomarkers to monitor the self repair process

IL - 6

Fractalkine

MMP 9

VEGF

V - CAM

Irisin

Myostatin

Follistatin

CAF 22

IGF - 1

BDNF

NT - 3

GFAP

S100 B

Chmielewska N et al., Neurol Neurochir Pol. 2018

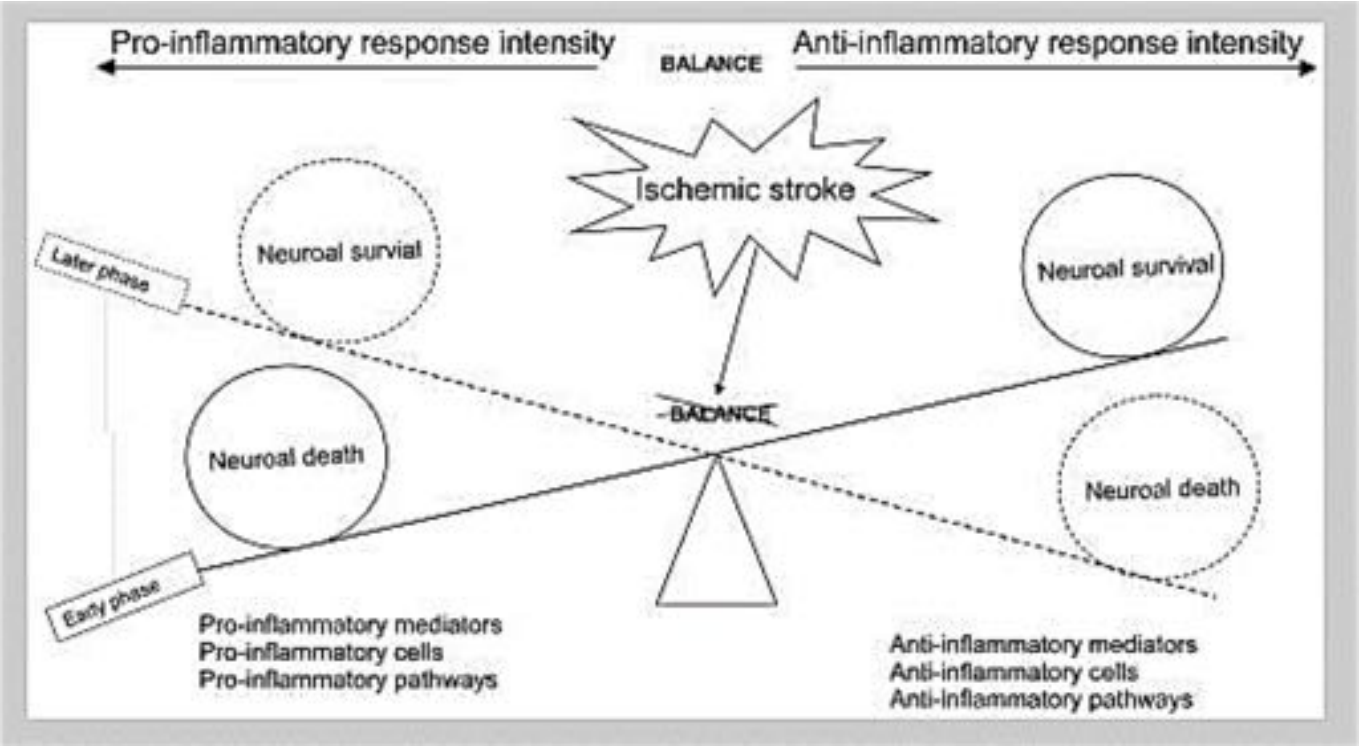
Inflammatory factors induce rescue

IL - 6

Fractalkine

T0: 72 H after stroke

T3: 5- 12 wk from rehabilitation



Jin, Rong, et al. *Journal of cardiovascular translational research* 6.5, 2013

Angiogenesis stimulates neurogenesis and vice versa

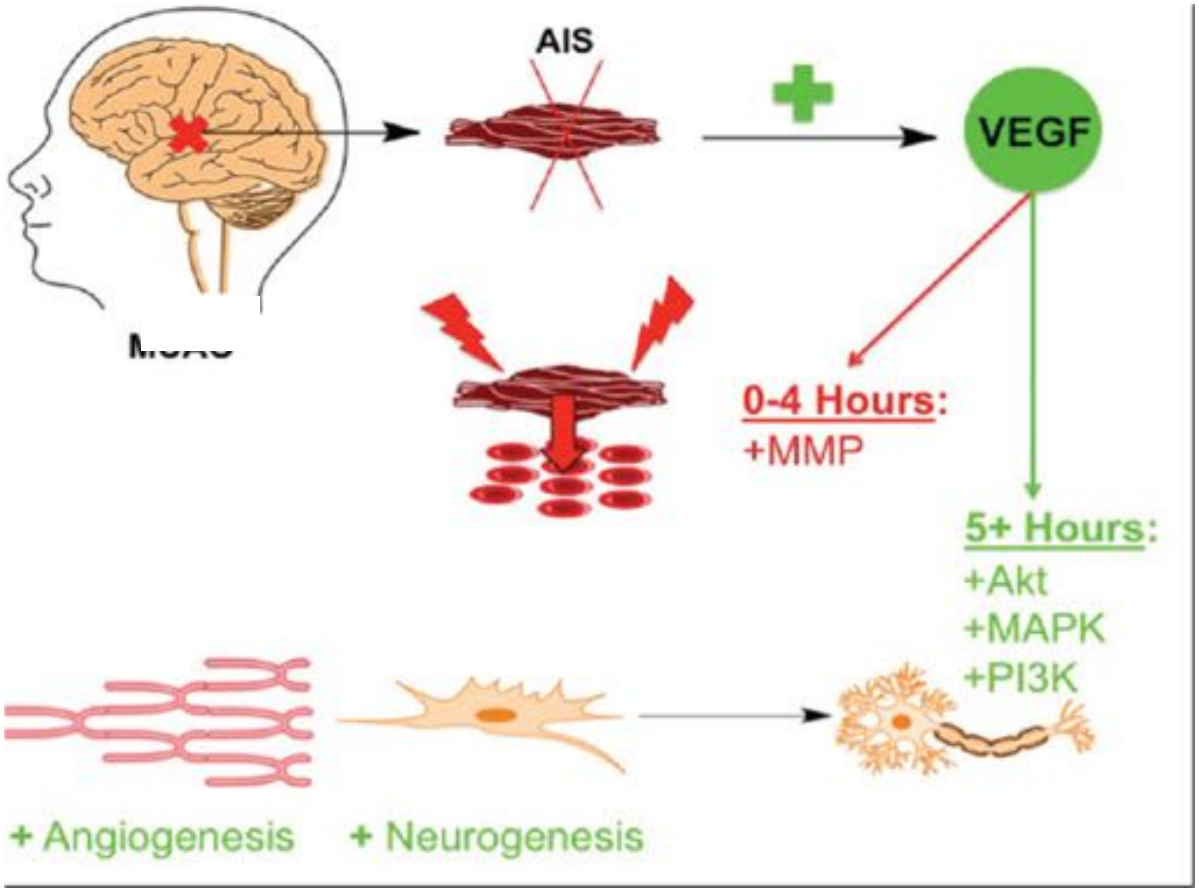
↑ ↑ VEGF

↑ 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

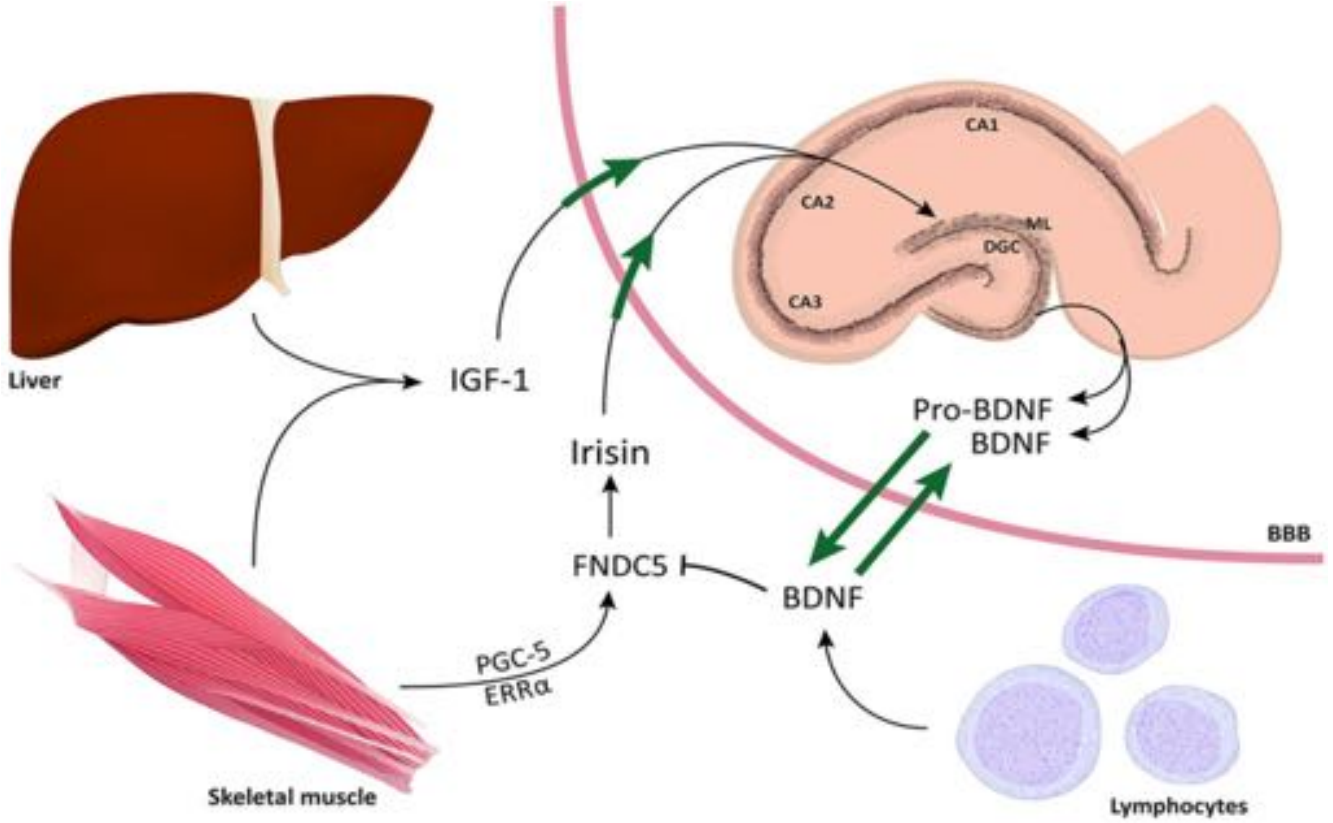
IGF - 1

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

BDNF

Neural survivor
Angiogenesis
Neurogenesis
Synaptic plasticity

Memory functions
Spatial learning
Learning speed

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

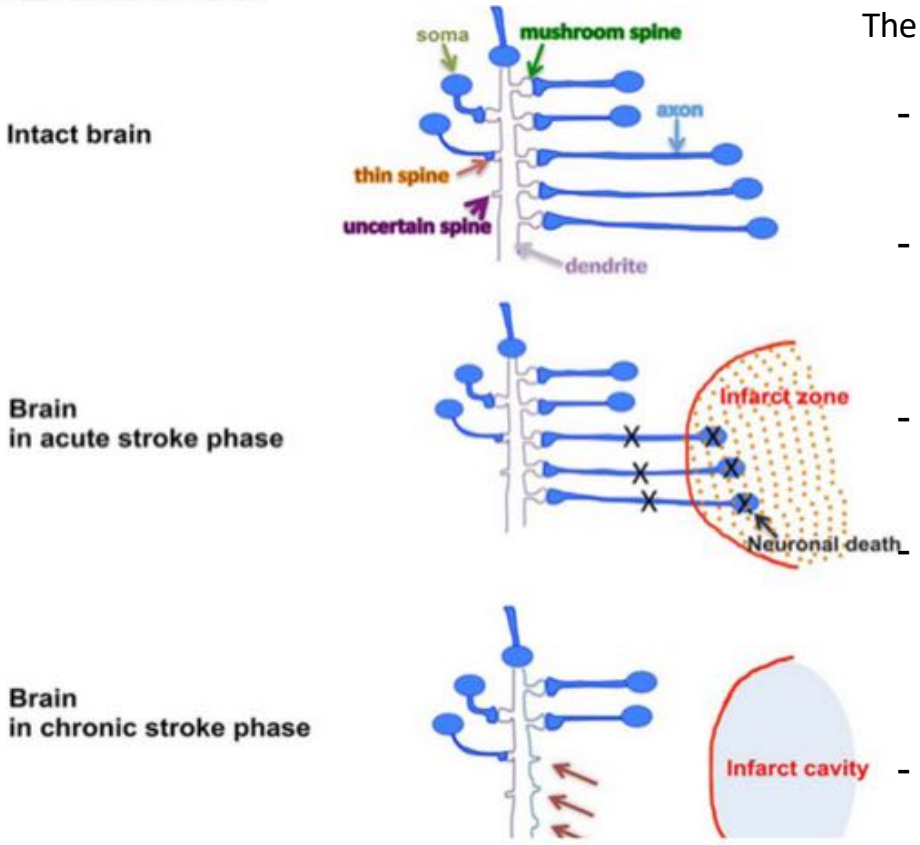
Conclusions

- 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!

Website: http://ita-slo.eu/it/memori-net
http://memorinet.eu

Neurons: the inflammatory paradox of cellular self-injury



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+) bythe 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.

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The Human Plasma Proteome

a reference platform for biomarker discovery

HUPO
Human Proteome Organization

http://www.plasmaproteomedatabase.org/

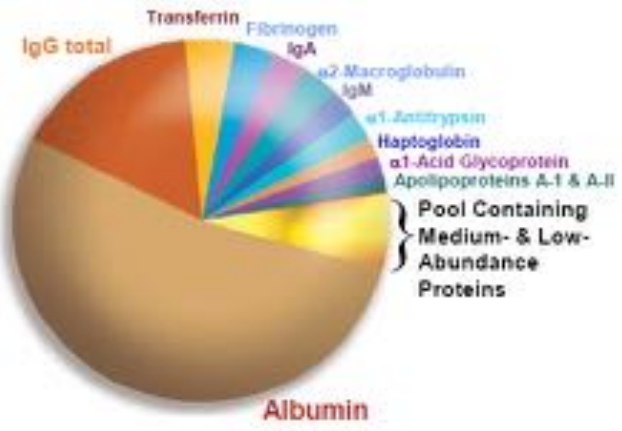
Statistics	
Proteins	**10,546**
Proteins with concentration	**1,278**
Proteins with MRM data	**279**
Proteins in extracellular vesicles	**318**
PubMed	**509**

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.

Nanjappa, V. *et al*. (2013). Plasma Proteome Database as a resource for proteomics research: 2014 update. *Nucleic Acids Research.*

Relative Abundance of plasma proteins



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