Scuola Superiore di Neurologia
Genova, 16-2-2016

Meccanismi di danno

Prof. Carlo Ferrarese

- Meccanismi patogenetici (geni+ambiente)
- Meccanismi di danno neuronale post-ischemico
- Meccanismi protettivi e riparativi
- Marcatori con fini diagnostici/prognostici
- Potenziali target terapeutici (neuroprotezione)
- Trombolisi e neuroprotezione

Metodologia

1. STUDI GENETICI
2. MODELLI ANIMALI
3. MODELLI CELLULARI
4. MARKERS BIOLOGICI NEI PAZIENTI
5. RICERCA TRASLAZIONALE

The five **Scientific Priorities** are to:

- Identify genes that predispose individuals to stroke and improve our understanding of the proteins produced by these genes.

- Define more completely the interactions between brain cells, blood vessel walls, and circulating blood elements in the healthy brain and before, during, and after stroke.

- Improve our understanding of normal and abnormal blood flow in the brain and how to safely re-establish blood flow after stroke.

- Develop effective combinations of therapies for stroke patients, especially those patients who cannot be treated with currently available clot-busting drugs.

- Clarify the cellular and molecular events that help the brain recover from stroke, and, using this information, develop new treatments to help restore function to patients.

- **Meccanismi patogenetici (geni+ambiente)**

  - **STUDI GENETICI**

BBA Clinical 3 (2015) 96–106

Review

Genetics of ischaemic stroke in young adults

Eva Terni, Nicola Giannini, Marco Brondi, Vincenzo Montano, Ubaldo Bonuccelli, Michelangelo Mancuso *

Department of Experimental and Clinical Medicine, Neurological Clinic, University of Pisa, 56126 Pisa PI, Italy

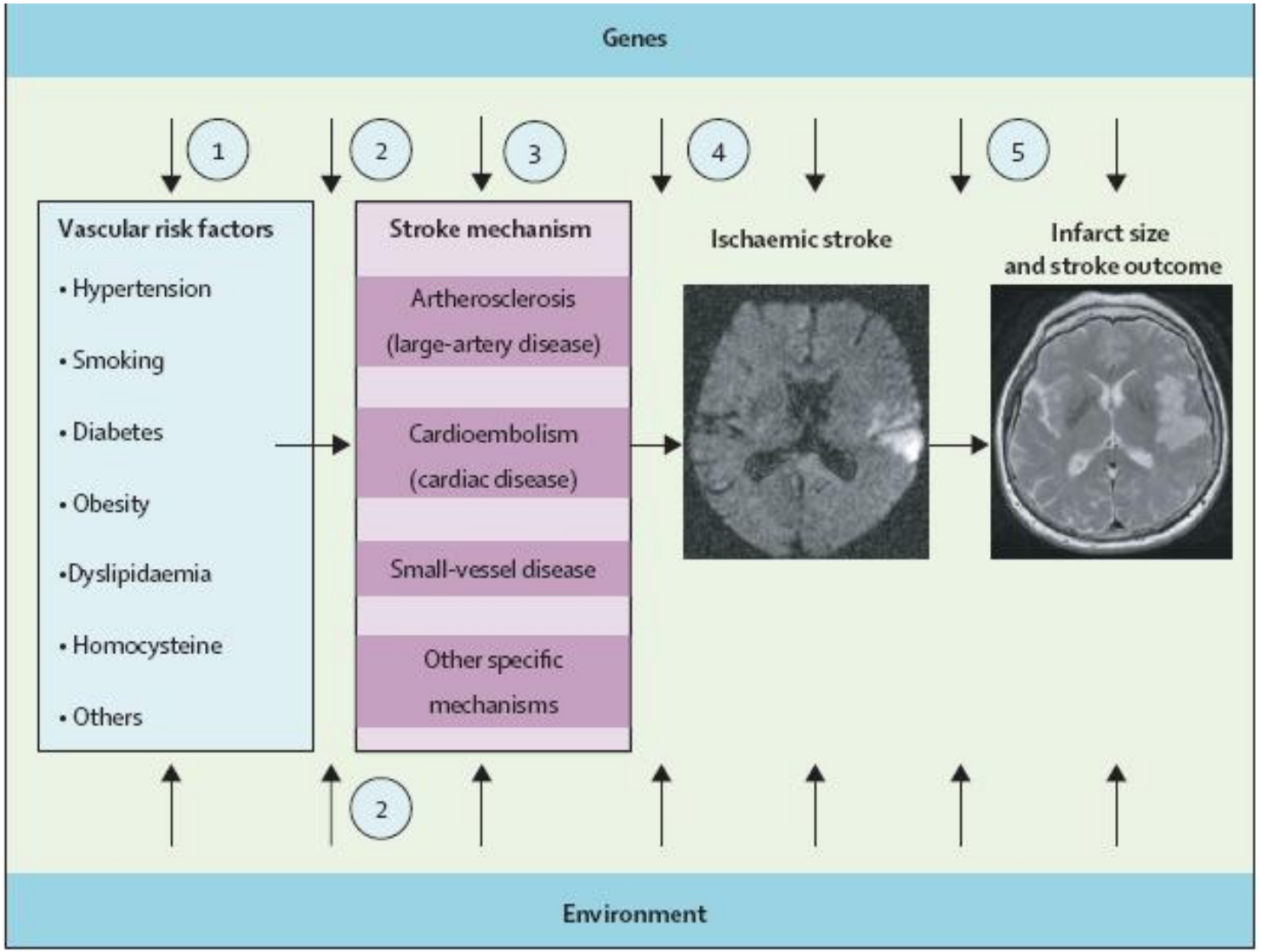
Common mutations in monogenic diseases for details see the text.

Monogenic diseases	Involved genes	Genes functions
MELAS	*tRNA (Leu) A3243G*	Mitochondrial tRNA
	tRNA (Leu) T3271C	Mitochondrial tRNA
	tRNA (Lys) A8344G	Mitochondrial tRNA
Familial hemiplegic migraine	*CACNA1A*	Encoding the alpha1A sub-unit of the voltage-gated calcium channels in neurons
CADASIL	*NOTCH3*	Unknown
CARASIL	*HTRA1*	Protease
FABRY	*α-GAL A*	Encoding α-galactosidase A enzyme
Small vessel disease	*COL4A1*	Encoding the α1[IV]-chain of type IV collagen
HERNS	*TREX1*	Encoding three-prime repair exonuclease 1
Stroke and vasculopathy with ADA2 mutations	*CECR1*	Encoding the ADA2 protein (important for endothelial and leukocyte development and differentiation)
Homocystinuria	*Multiple genes encoding different enzymes*	Deficiencies of this enzymes can cause very high plasma concentrations of homocysteine and homocystinuria
Sickle cell disease	*Haemoglobin beta chain gene*	Encoding for beta chain of normal haemoglobin (mutation of this gene causes polymerization or aggregation of abnormal hemoglobin -. HbS - within red blood cells)
Vascular Ehlers-Danlos syndrome	*COL3A1*	Encoding collagen type III
Marfan syndrome	*FBN1*	Encoding fibrillin 1
Pseudoxanthoma elasticum	*ABCC6*	ATP-binding cassette C6

Common variants in polygenic diseases. For details see the text.

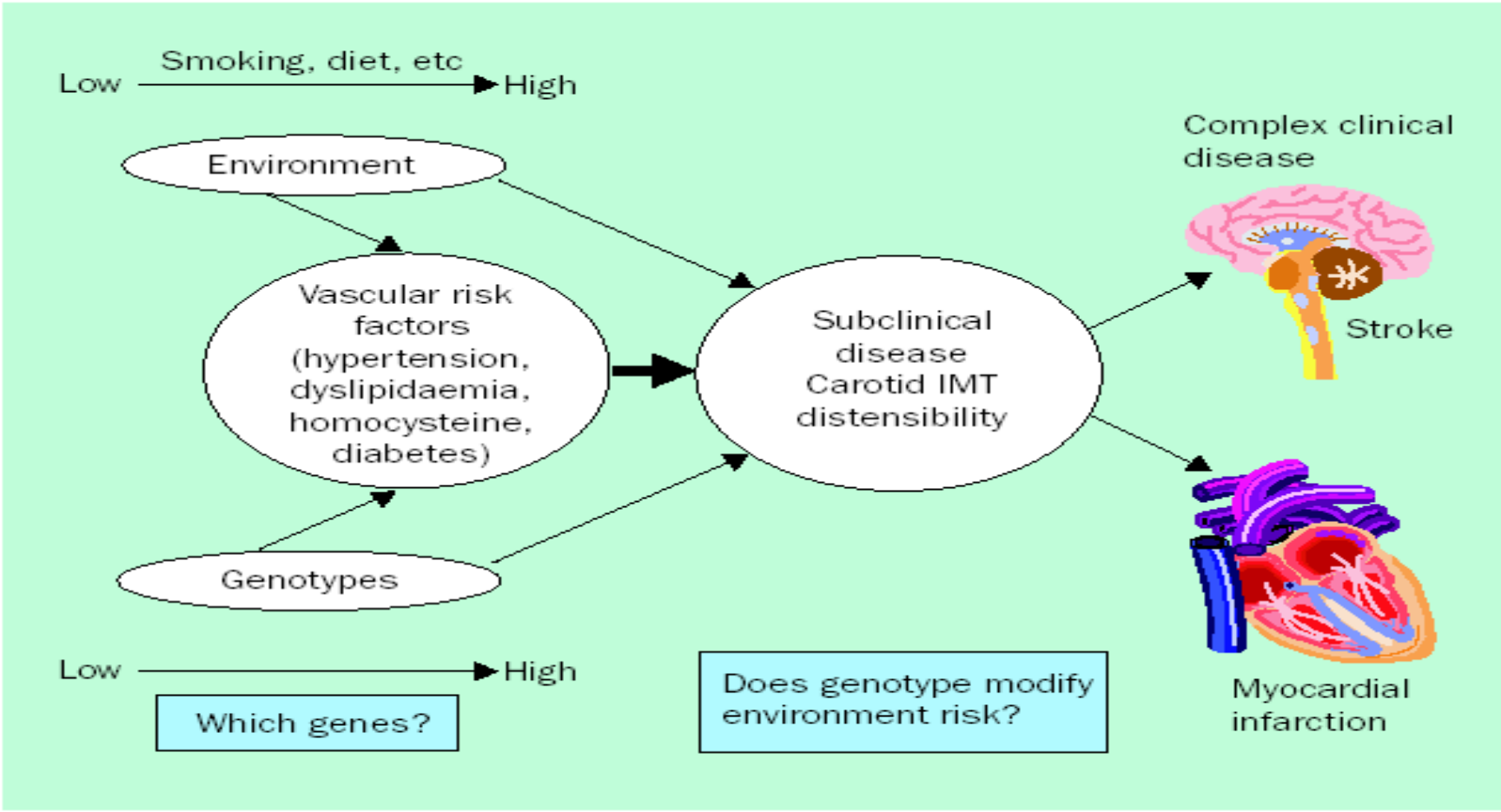
Gene	Polymorphism	Genes functions
MTHFR	*C677T*	This polymorphism is associated with high levels of plasmatic homocysteine
ACE	*I/D situated in intron 16*	ACE is a membrane-bound enzyme which plays an important role within the renin-angiotensin aldosterone system
AGT	*M235T*	AGT is a glycoprotein substrate for renin action. This polymorphism is associated with increased blood pressure, carotid plaques and white matter lesions
Prothrombin gene	*c.20210G4A*	This mutation is associated with increased prothrombin levels
Fibrinogen gene	*c.455G4A*	Possible association between fibrinogen polymorphisms, high fibrinogen levels and arterial thrombosis
FV	*c.1691G4A*	This mutation leads to a p.Arg506Gln amino- acid change, which determines a resistance to aPCR (activated protein C), a stroke predisposing condition
PAI-1 (*SERPINE1*)	*4G/5G*	PAI-1 is a fast acting inhibitor of tissue plasminogen (t-PA), which plays a key role in fibrinolytic homeostasis (mutations cause increased thrombotic risk)
APOE	*ε2/ε3/ε4*	This polymorphisms may have an impact on total cholesterol, LDL and apoE plasma levels
PON1	*Q192R*	Alteration of enzyme activity associated with this SNP may influence the formation of atheromas
MMP-3	*5A/6A*	Homozygosity for 6A allele is responsible of a lower proteolytic activity with an increased deposition of extracellular matrix and a faster progression of the atherosclerotic plaque
EAAT2	*A-to-C change at 181 bp from the transcriptional start site*	The mutant genotype is associated with increased plasma glutamate concentrations

Stroke: complex disease



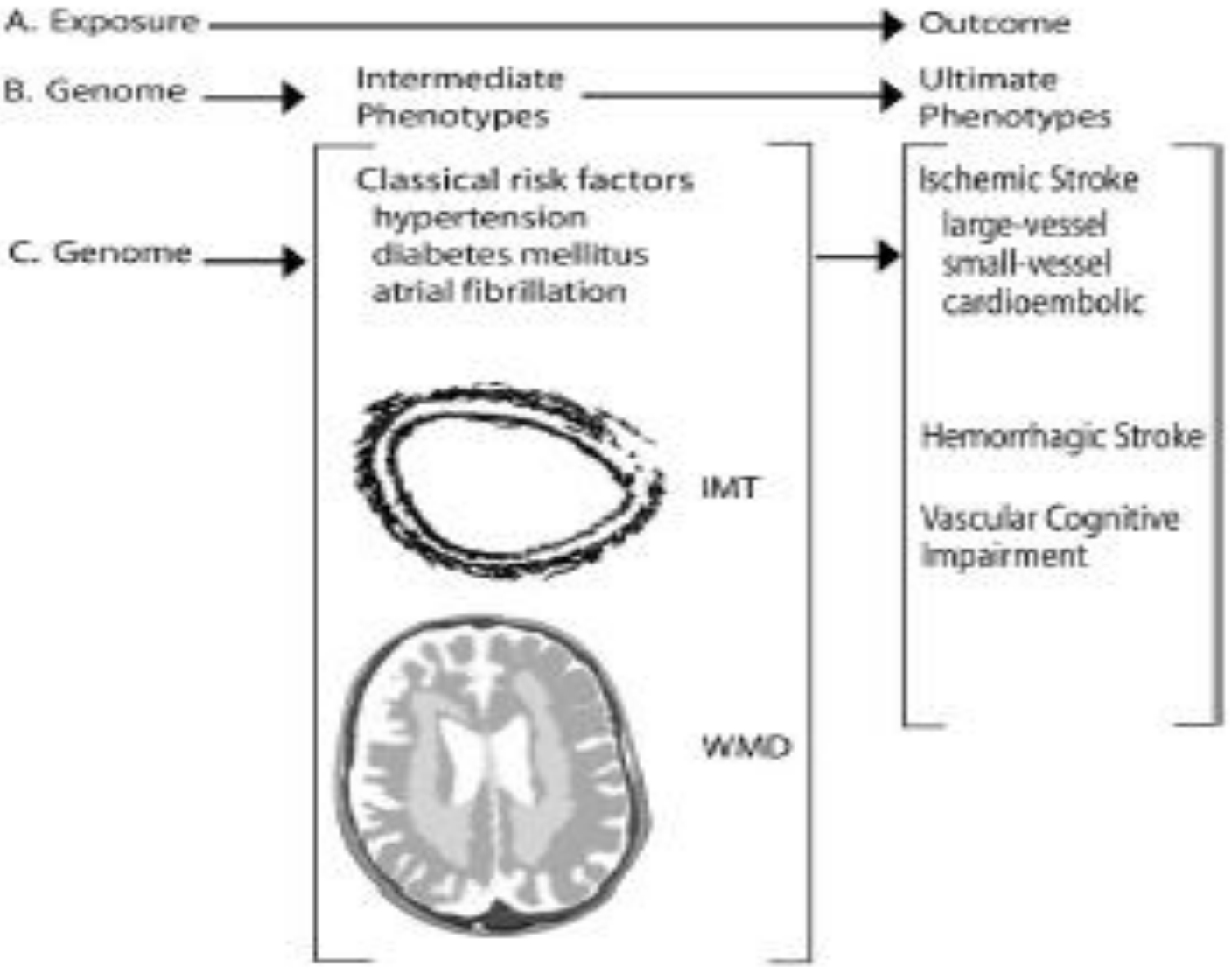
Dichgans 2007

Interazioni geni-ambiente nell'eziologia dell'ictus



THE LANCET Neurology 2004;3:227-236

Modelli di studio di “fenotipi complessi”



Stroke 2004;35:2735_39)

Il problema del “silent stroke”

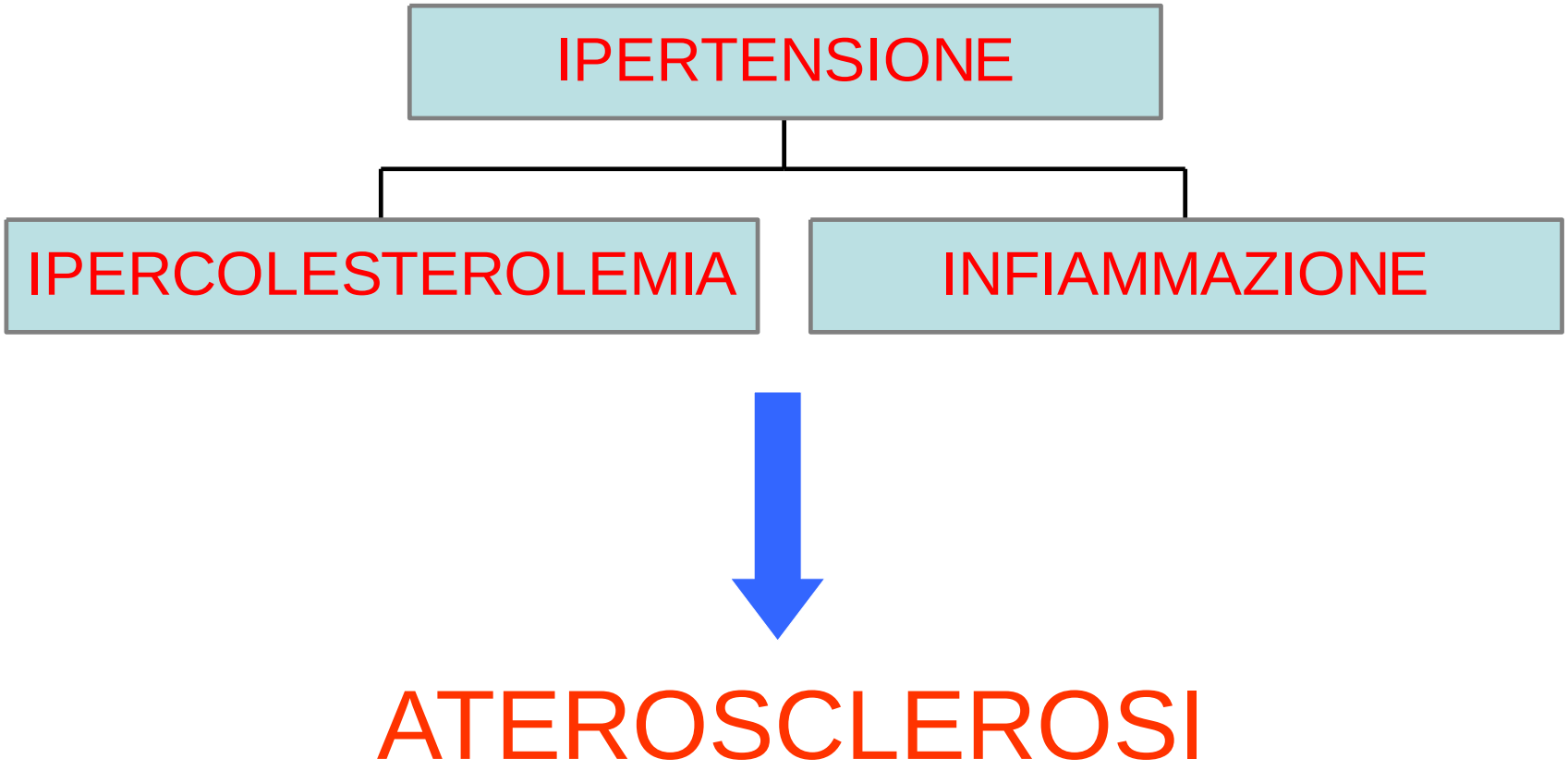
Table 1. Definition of silent (asymptomatic) stroke

Absence of neurological symptoms and signs including laterality of deep tendon reflex and vascular dementia
Absence of prior stroke including transient ischaemic attack
Presence of brain parenchymal lesion of vascular origin confirmed by computed tomography or magnetic resonance imaging

If a lesion without any corresponding focal sign is detected in a symptomatic stroke patient, the term ‘silent (asymptomatic) cerebrovascular lesion’ is applied. Diffuse white matter changes (periventricular white matter hyperintensities and leukoaraiosis) and brain atrophy are not included unless any focal parenchymal lesion is identified.

Curr Opin Neurol 14:77-82, 2001

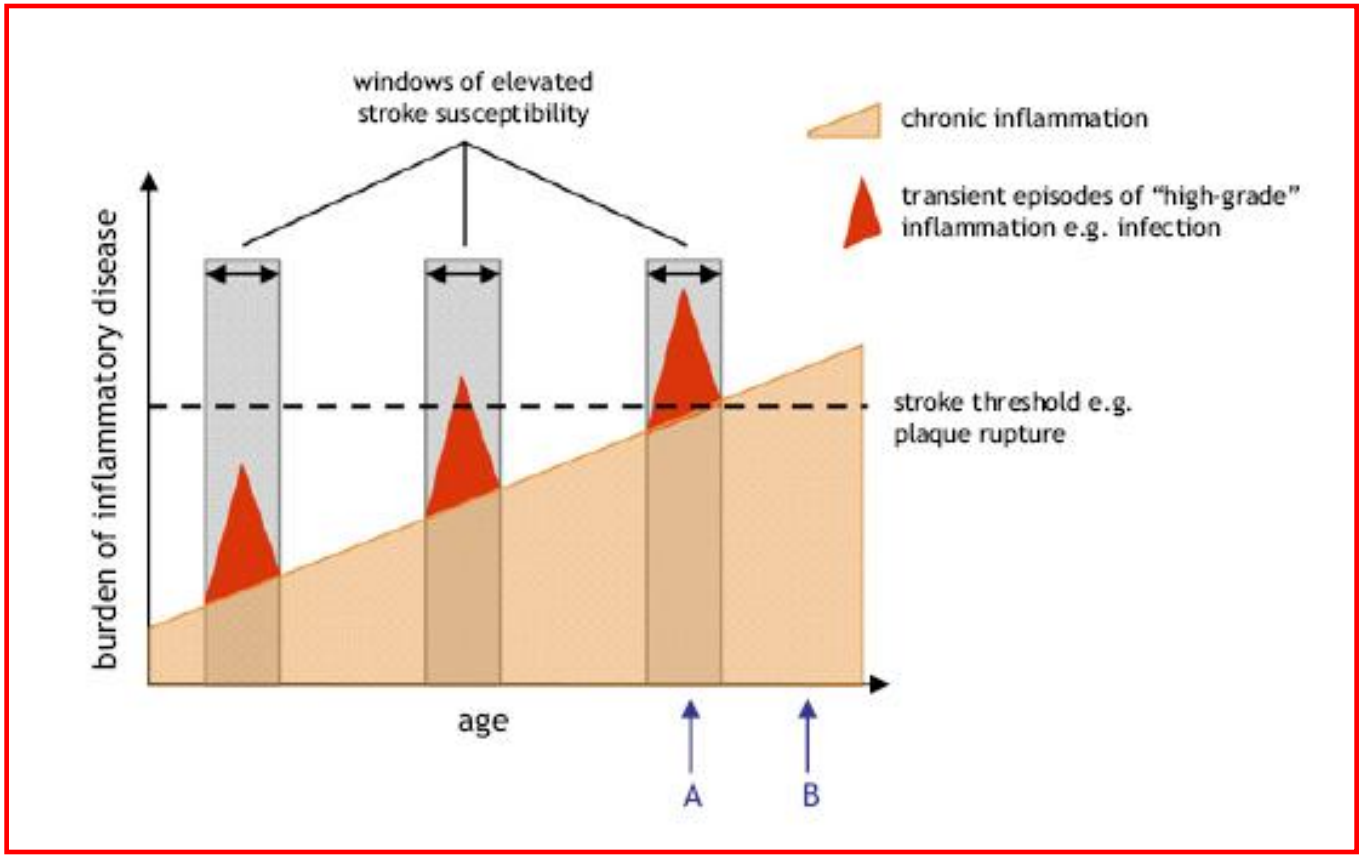
INTERAZIONI TRA I FATTORI DI RISCHIO



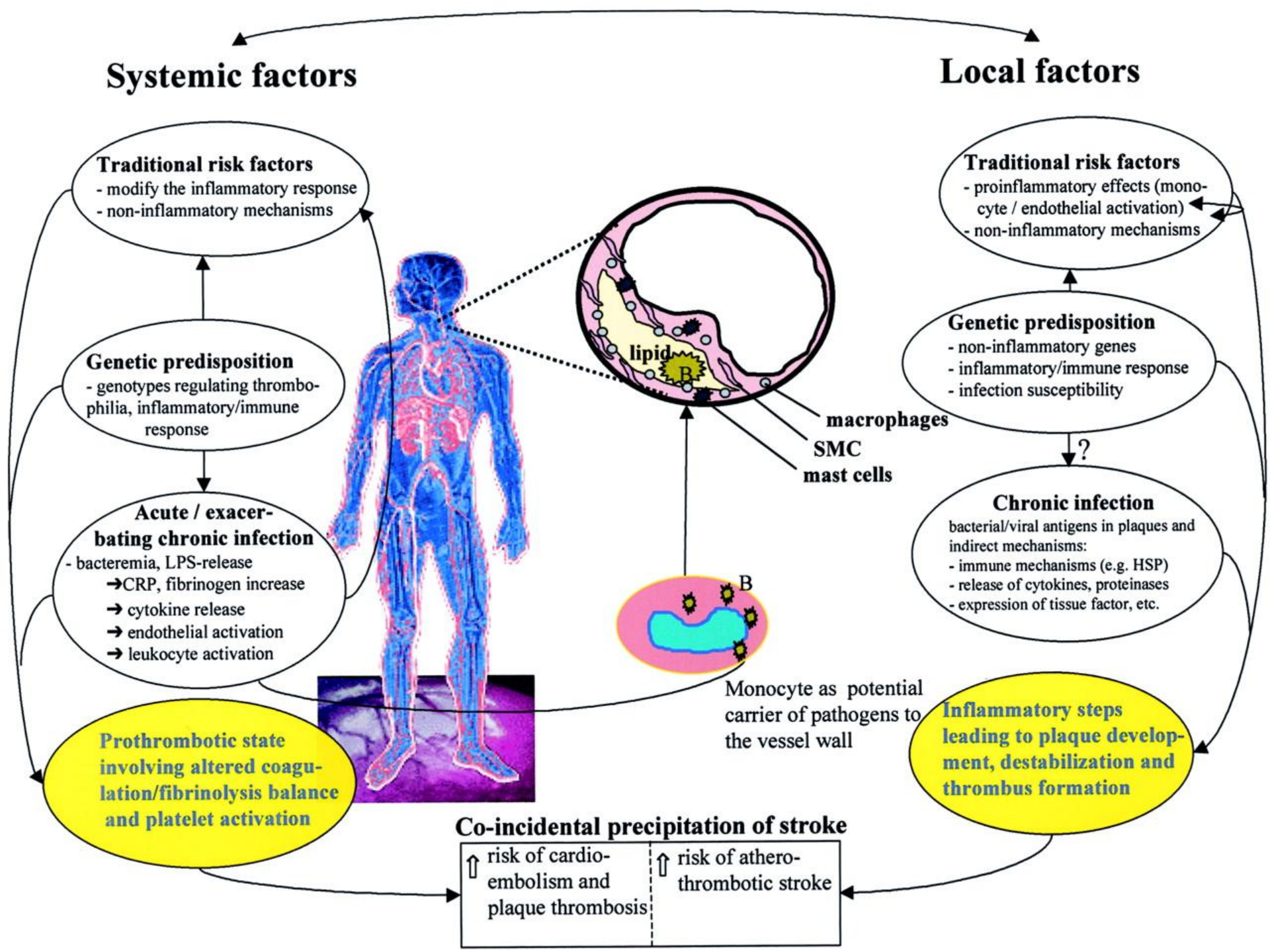
ATEROGENESI=INFIAMMAZIONE

- Danno endoteliale (ipertensione, ipercolesterolemia, fumo)
- Aumento di permeabilità endoteliale a lipoproteine (LDL)
- Ossidazione di LDL
- Reclutamento di Monociti
- Rilascio di citochine
- Attivazione di PCR
- Trasformazione in macrofagi (foam cells)
- Attivazione piastrinica
- Rilascio di fattori piastrinici

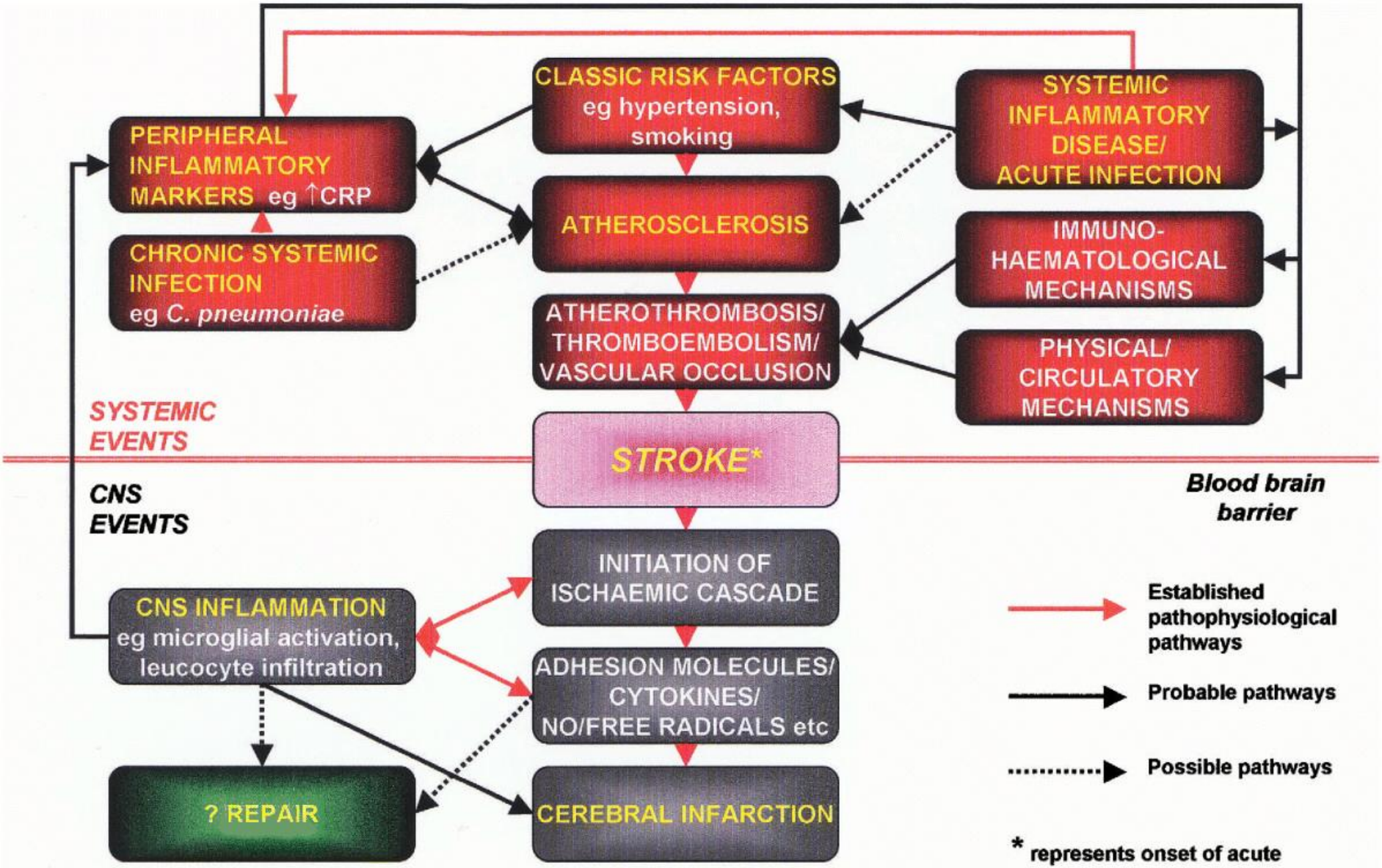
INFLAMMAGING E INFEZIONI: STROKE TRESHOLD



McColl et al. Neuroscience 2009

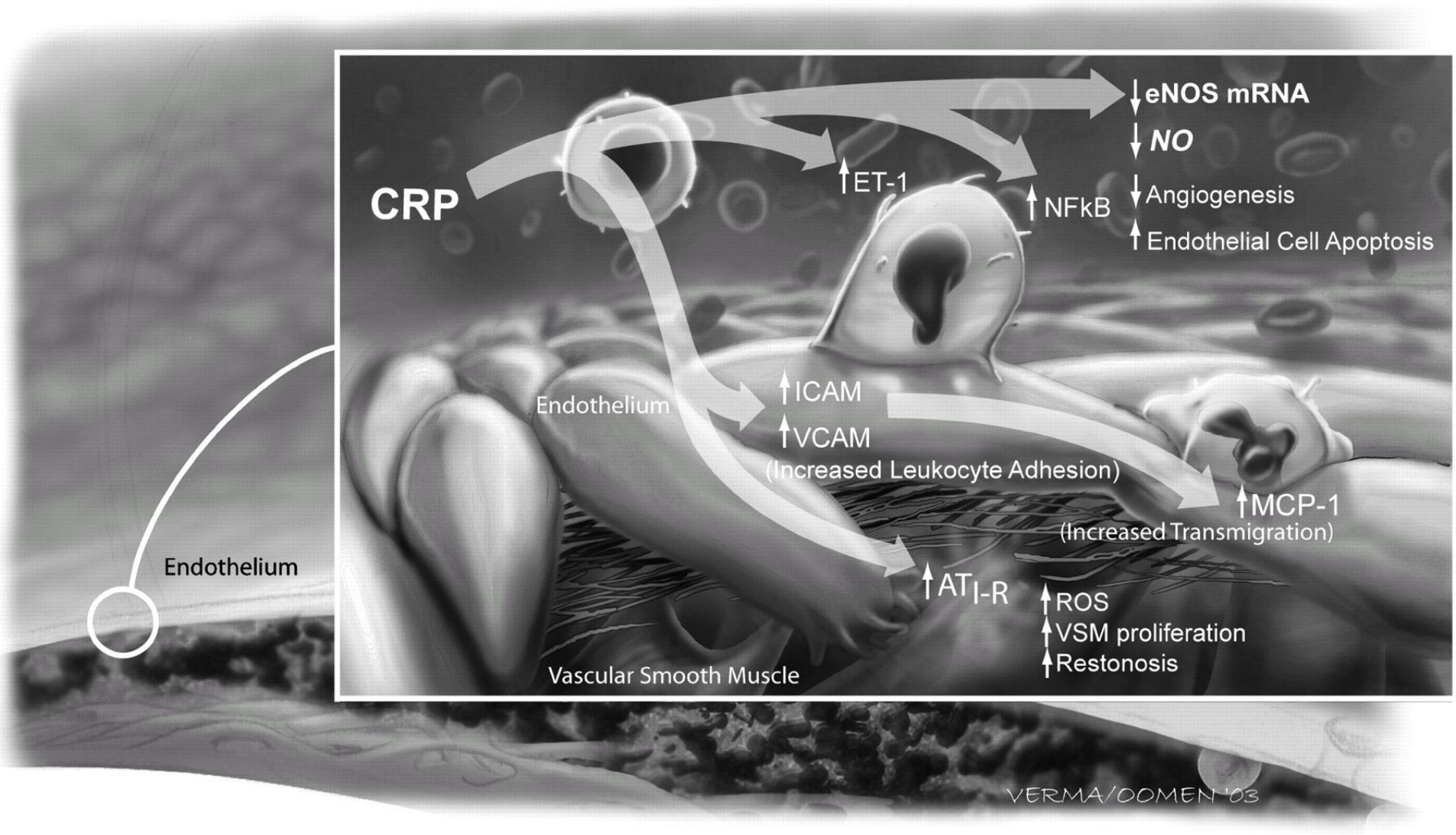

Systemic factors
Local factors
Traditional risk factors
- modify the inflammatory response
- non-inflammatory mechanisms
Genetic predisposition
- genotypes regulating thrombophilia, inflammatory/immune response
Acute / exacerbating chronic infection
- bacteremia, LPS-release
➔CRP, fibrinogen increase
➔ cytokine release
➔ endothelial activation
➔ leukocyte activation
Prothrombotic state involving altered coagulation/fibrinolysis balance and platelet activation
lipid
B
macrophages
SMC
mast cells
B
Monocyte as potential carrier of pathogens to the vessel wall
Traditional risk factors
- proinflammatory effects (monocyte / endothelial activation)
- non-inflammatory mechanisms
Genetic predisposition
- non-inflammatory genes
- inflammatory/immune response
- infection susceptibility
?
Chronic infection
bacterial/viral antigens in plaques and indirect mechanisms:
- immune mechanisms (e.g. HSP)
- release of cytokines, proteinases
- expression of tissue factor, etc.
Inflammatory steps leading to plaque development, destabilization and thrombus formation
Co-incidental precipitation of stroke
⇧ risk of cardio-embolism and plaque thrombosis
⇧ risk of athero-thrombotic stroke

Infiammazione come fattore di rischio e meccanismo patogenetico nell'ictus: eventi sistemici e cerebrali

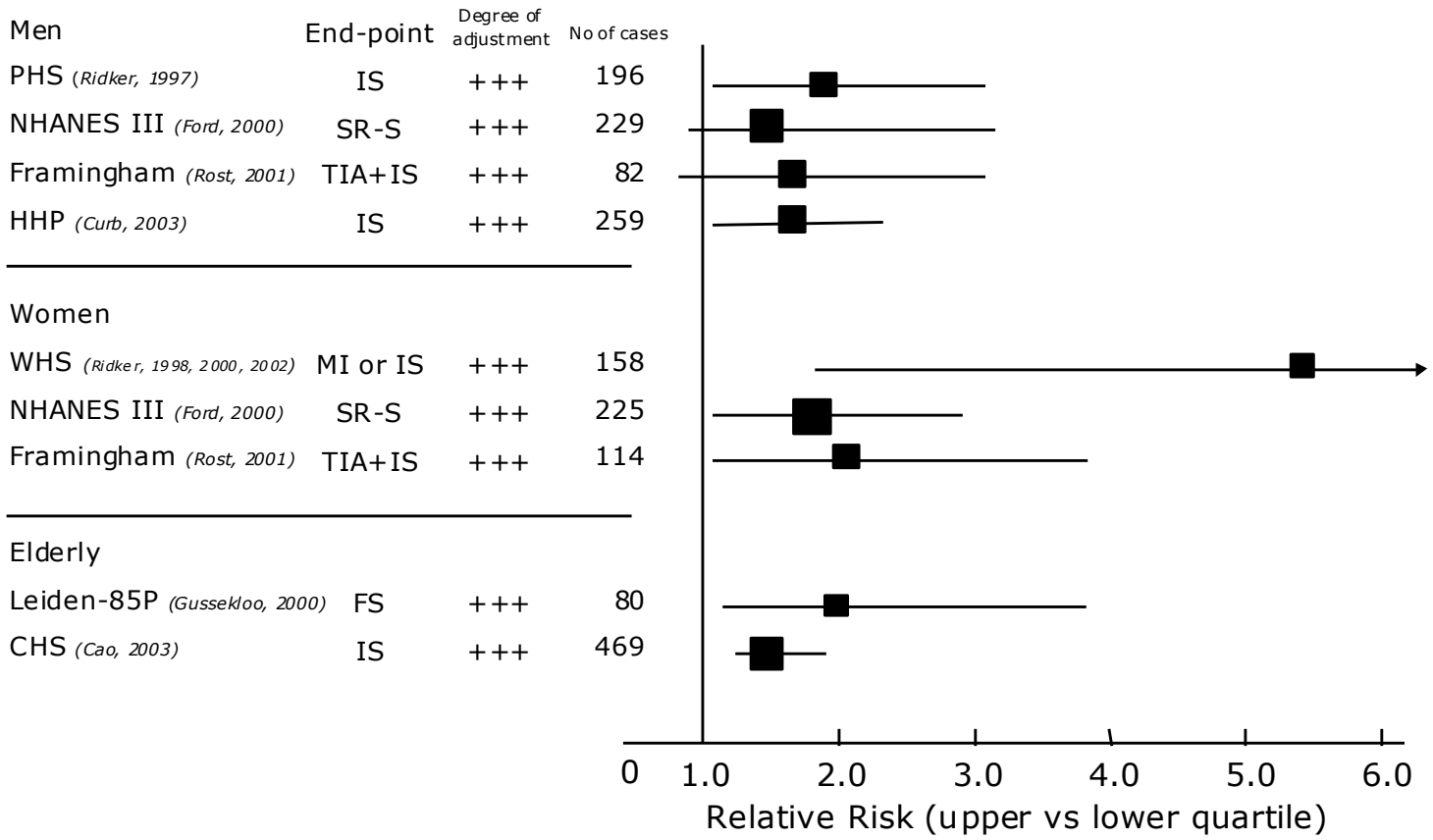


J Cer Blood Flow Metab 2002;22:1399

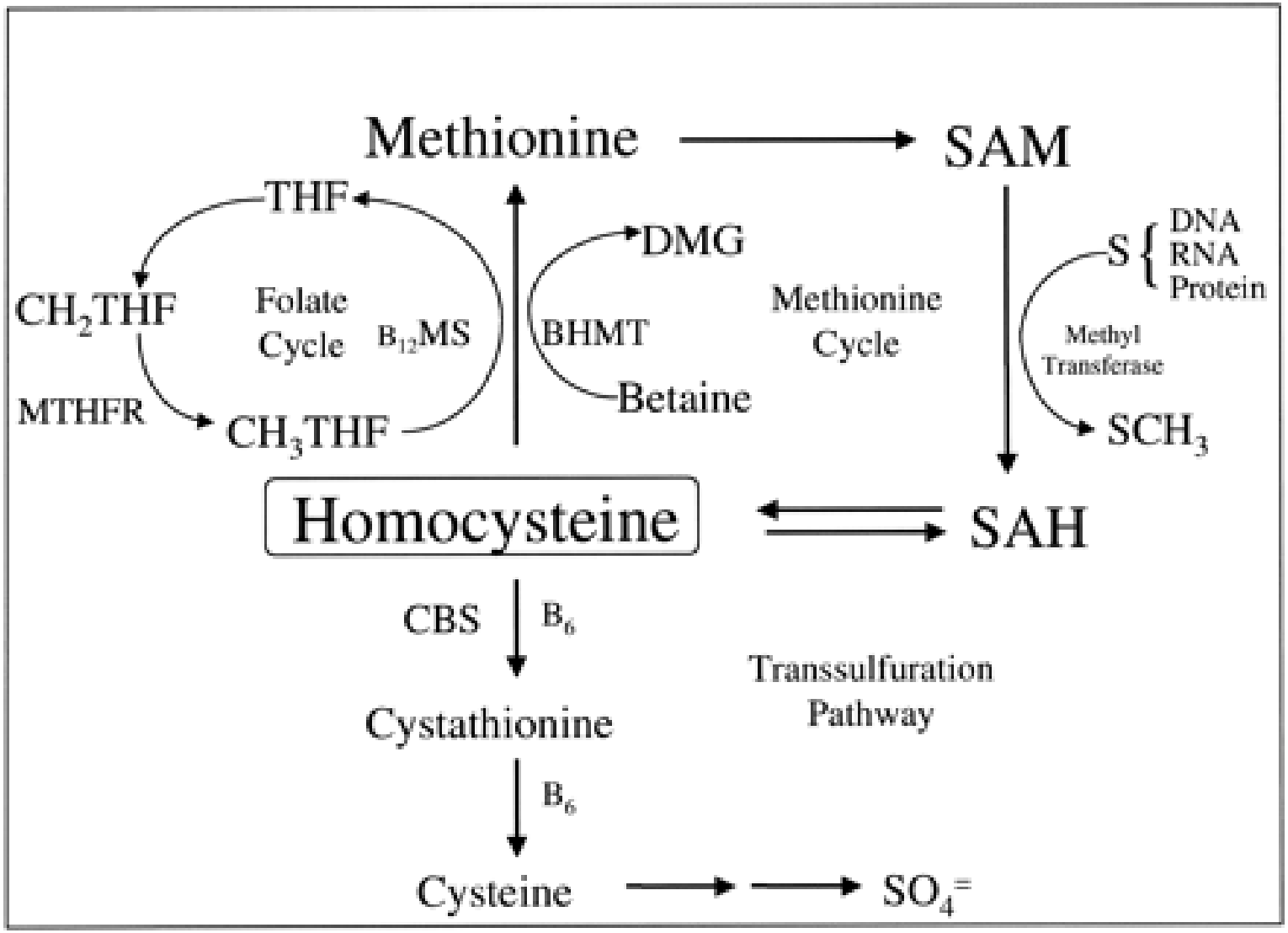
Proatherogenic effect of CRP



Prospective studies relating baseline CRP levels to the risk of first cerebrovascular event



Homocysteine



OMOCISTEINA ED ICTUS

- Rare mutazioni omozigoti CBS: omocistinuria
- Mutazioni eterozigoti: aumentato rischio?
- Poolimorfismi omozigoti MTHFR (10% popolazione): ridotta attività enzimatica (variante termolabile)
- Polimorfismi MTHFR (lieve iperomocisteinemia se associati a deficit di folati): rischio?
- *Aumento di omocisteina dopo l'ictus*

- FATTORE DI RISCHIO PER AD E VD

Table 1. Factors that increase plasma homocysteine levels

Type of factor	Examples
Genetic defects in the enzymes involved in homocysteine metabolism	Trans-sulphuration: cystathionine β-synthase deficiency
	Remethylation: N^5,N^{10}-methylene tetrahydrofolate reductase deficiency or thermolabile variant; methionine synthase defiency
Nutritional	Folate deficiency
	Vitamin B_{12} (combalamin) deficiency (an essential cofactor for methionine synthase)
	Vitamin B_6 (pyridoxine) deficiency (an essential cofactor for cystathionine β-synthase)
	Increased methionine intake (animal protein)
Diseases	Pernicious anaemia
	Renal impairment
	Hypothyroidism
	Malignancy: acute lymphoblastic leukaemia; carcinoma of the breast, ovary and pancreas
	Severe psoriasis
	Transplant recipients
Medications/toxins	Folate antagonists: methotrexate; phenytoin; carbamazepine
	Vitamin B_6 antagonists: theophylline; azaribine; oestrogen-containing oral contraceptives; cigarette smoking
Age/sex	Advanced age
	Male sex
	Postmenopausal women

Meccanismi patogenetici dell'omocisteina

- Aumentata trombogenicità dell'endotelio (inibiz trombomodulina e prot. C, aumentata adesione piastrinica)
- Interazione con NO e sua deplezione
- Aumentati ROS endoteliali: stress ossidativo
- Deficit di metilazione DNA e istoni: alterata espressione genica

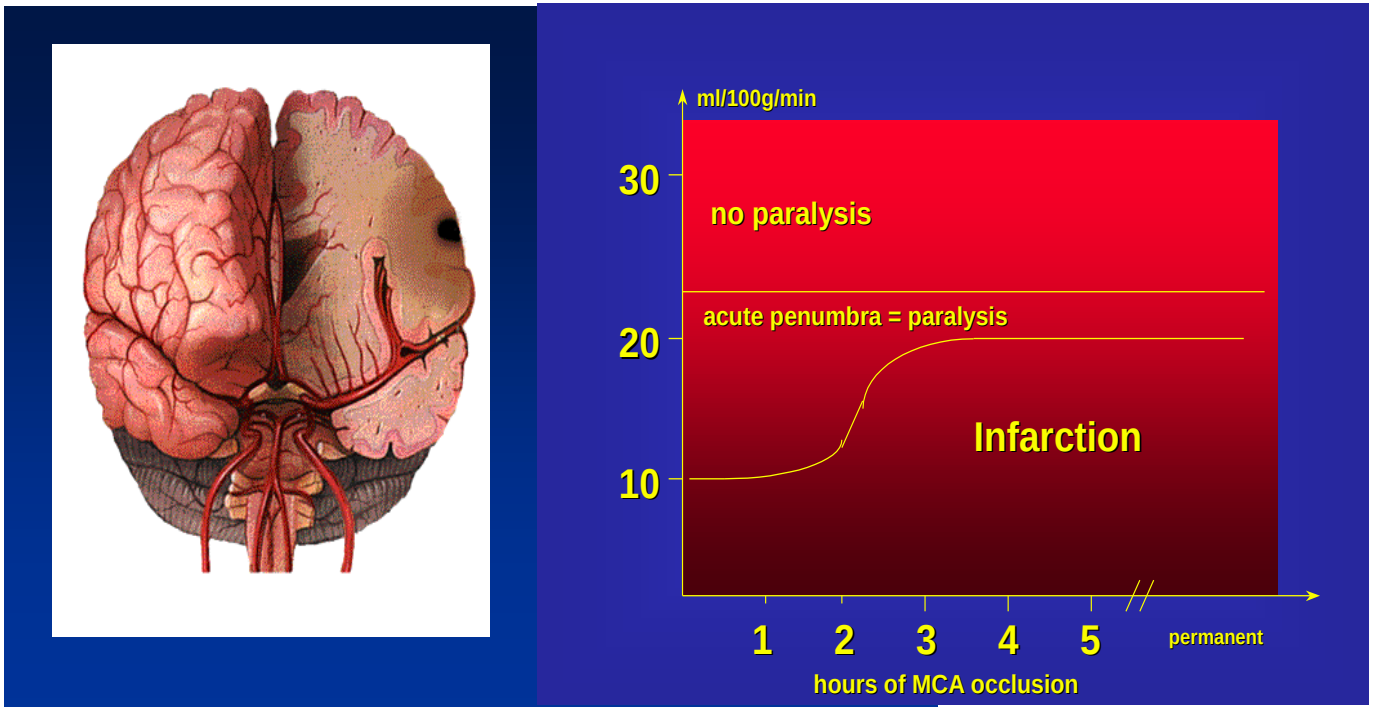
The role of platelets

- Platelets attach to dysfunctional endothelium, macrophages, and exposed collagen.
- The activated platelets release granules containing cytokines and growth factors, causing conversion of arachidonic acid to both thromboxane A2, leading to further platelet aggregation, and leukotrienes, thereby amplifying the inflammatory process.
- Platelets also can be activated by platelet-activating factor (PAF), which is produced by monocytes, endothelial cells, and neutrophils. PAF causes platelet aggregation and degranulation, and also can promote leukocyte activation.
- Platelets release serotonin and glutamate

- **Meccanismi di danno neuronale post-ischemico**

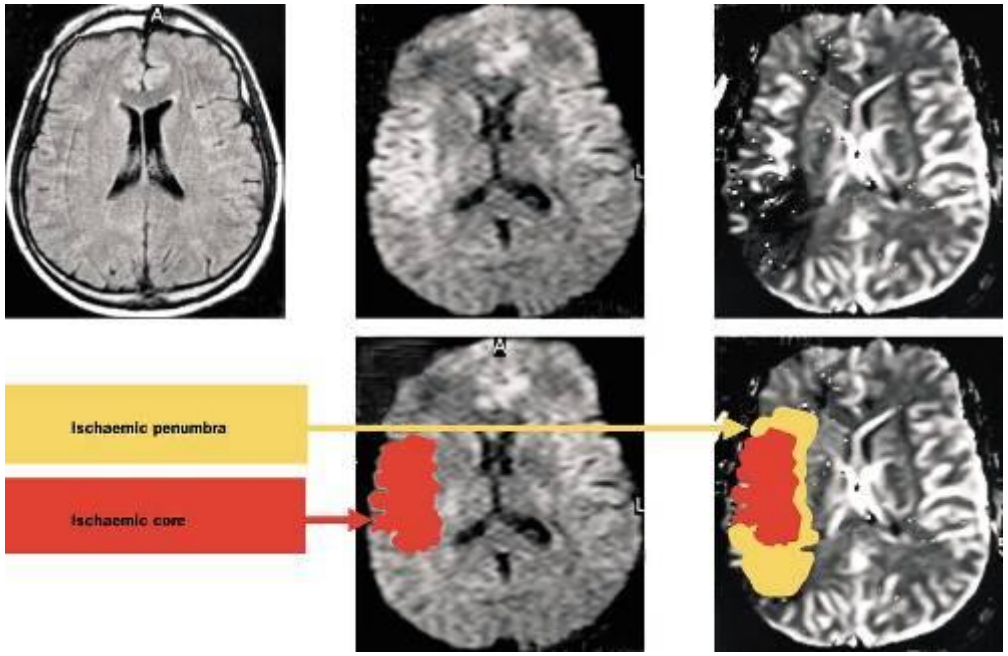
Progressione di eventi legati alla riduzione del flusso ematico cerebrale

(PENOMBRA ISCHEMICA-FINESTRA TERAPEUTICA)



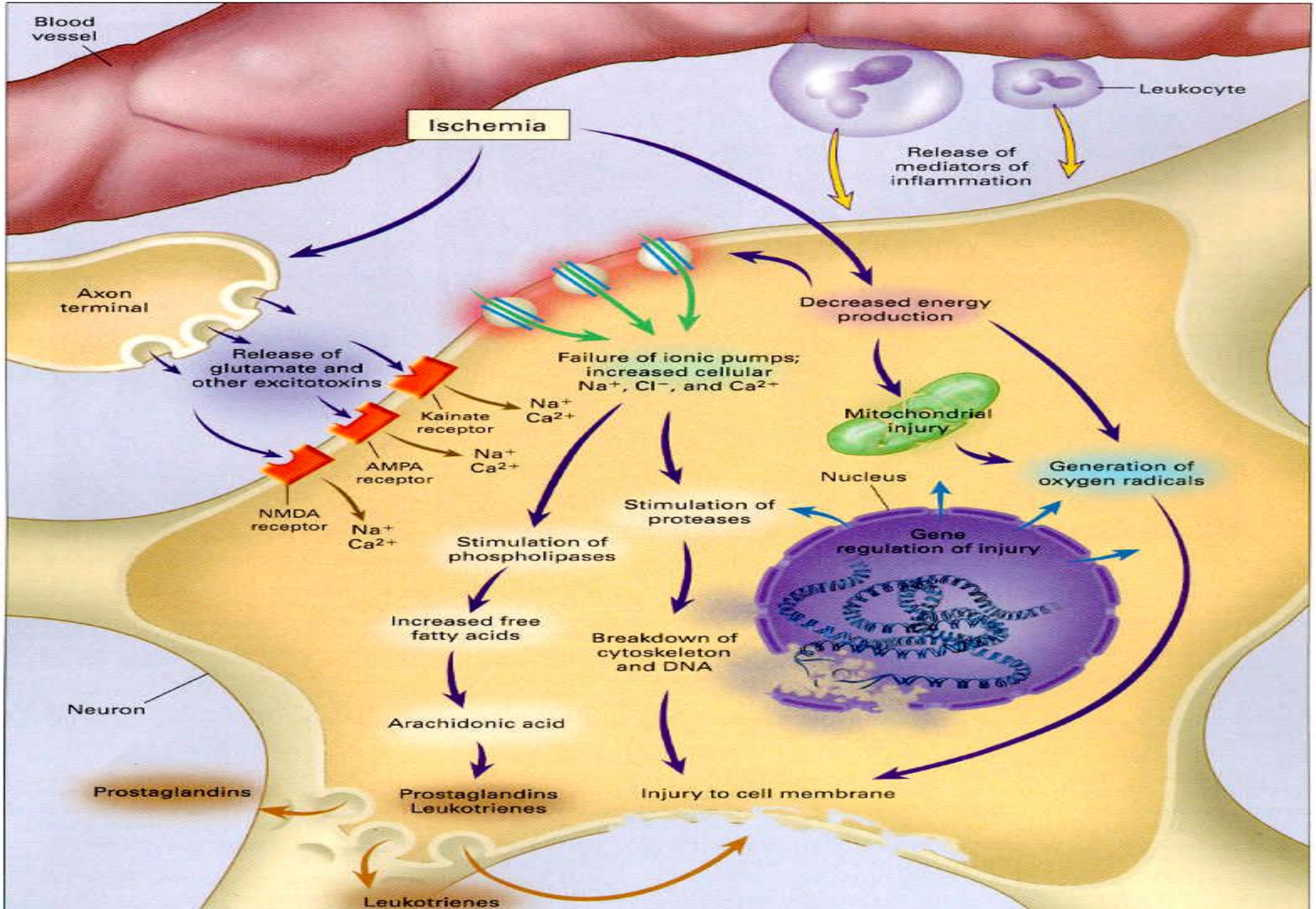
IMPORTANZA DEI CIRCOLI COLLATERALI!

Penumbra-based treatment

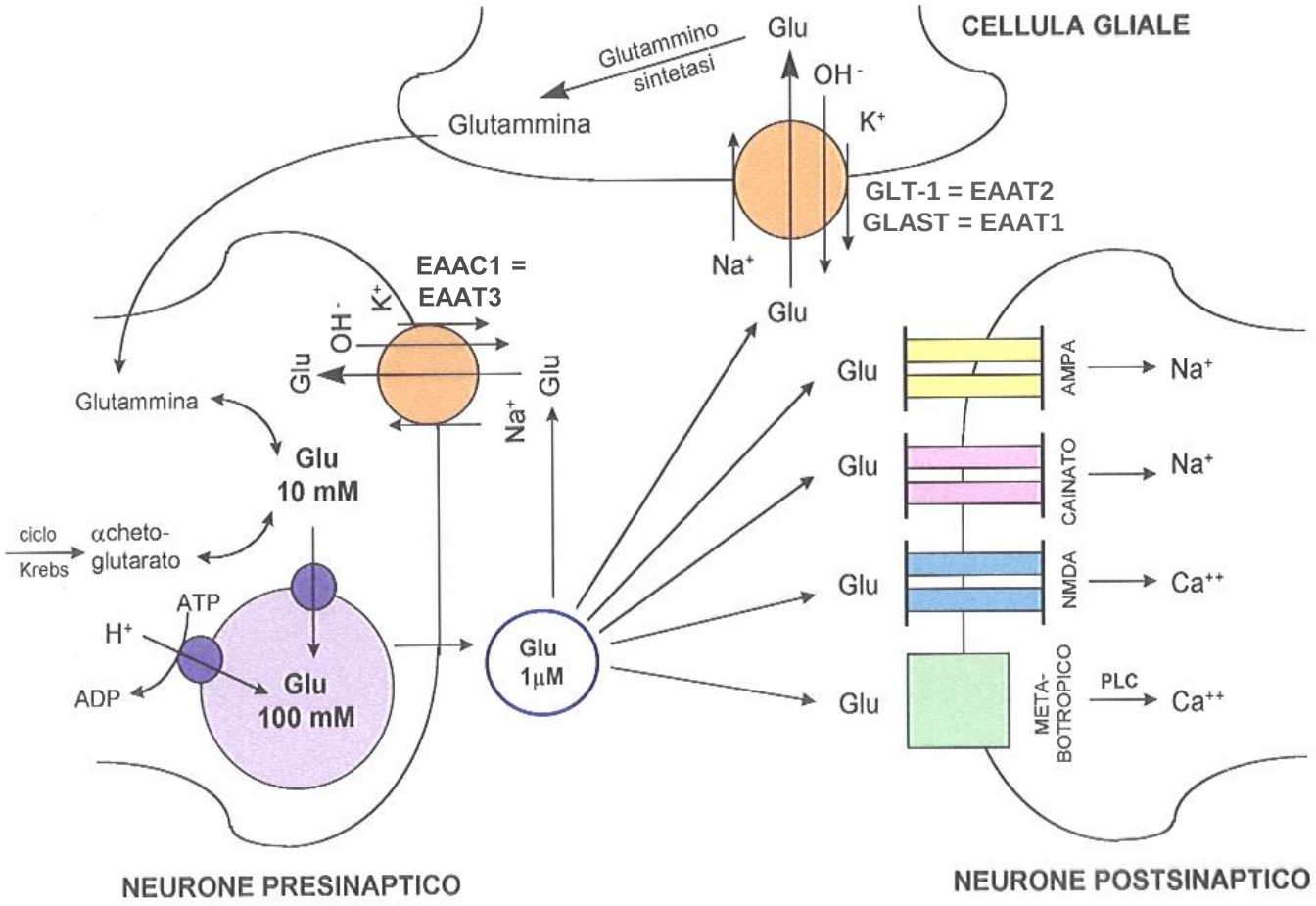


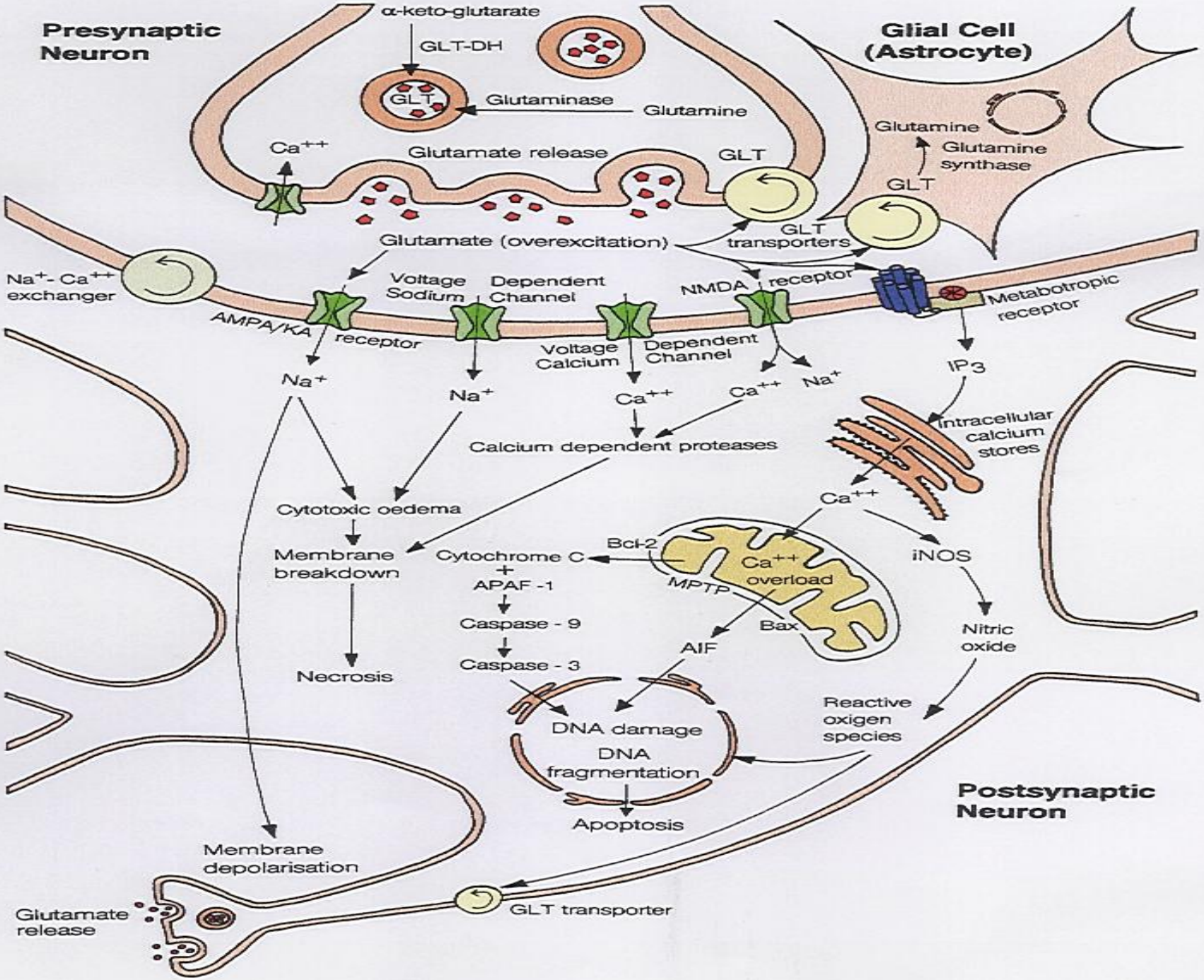
« Time is brain » → « Penumbra is brain »

La “cascata ischemica”



Neurotrasmissione glutammatergica

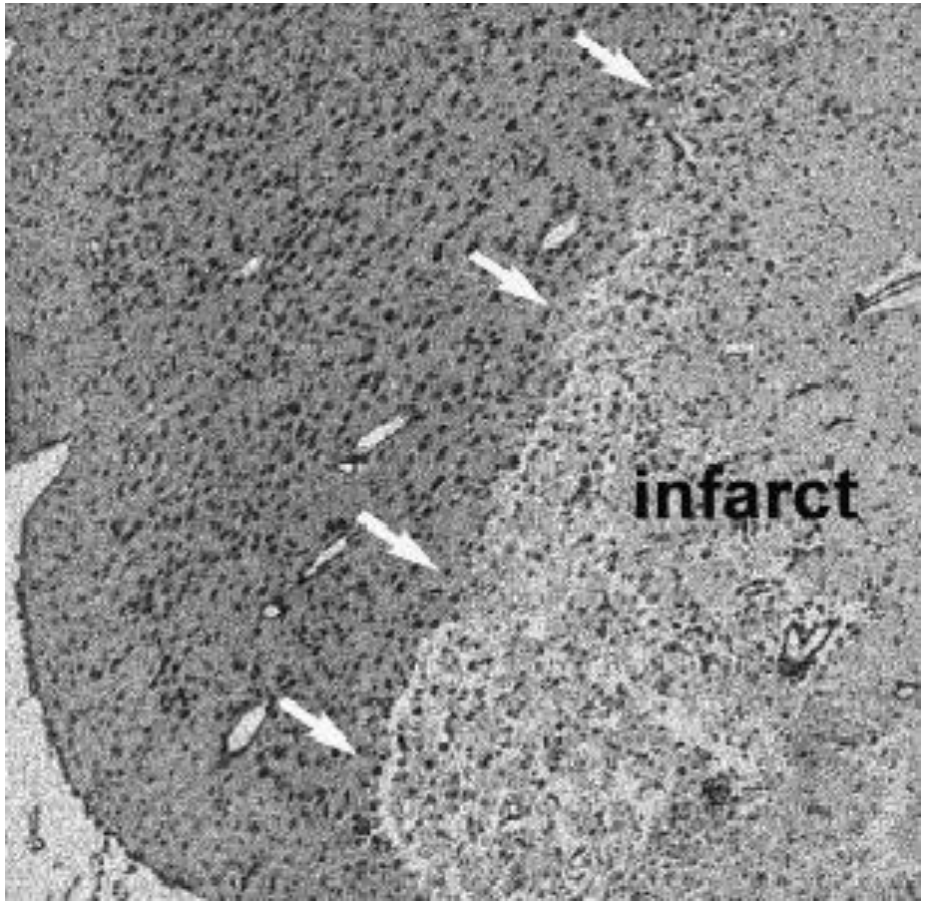


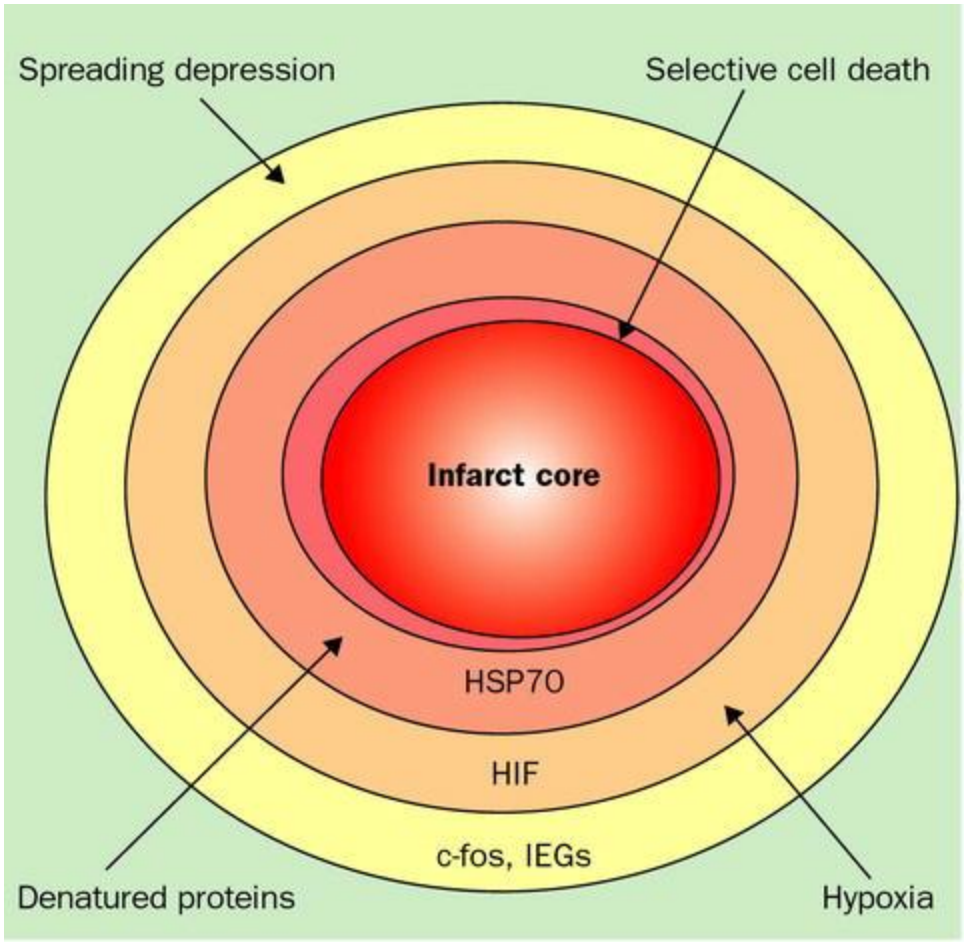

Presynaptic Neuron
α-keto-glutarate
GLT-DH
GLT
Glutaminase
Glutamine
Ca++
Glutamate release
GLT
Glial Cell (Astrocyte)
Glutamine
Glutamine synthase
GLT
GLT transporters
Glutamate (overexcitation)
Na+- Ca++ exchanger
Voltage Sodium Dependent Channel
NMDA receptor
Metabotropic receptor
AMPA/KA receptor
Voltage Calcium Dependent Channel
IP3
Na+
Na+
Ca++
Ca++
Na+
Calcium dependent proteases
intracellular calcium stores
Cytotoxic oedema
Ca++
Membrane breakdown
Cytochrome C + APAF -1
Bcl-2
Ca++ overload
MPTP
Bax
iNOS
Caspase - 9
Caspase - 3
AIF
Nitric oxide
Necrosis
Reactive oxigen species
DNA damage
DNA fragmentation
Apoptosis
Postsynaptic Neuron
Membrane depolarisation
Glutamate release
GLT transporter

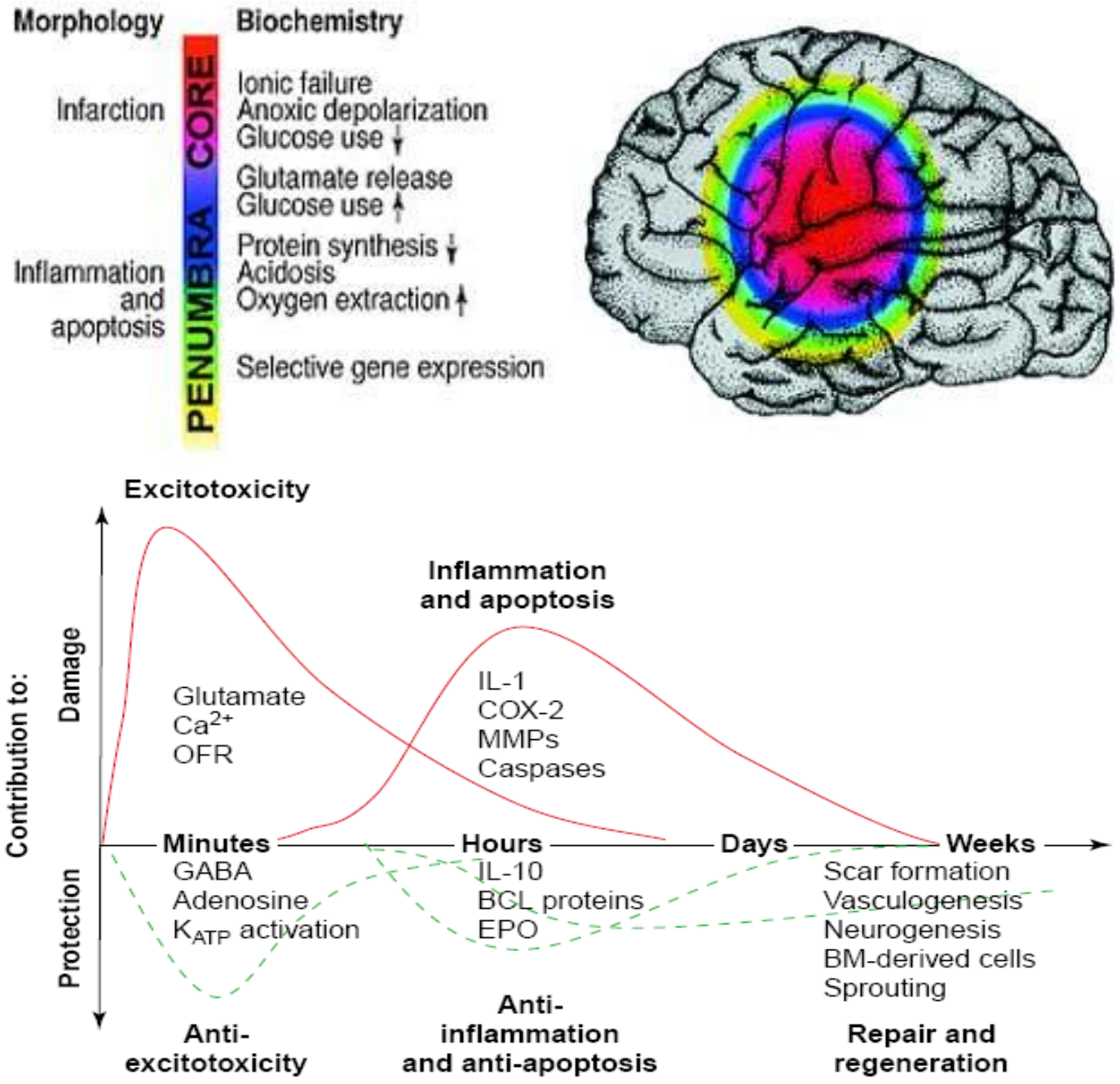
The “Penumbra”

Area of “silent”, yet viable, tissue

“Molecular” Penumbra






Morphology
Biochemistry
Infarction
Inflammation and apoptosis
PENUMBRA
CORE
Ionic failure
Anoxic depolarization
Glucose use ↓
Glutamate release
Glucose use ↑
Protein synthesis ↓
Acidosis
Oxygen extraction ↑
Selective gene expression
Excitotoxicity
Inflammation and apoptosis
Contribution to:
Damage
Protection
Glutamate
Ca2+
OFR
IL-1
COX-2
MMPs
Caspases
Minutes
Hours
Days
Weeks
GABA
Adenosine
KATP activation
IL-10
BCL proteins
EPO
Scar formation
Vasculogenesis
Neurogenesis
BM-derived cells
Sprouting
Anti-excitotoxicity
Anti-inflammation and anti-apoptosis
Repair and regeneration
TRENDS in Neurosciences

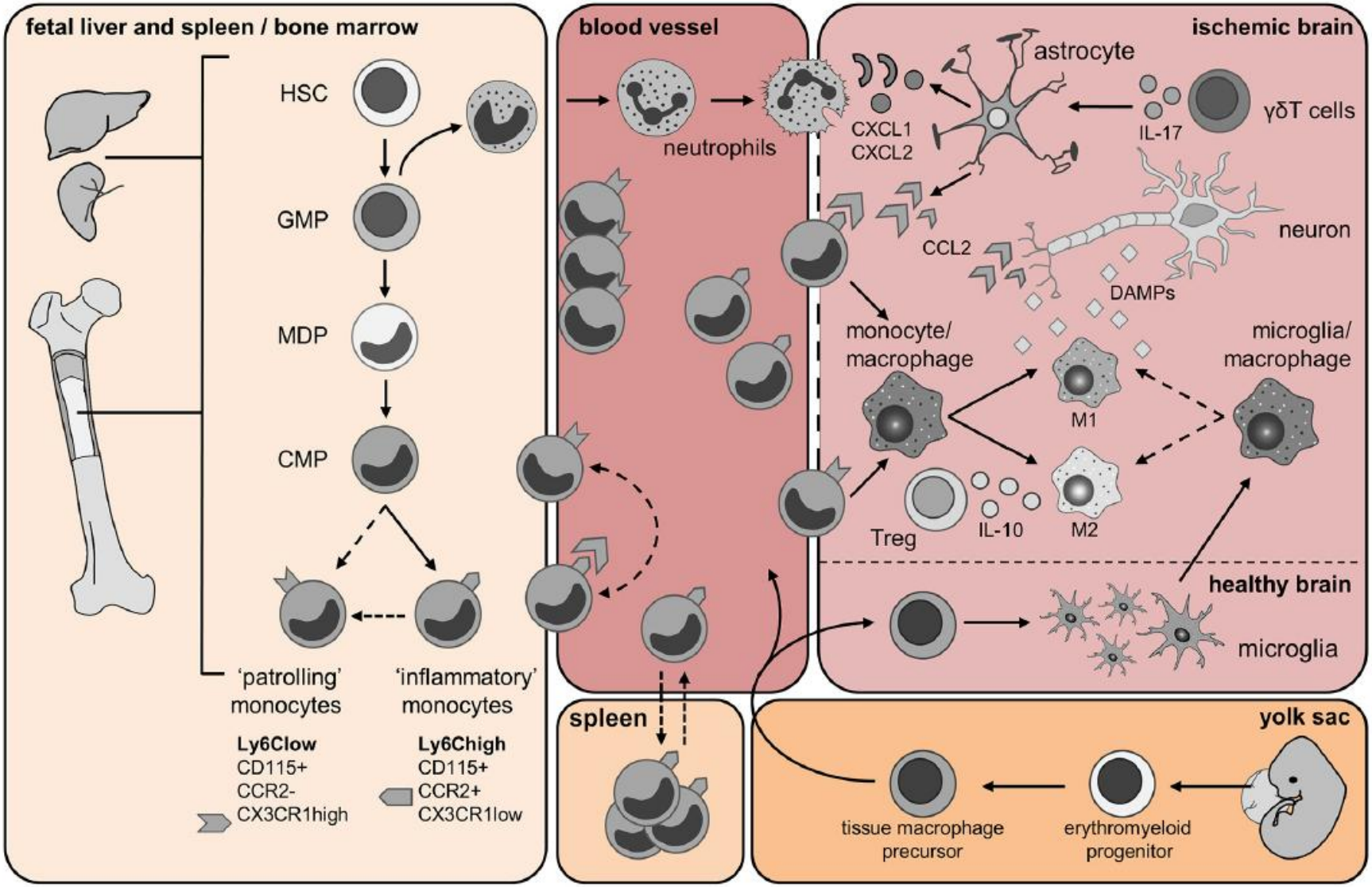
frontiers in
CELLULAR NEUROSCIENCE

REVIEW ARTICLE
published: 14 January 2015
doi: 10.3389/fncel.2014.00461

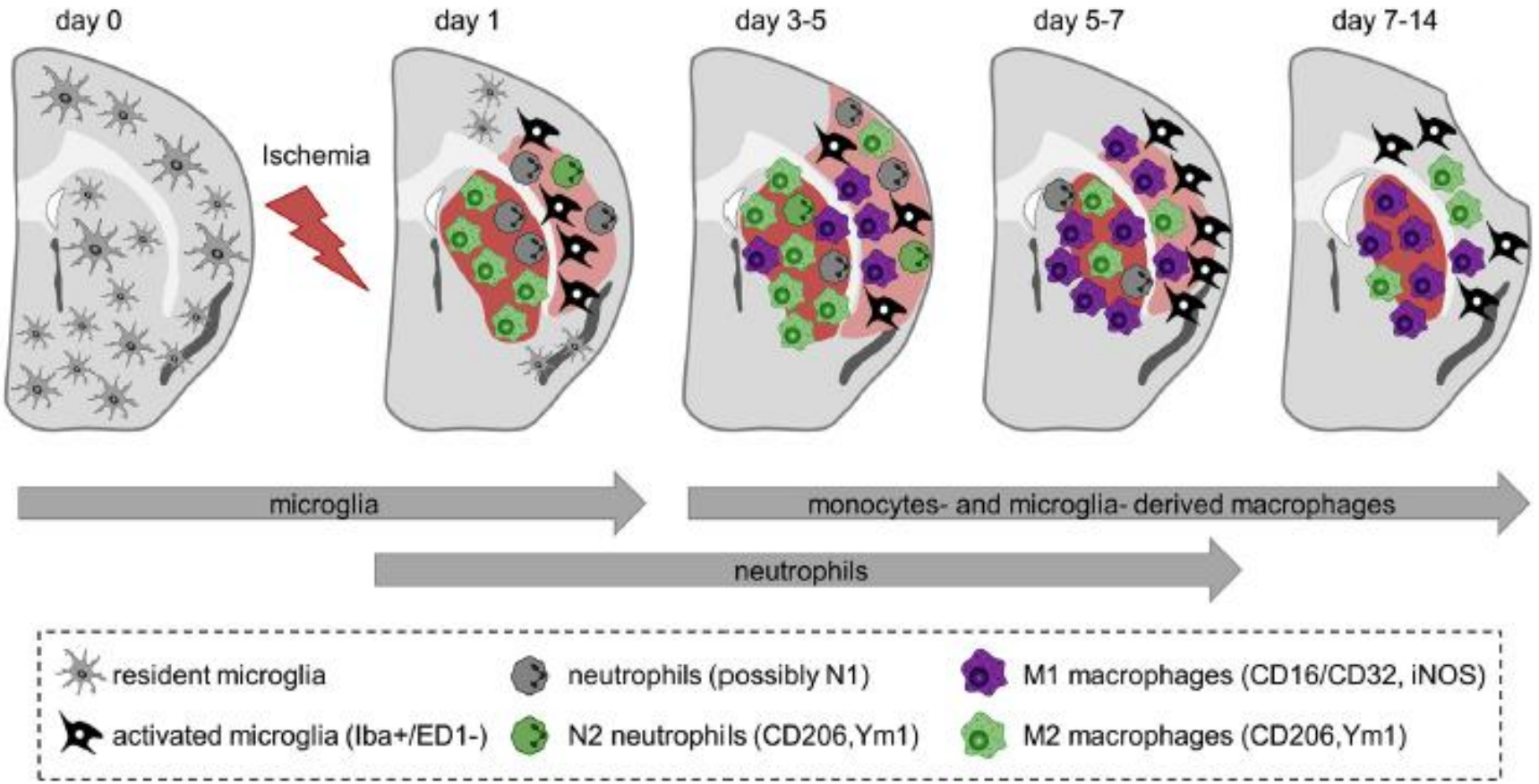
The role of microglia and myeloid immune cells in acute cerebral ischemia

*Corinne Benakis, Lidia Garcia-Bonilla, Costantino Iadecola and Josef Anrather**

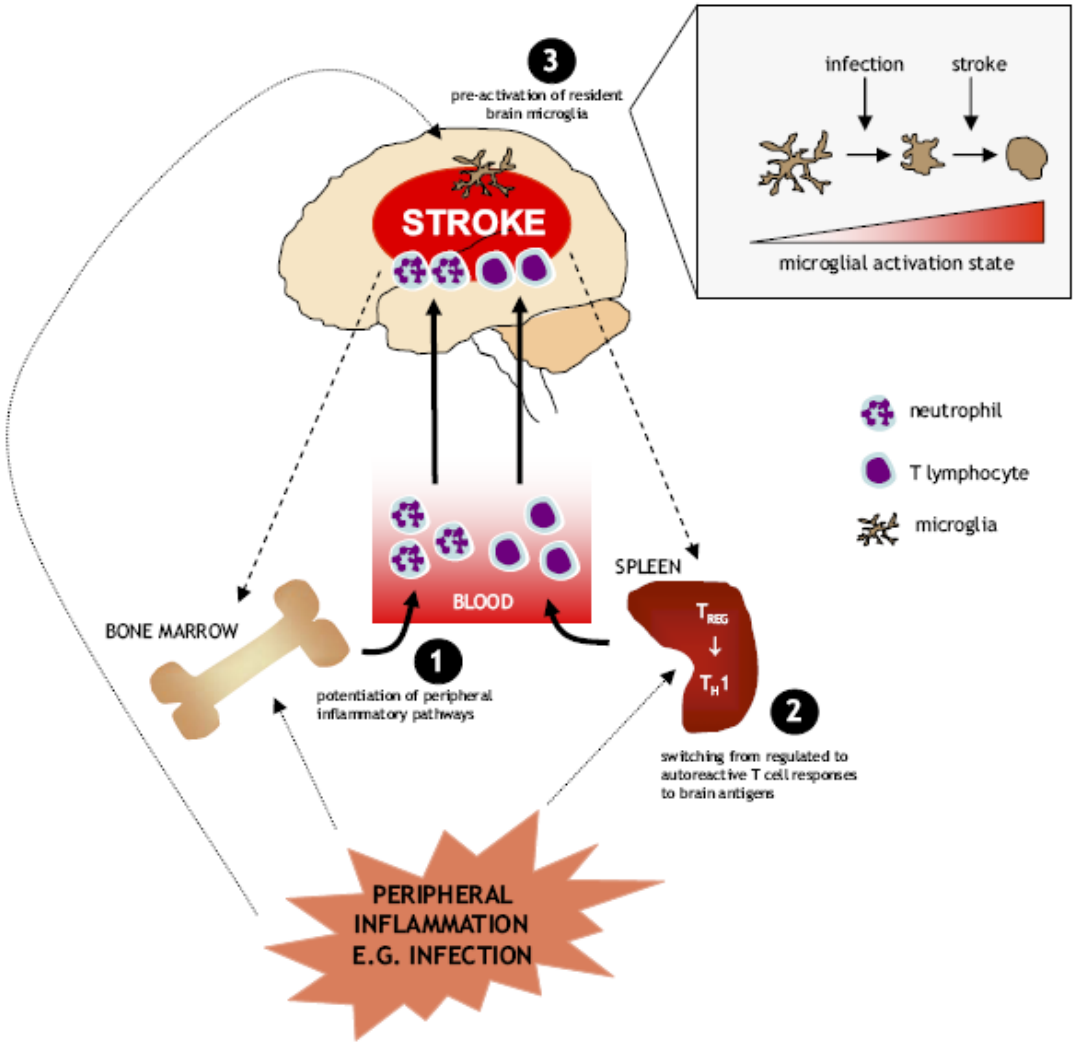
Feil Family Brain and Mind Research Institute, Weill Cornell Medical College, New York, NY, USA



Spatio-temporal profile of microglia and myeloid immune cells and their polarization state in transient brain ischemia

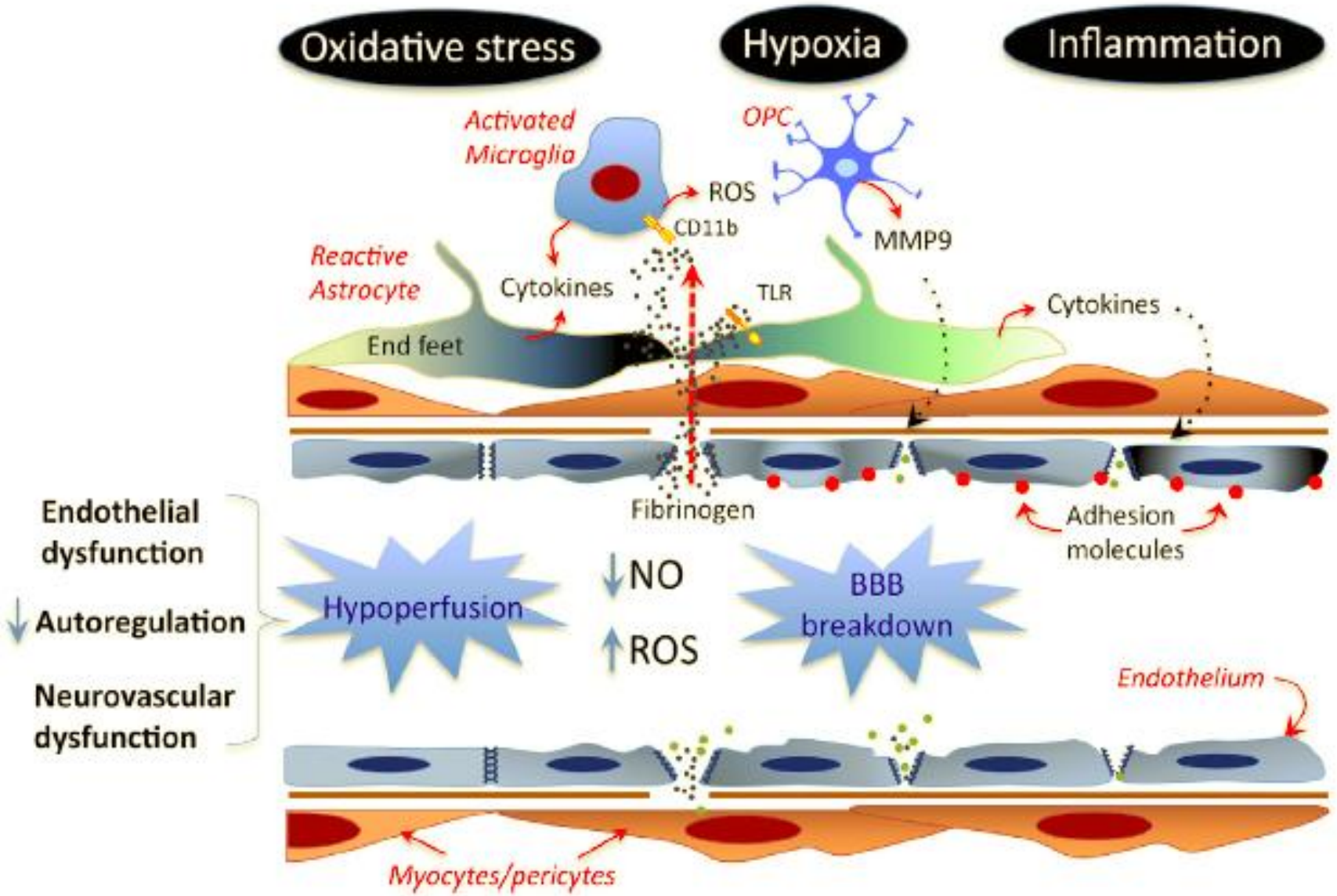


INFLAMMAZIONE SISTEMICA-INFEZIONI E DANNO POST-ISCHEMICO

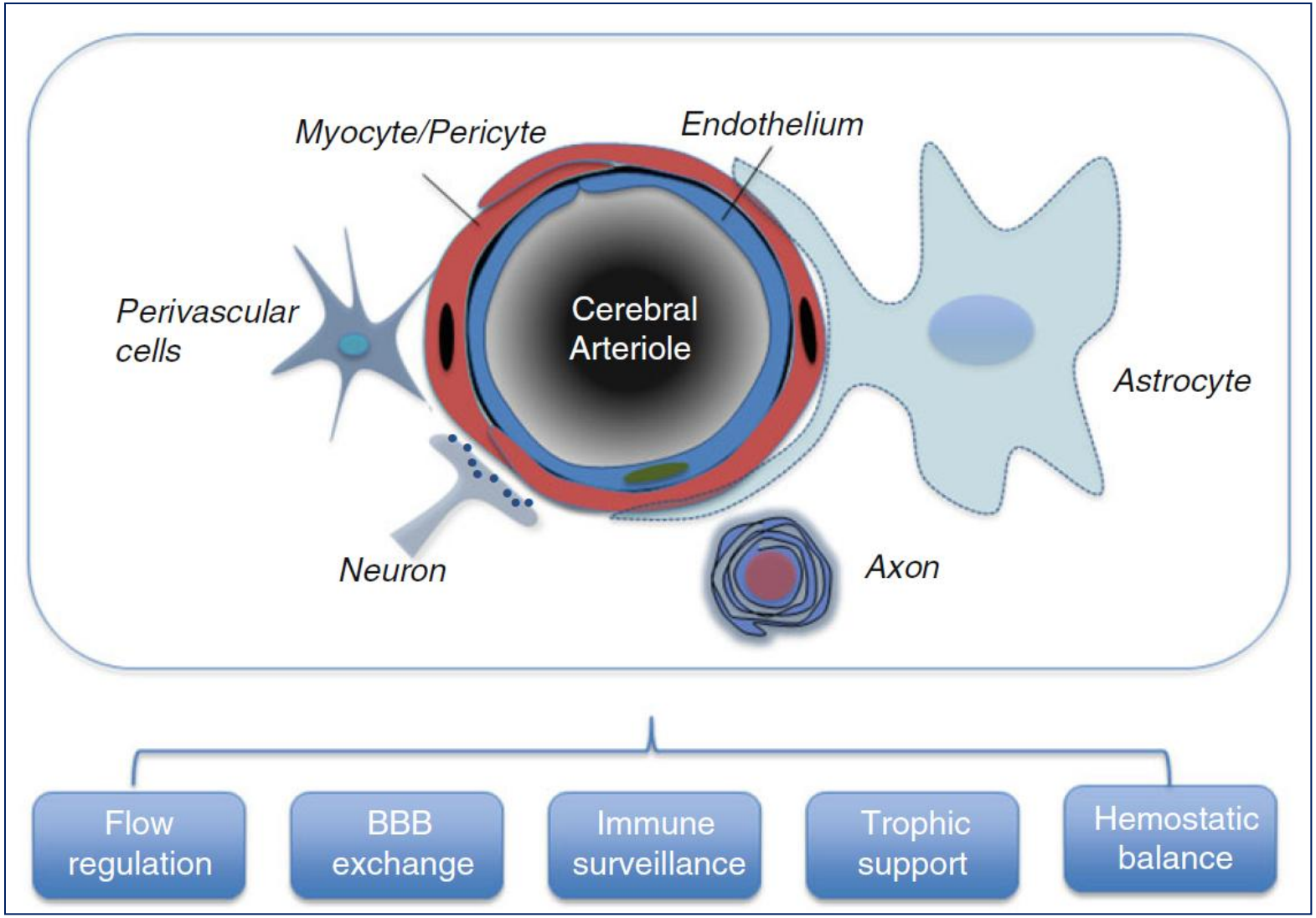


1. Systemic inflammation can potentiate the cellular inflammatory responses to stroke originating in peripheral organs.
2. Systemic inflammation can convert the regulated T-cells responses to exposed brain antigens to autoreactive TH1 responses
3. Systemic inflammation can prime microglia via immune-brain signaling pathways which could alter their subsequent response to an ischemic insult

INFIAMMAZIONE E DANNO DELLA BARRIERA EMATO-ENCEFALICA

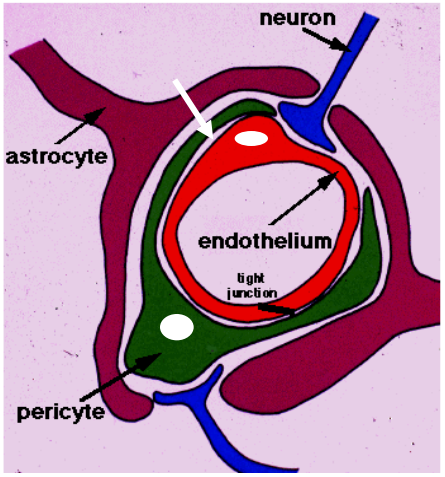


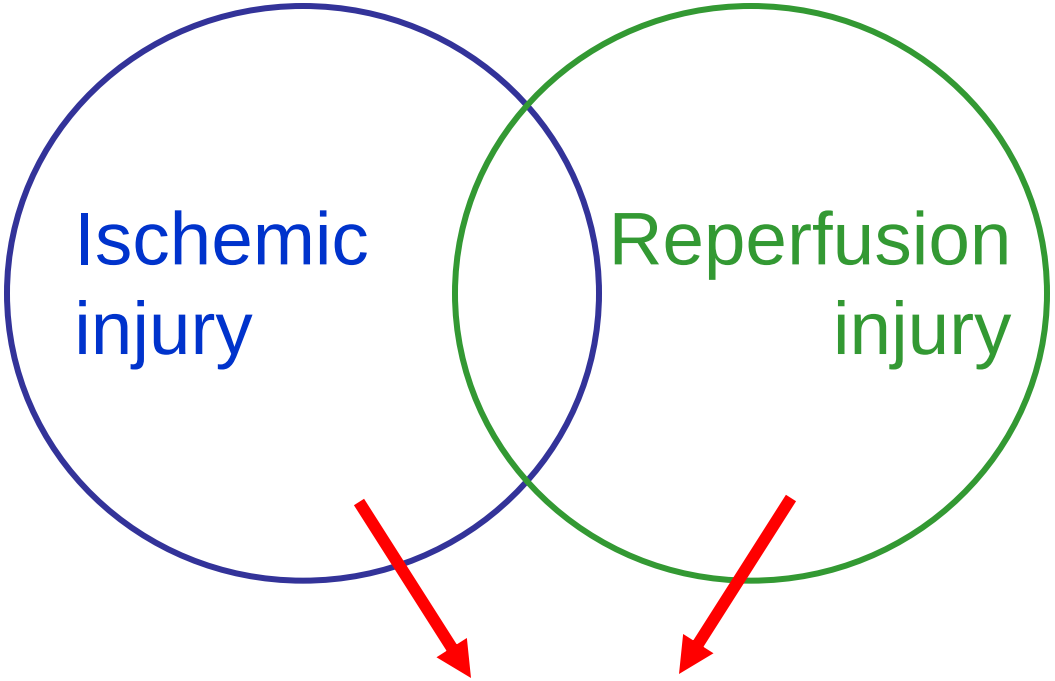
The neurovascular unit



Iadecola C. Acta Neuropathol 2010;120:287–296

Limit the damage to the neurovascular unit



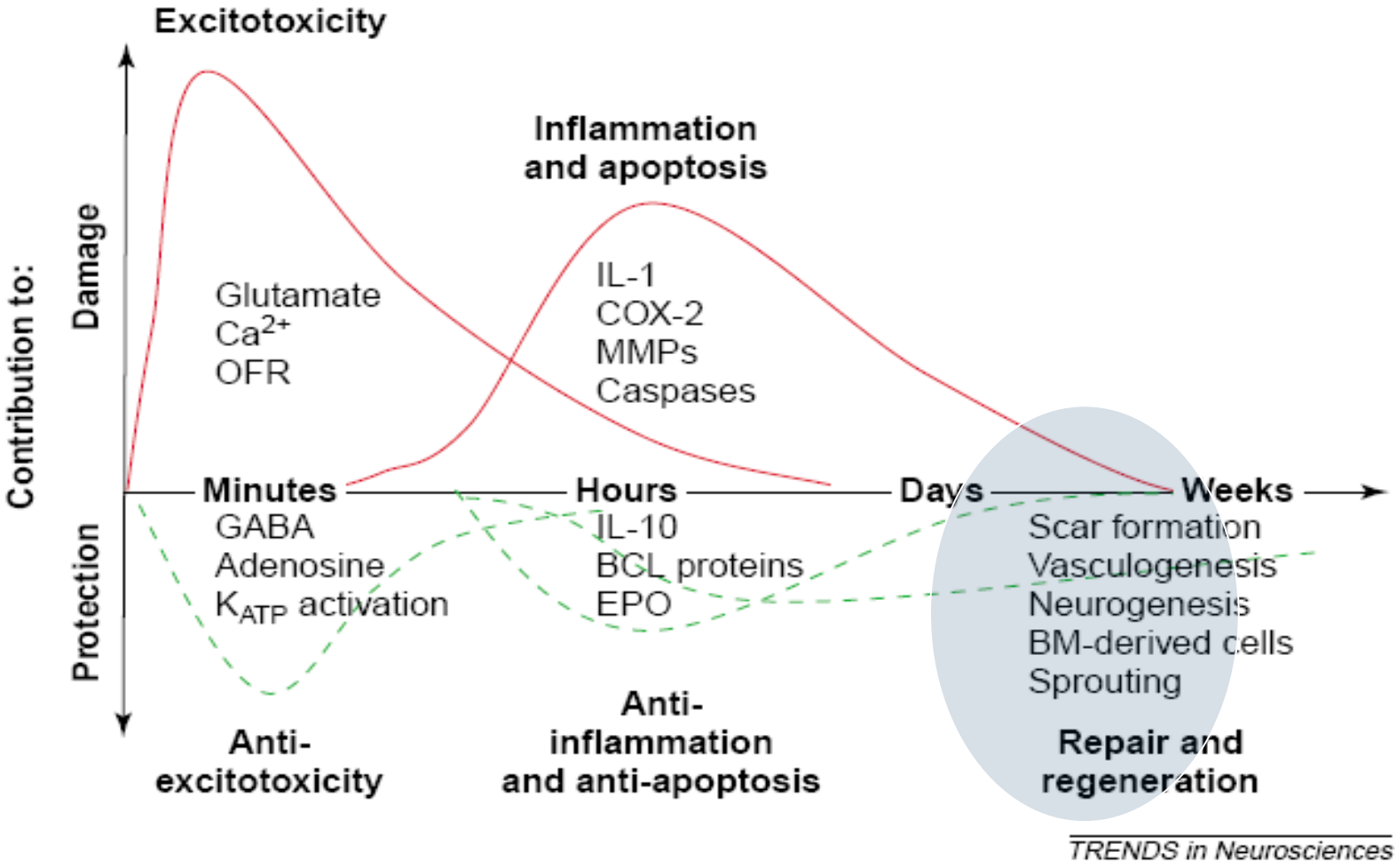


Neuro-toxicity
Glial toxicity
Endothelial toxicity

Haemorrhage

- **Meccanismi protettivi e riparativi**

Focal cerebral ischemia induces a complex series of mechanisms



Dirnagl, Simon, and Hallenbeck. TINS 2003

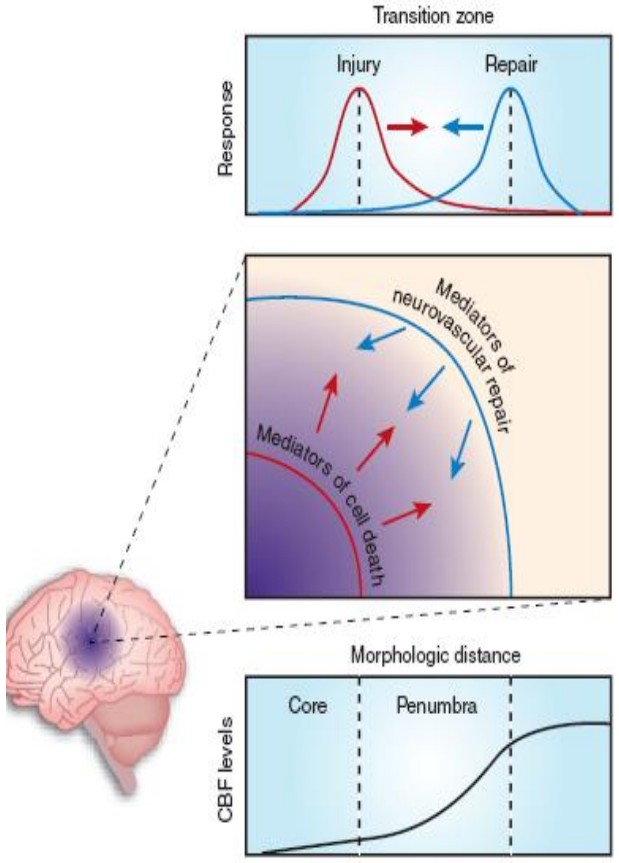
PERSPECTIVE

nature medicine

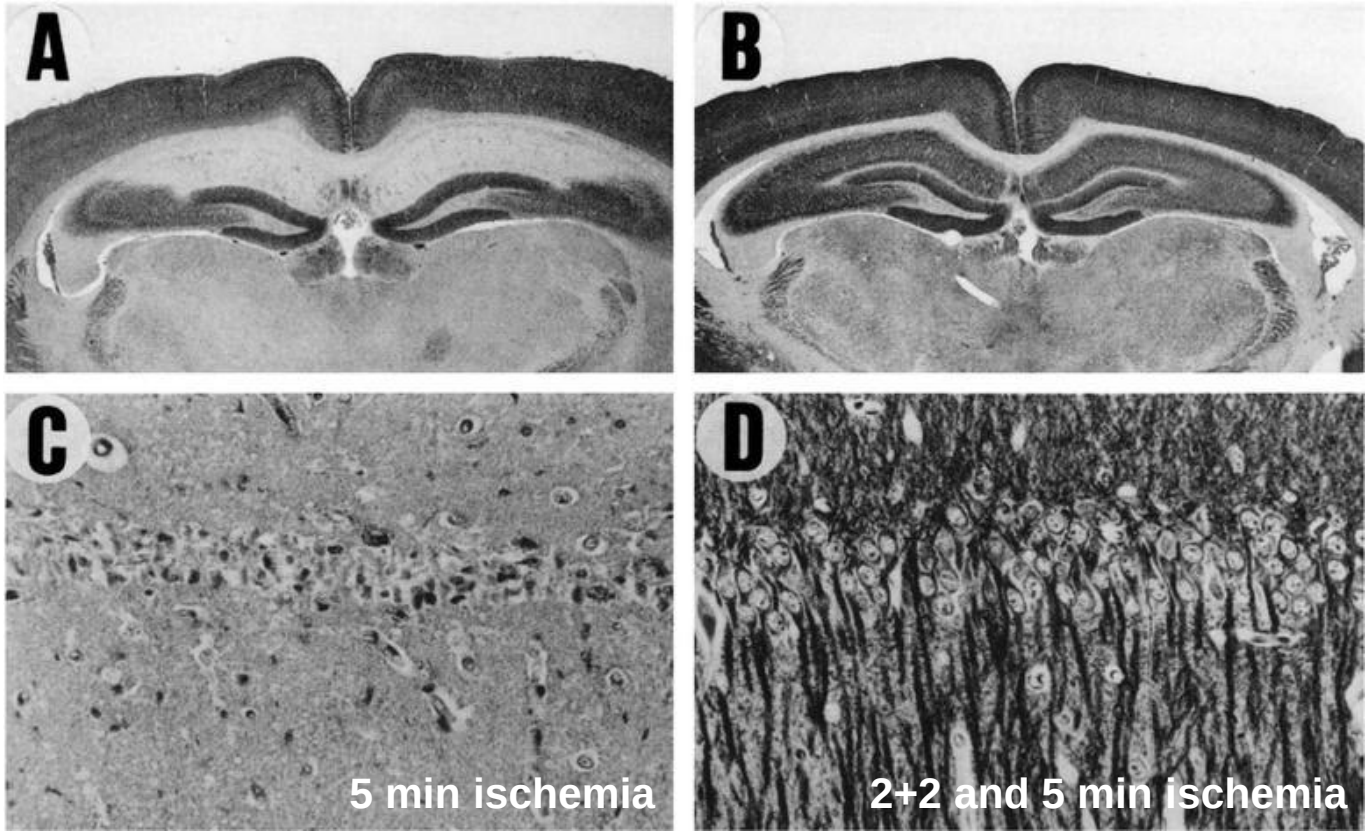
A new penumbra: transitioning from injury into repair after stroke

Eng H Lo

paradigm shift is needed. Most molecular targets for therapy have biphasic roles in stroke pathophysiology. During the acute phase, these targets mediate injury. During the recovery phase, the same mediators contribute to neurovascular remodeling. It is this boundary zone that comprises the new penumbra, and future investigations should dissect where, when and how damaged brain makes the transition from injury into repair.



Global Ischemic Tolerance



Kitagawa K et al, 1990

Attenuated Stroke Severity After Prodromal TIA

A Role for Ischemic Tolerance in the Brain?

M. Weih, MD; K. Kallenberg; A. Bergk; U. Dirnagl, MD; L. Harms, MD;
K.D. Wernecke, PhD; K.M. Einhäupl, MD

Background and Purpose—Ischemic tolerance has been extensively studied in experimental models of heart and brain ischemia. While there is some clinical evidence of ischemic tolerance in the heart, it is not known whether the same is true for the human brain.

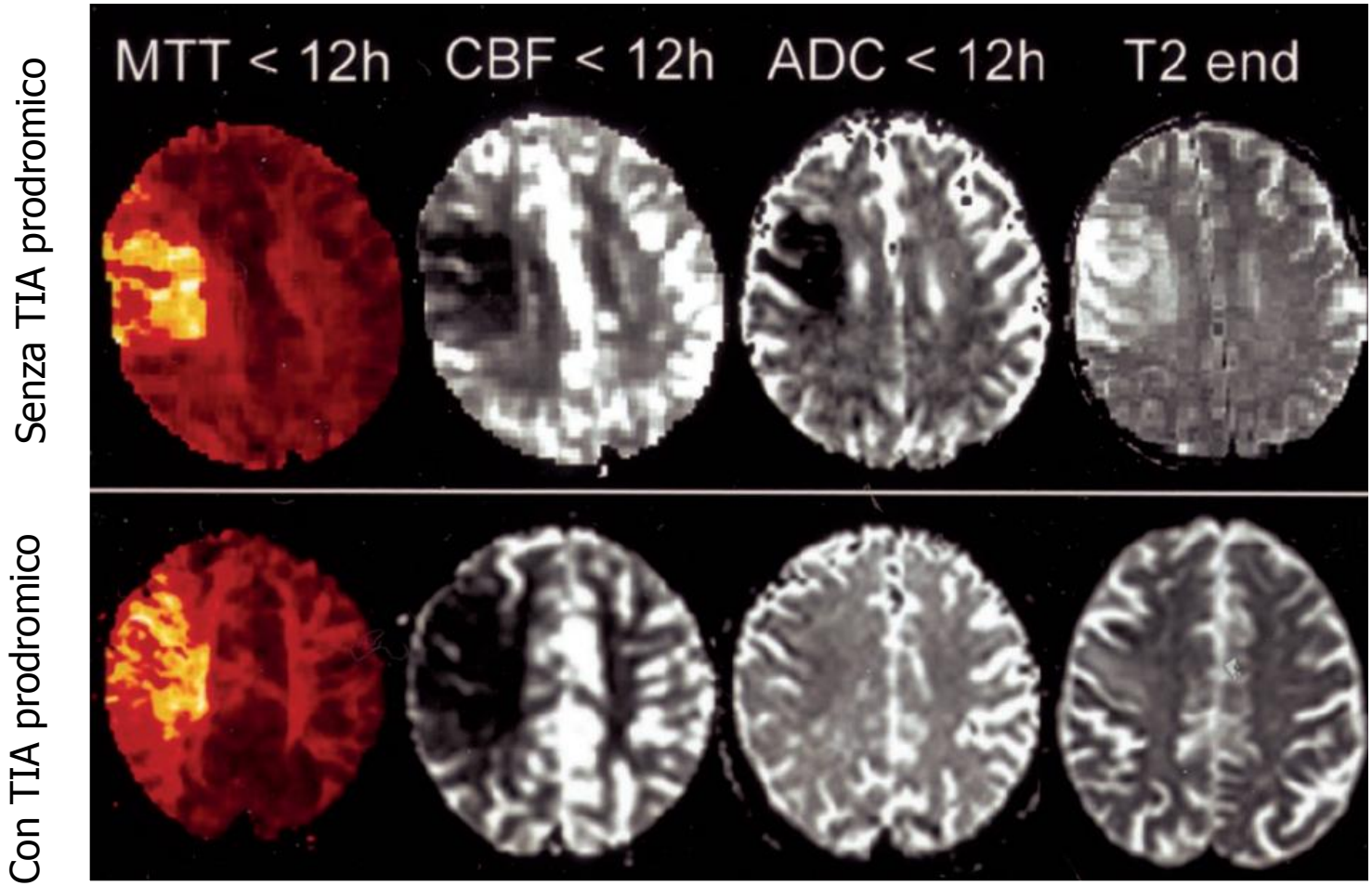
Methods—We conducted a retrospective case-control study in 148 stroke patients with and without antecedent TIA.

Results—Despite no significant differences in baseline characteristics, independence (Rankin scale score of 0 to 1) and favorable outcome (Glasgow Coma Scale score of 5) were significantly associated with prior TIA in univariate analysis. After correction for other cardiovascular risk factors, TIA before stroke also was an independent predictor of mild stroke (Canadian Neurological Scale score of ≥6.5) in multivariate models (absolute difference 21.6%; $P=0.01$).

Conclusions—Assuming that a TIA represents an adequate stimulus to elicit ischemic tolerance, our results suggest that ischemic tolerance might occur in the human brain. (***Stroke.*** **1999;30:1851-1854.)**

TIA	Cases	Controls	OR (95% CI)
	Severe stroke (n=40)	Mild stroke (n=108)	
No (n=111)	36	75	
Yes (n=37)	4	33	3.96 (1.38–11.38)
	Dependent, n=52	Independent, n=56	
No (n=81)	45	36	
Yes (n=27)	7	20	3.57 (1.39–9.14)
	Unfavorable, n=73	Favorable, n=54	
No (n=93)	59	34	
Yes (n=34)	14	20	2.48 (1.12–5.49)

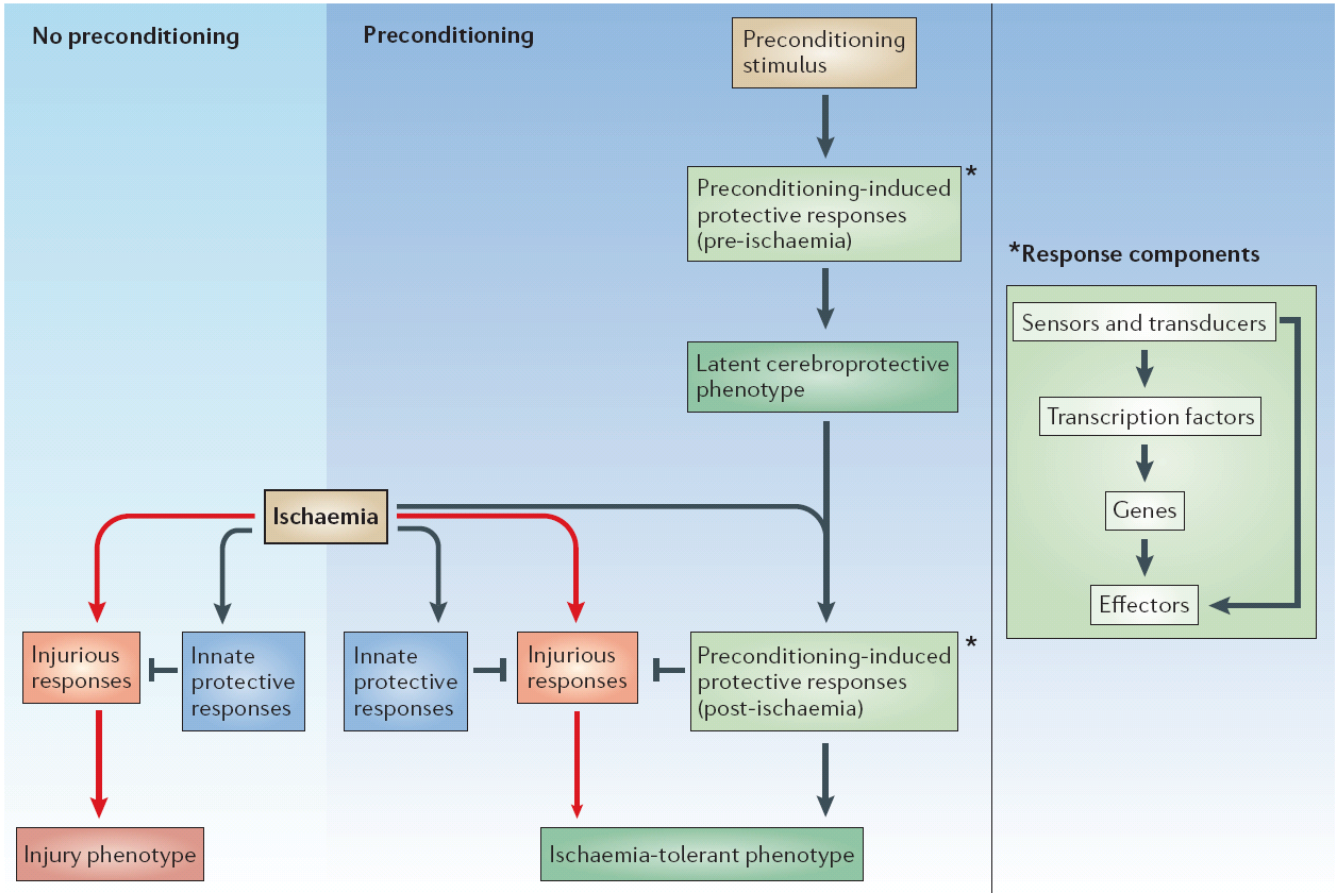
Independent indicates Rankin scale score 0–1; favorable outcome, Glasgow Coma Scale score 5; and unfavorable outcome, Glasgow Coma Scale score 1–4.



Il paziente con TIA prodromico ha un infarto nel territorio dell'ACM di minori dimensioni rispetto al paziente senza TIA prodromico

Stroke 2004;35:616-621

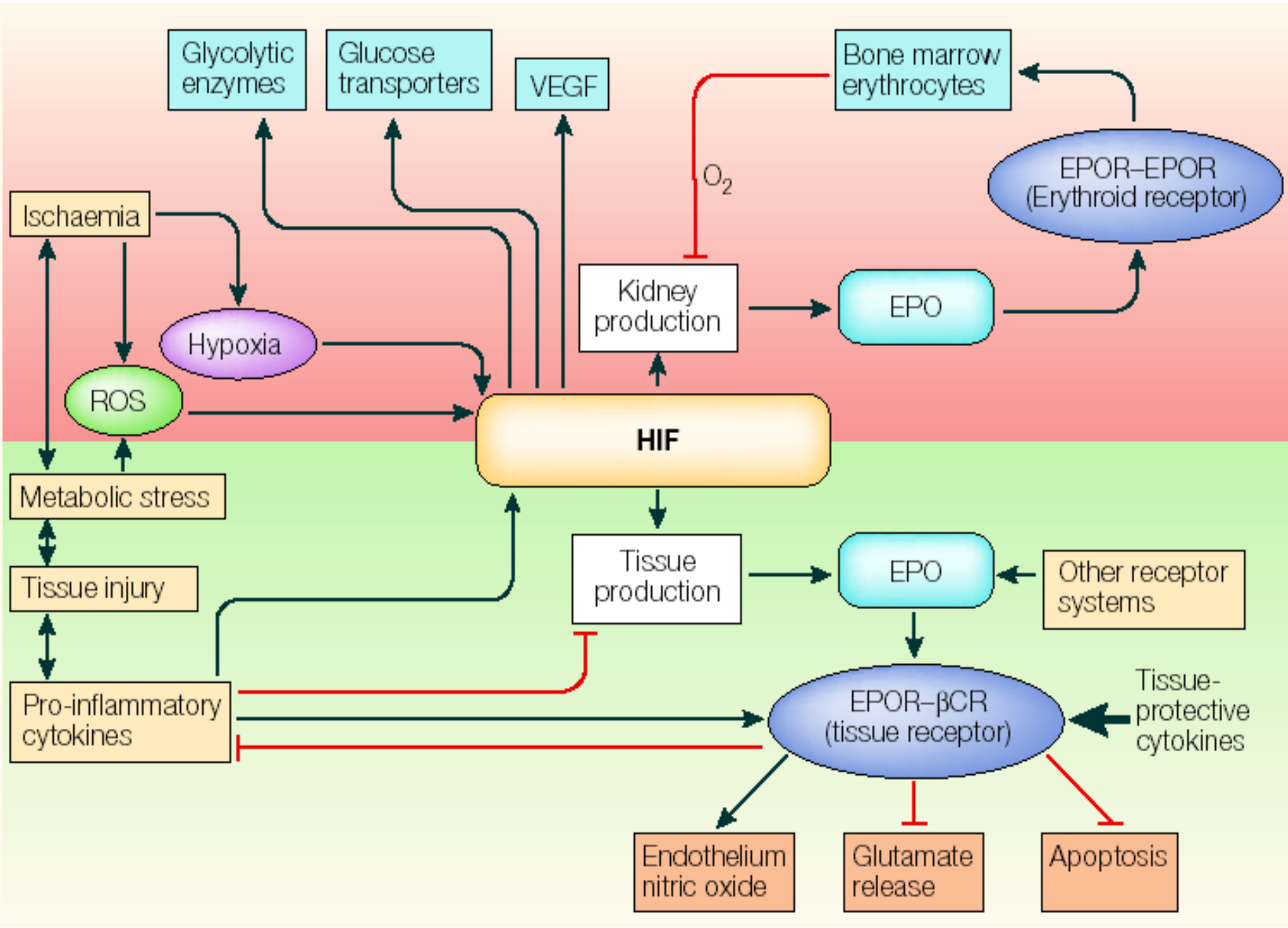
Protezione cerebrale da precondizionamento



Nat Rev 2006;7:437-448

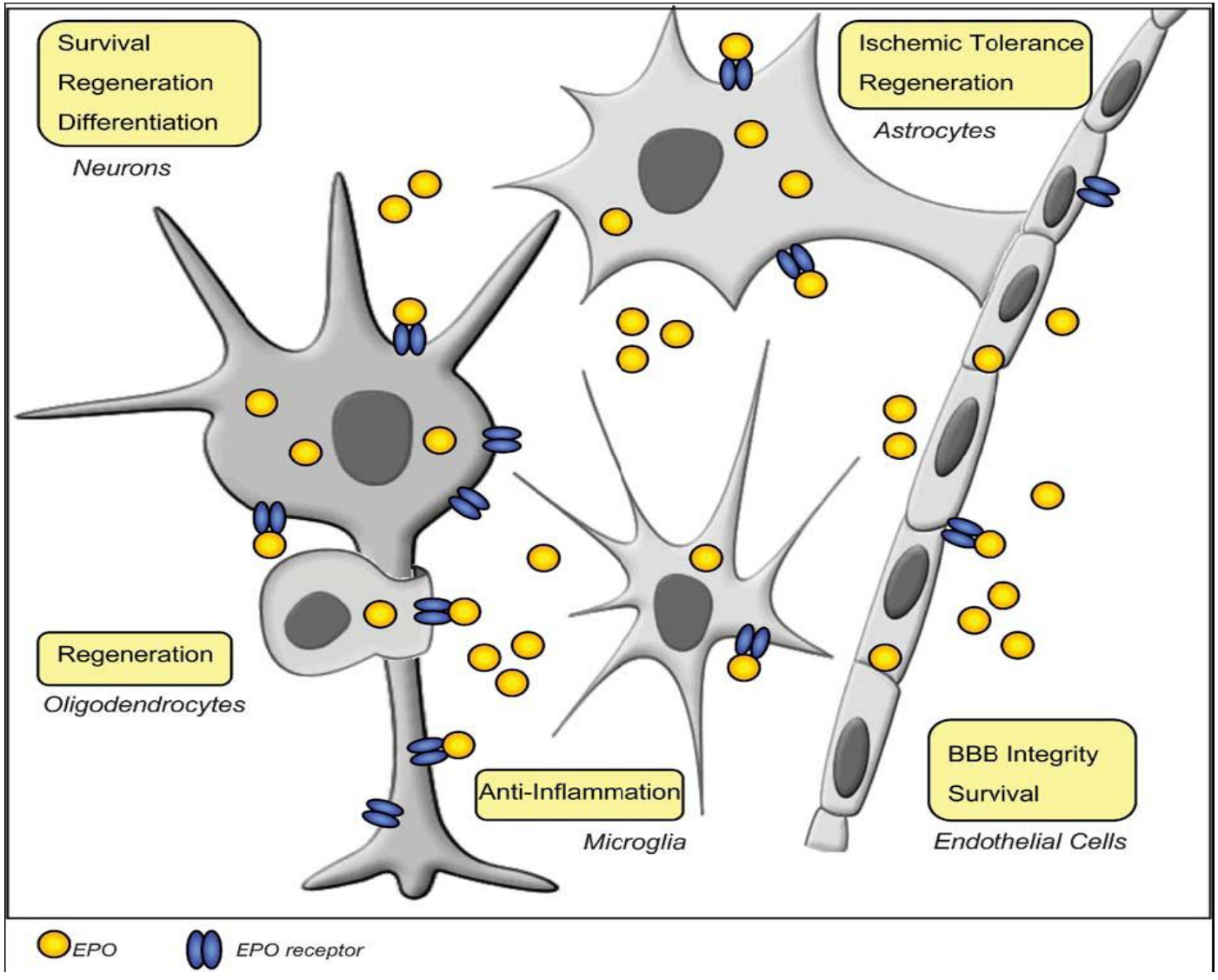
FASI DEL PRECONDIZIONAMENTO

- Induction phase: activation of transcription factors
- Transduction phase: amplification of signals through intracellular mediators (protein kinases)
- Effector phase: activation of protective mechanisms (antiapoptotic, antioxidative, antiinflammatory)

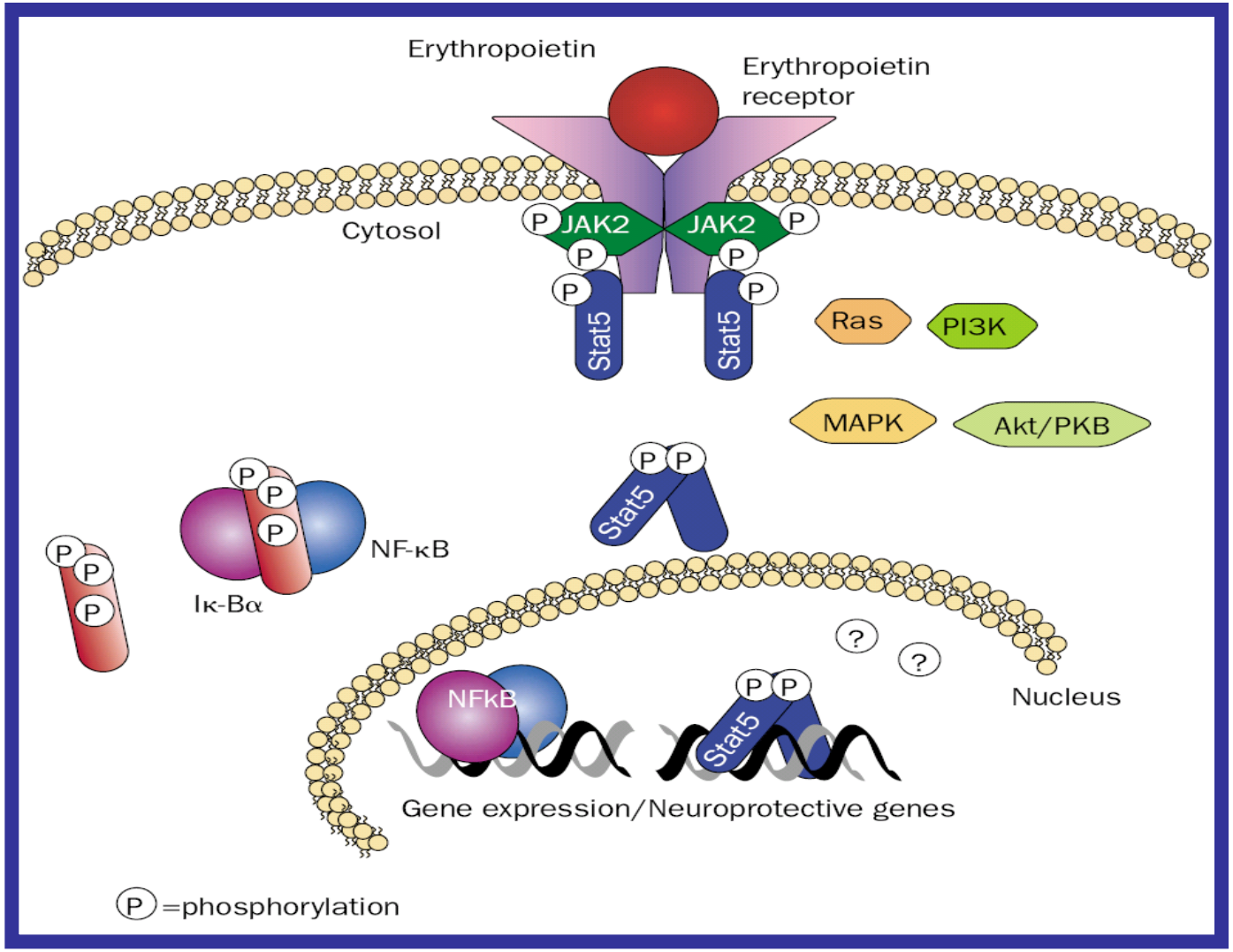

Glycolytic enzymes
Glucose transporters
VEGF
Bone marrow erythrocytes
O_2
EPOR–EPOR (Erythroid receptor)
Ischaemia
Kidney production
EPO
Hypoxia
ROS
HIF
Metabolic stress
Tissue injury
Tissue production
EPO
Other receptor systems
Pro-inflammatory cytokines
EPOR–βCR (tissue receptor)
Tissue-protective cytokines
Endothelium nitric oxide
Glutamate release
Apoptosis

FONTI E PLEIOTROPISMO DELL'EPO

EPO and EPO receptors are expressed by :
astrocytes, neurons, oligodendocytes, microglial cells, and brain endothelial cells



Trasduzione del segnale dell'eritropoietina



Lancet 2002;359:96-97

Possibili applicazioni cliniche…

La comprensione dei meccanismi cellulari della tolleranza ischemica offre la speranza di *identificare nuovi fattori neuroprotettivi* che potrebbero essere impiegati per trattare oltre all'ictus anche patologie neurodegenerative

L'impiego di agenti precondizionanti potrebbe anche consentire l'*ampliamento della finestra terapeutica per la trombolisi*

Marcatori con fini diagnostici/prognostici

RUOLO E OBIETTIVI DEI BIOMARKERS NELL'ICTUS

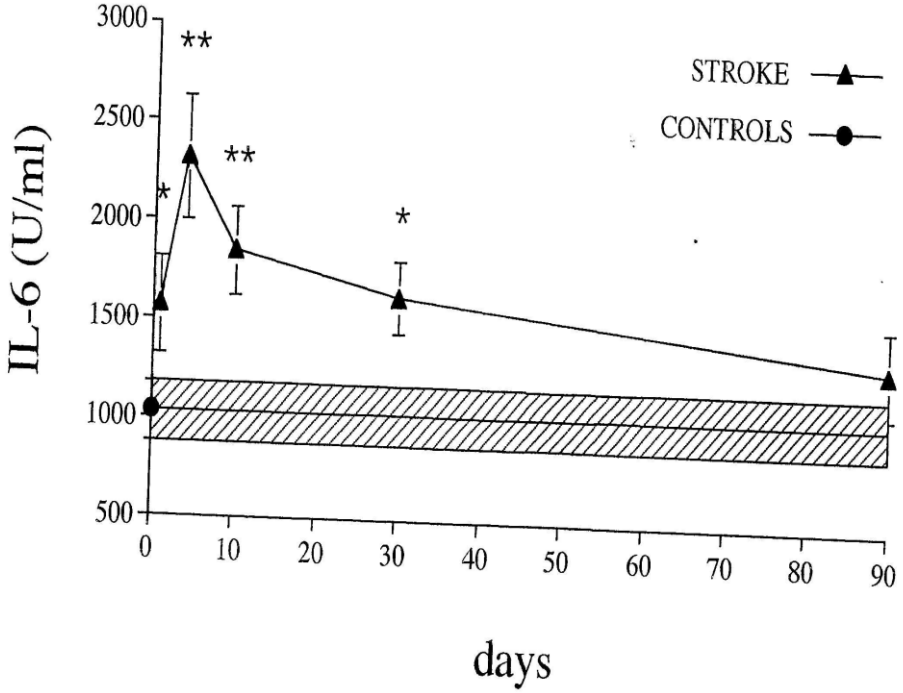
- **Migliorare l'accuratezza diagnostica**
- **Valore prognostico**
- **Scelta del trattamento più appropriato**
- **Monitoraggio dell'efficacia della terapia**

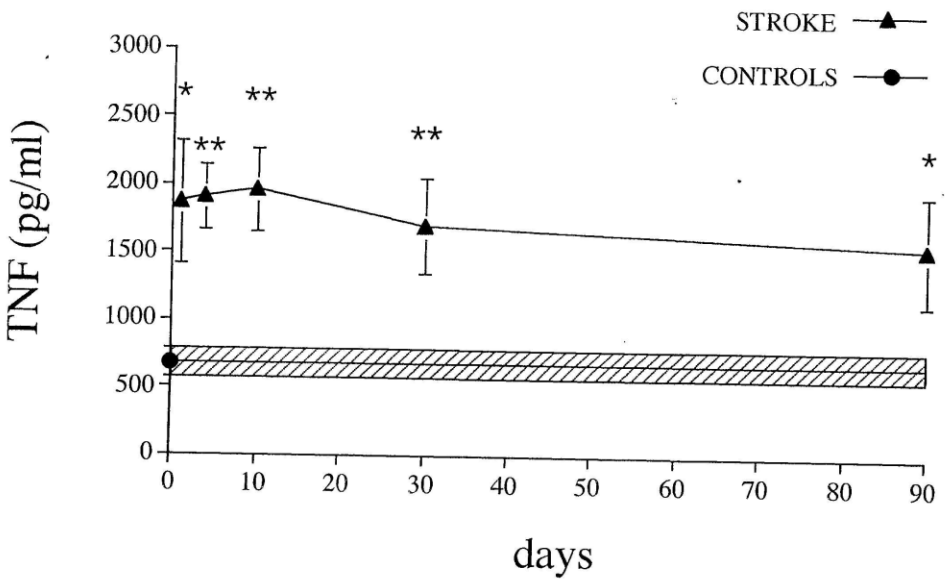
J Cereb Blood Flow Metab 1999 Sep;19(9):1004-9

Increased cytokine release from peripheral blood cells after acute stroke.

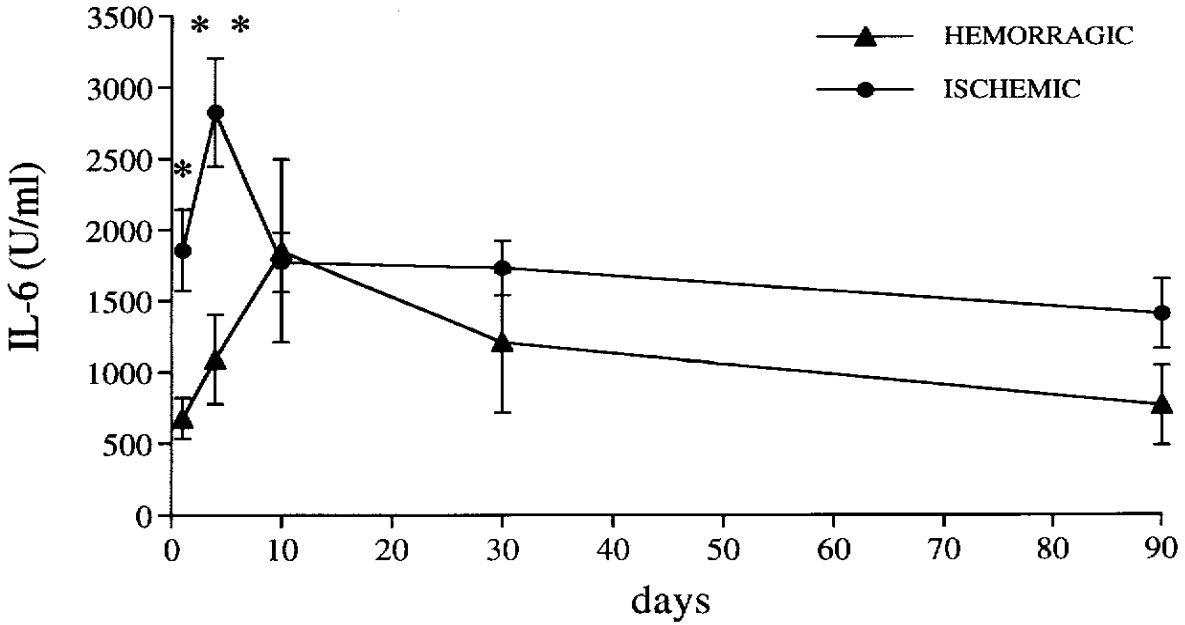
Ferrarese C, Mascarucci P, Zoia C, Cavarretta R, Frigo M, Begni B, Sarinella F, Frattola L, De Simoni MG.

Department of Neurology, University of Milan, Ospedale San Gerardo, Monza, Italy.





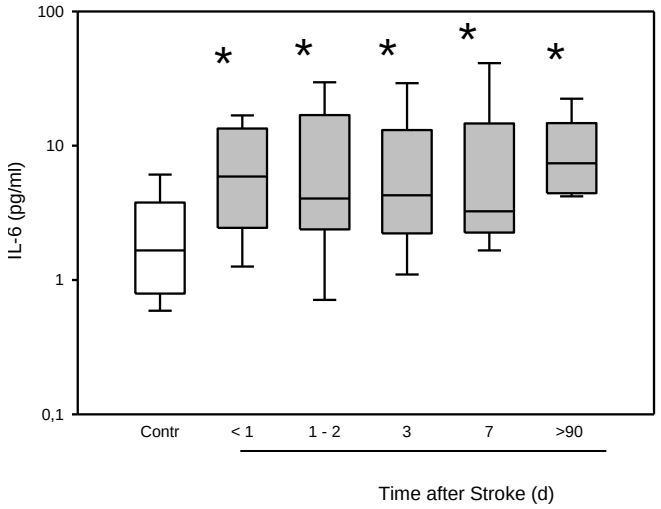
Rilascio *ex vivo* di IL-6 da cellule ematiche di pazienti con ictus: paragone tra ischemici e emorragici (* = p<0.05; ** = p<0.01).



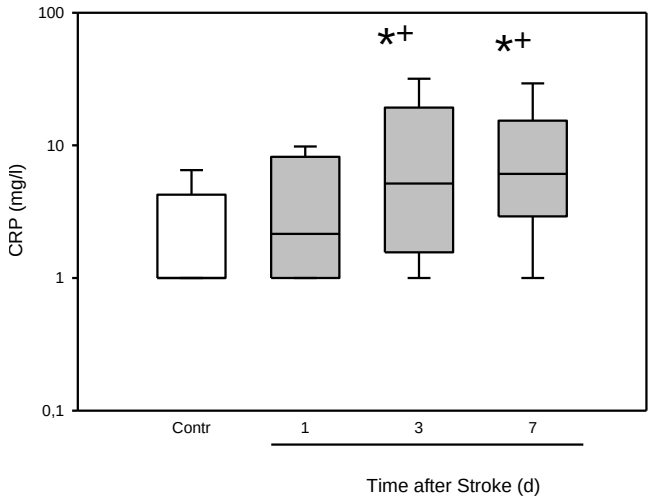
Ferrarese C , et al. J Cer Bl Fl Metab 1999;19:1004-9

L‘aumento di IL-6 precede quello di PCR

IL-6



CRP



CRP and outcome in ischemic stroke

Author	Patients	(Primary) endpoint	Follow-up	Detection limit	Time of CRP determination	Results
Canova et al., 1999	138 patients with TIA, ischemic or hemorrhagic stroke	Neurologic deficit	?	2.4 mg/l	On admission, < 120 h after onset of symptoms	No significant correlation between CRP values and prognosis
Muir et al. , 1999	228 patients with acute ischemic stroke	survival	959 days (average)	2.5 mg/l	< 72 h after onset of symptoms	CRP is an independent predictor of survival
Di Napoli et al., 2001	193 patients with ischemic stroke	Combined vascular endpoint (death or any new vascular event)	12 months	0.175 mg/l	< 24 h, 48 - 72 h after onset of symptoms, at discharge	CRP is an independent risk factor for a bad outcome, determination at discharge has the highest predictive value
Anuk et al., 2002	60 patients with first acute ischemic event	NIHSS, mRS	8 - 12 months		Hs, Dade Beringer nephelometer, lit. Bestellt, within 24 h after acute event	CRP on admission correlated with neurologic deficit on follow-up
Winbeck et al., 2002	127 first ischemic stroke	Barthel index and mRS, combined vascular endpoint	12 months	0.1 mg/l	< 24 h after onset of symptoms	CRP within 24 h predicts unfavorable outcome
Ceccarelli et al., 2002	Retrospective analysis of 288 elderly patients with acute stroke	mRS, length of hospital stay, mortality, rate of rehospitalization	12		< 12 h after admission	CRP on admission correlated with 30 day mortality, disability at discharge, and the rate of rehospitalization for secondary stroke
Arenillas et al., 2003	71 patients with first ischemic event plus intracranial stenosis	Cerebral ischemic events, myocardial infarction	12	0.175 mg/l	> 8 months after acute vascular event	CRP levels above 14 mg/l were associated with increased risk for ischemic events related intracranial large artery occlusive disease

PCR E IL6 PREDICONO OUTCOME SFAVOREVOLE A 30 GIORNI

Table 4. Area under ROC curves for D-dimer, CRP and IL-6, along with case selections, sensitivities and specificities for poor outcome at selected cutoffs

	Area under ROC	Cutoff	Sensitivity, %	Specificity, %	Cases selected, %
D-dimer	0.71 (0.63–0.78)	100 ng/ml	87	40	74
		200 ng/ml	58	64	46
		300 ng/ml	46	74	36
CRP	0.67 (0.59–0.75)	2 mg/l	82	40	71
		6 mg/l	60	68	46
		16 mg/l	32	82	25
IL-6	0.73 (0.66–0.81)	3 pg/ml	90	34	78
		7 pg/ml	67	69	49
		15 pg/ml	35	87	24

95% CI in parentheses.

180 pz con ictus ischemico < 24 ore

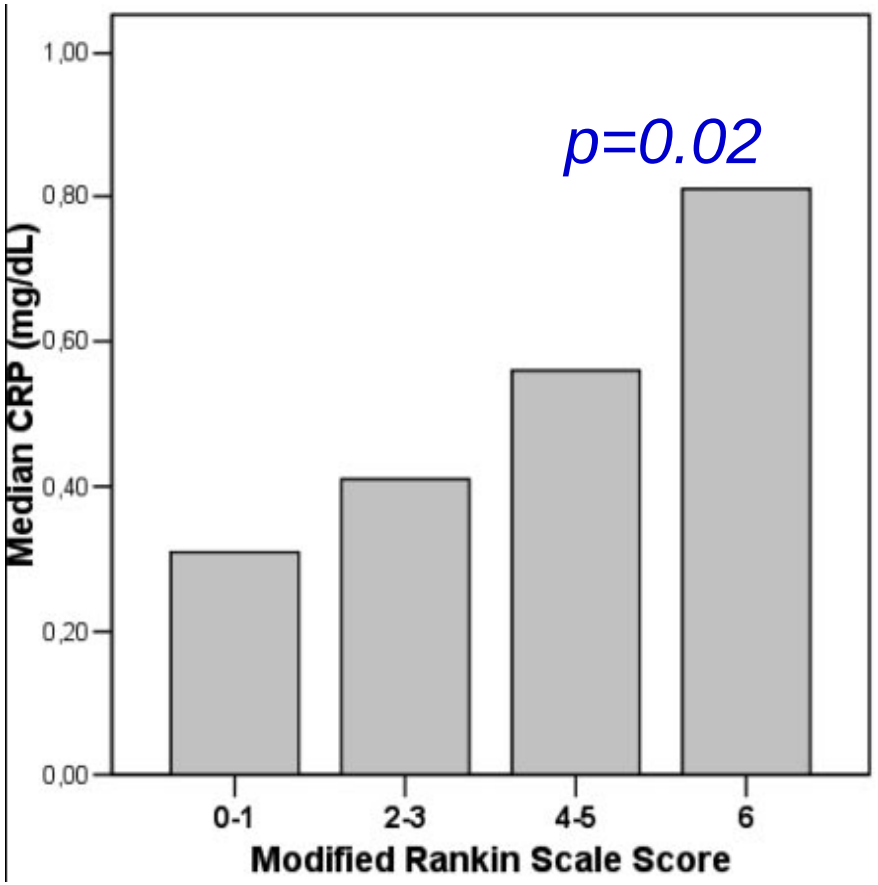
- 52% DISABILI O MORTI A 30 GG (mRs>2)

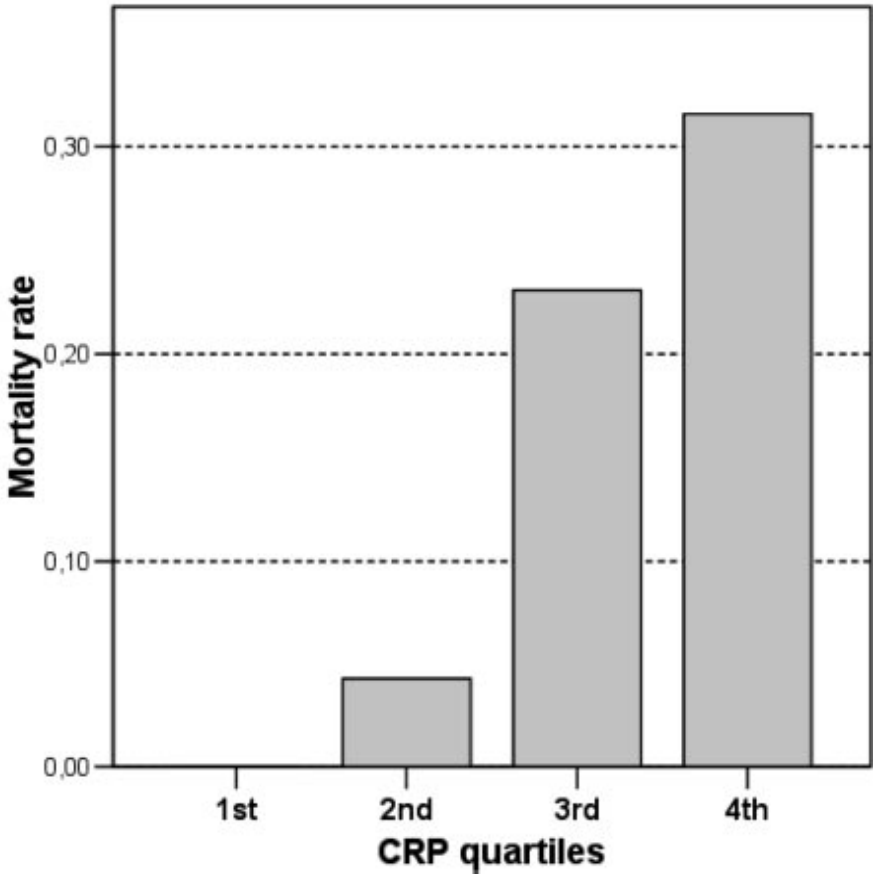
PCR → p= 0.004

IL-6 → p< 0.001

(Welsh P, Cerebrovasc Dis 2009)

PCR CORRELA CON OUTCOME CLINICO DOPO tPA





94 pazienti ictus acuto da occlusione prossimale CMA trattati con tPA entro 3 ore. Follow-up a 3 mesi con mRs.

(Montaner et al Stroke 2006)

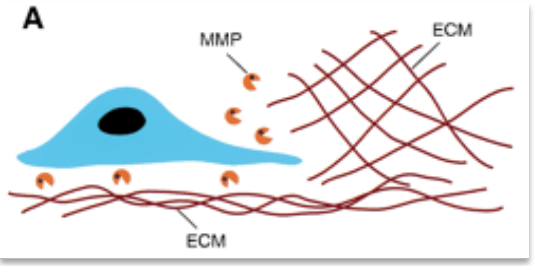
Complicazione emorragica nel caso di terapia trombolitica

- **MMP-9** è una metallo-proteinasi che degrada la matrice cellulare, presente nell'unità neurovascolare
(negativa nella fase acuta; positiva nella fase post-acuta di remodeling)

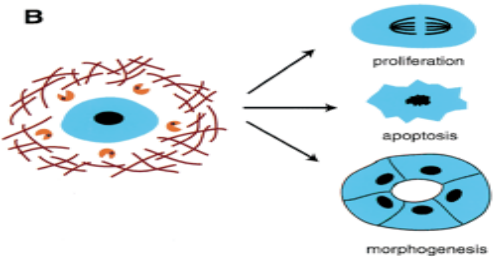
Zhao, Nature Medicine 2006

- **c-Fn** (cellular fibronectin) è un componente della lamina basale presente nell'endotelio cerebrale e la sua presenza nel siero è un indicatore di disfunzione della barriera ematoencefalica

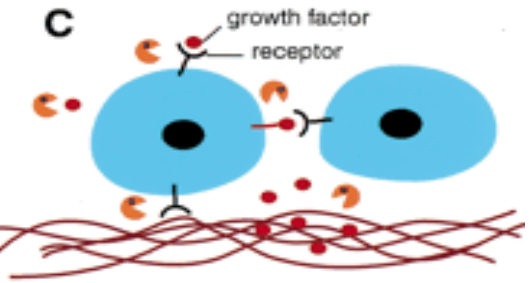
FUNZIONI FISIOLOGICHE delle METALLOPROTEINASI



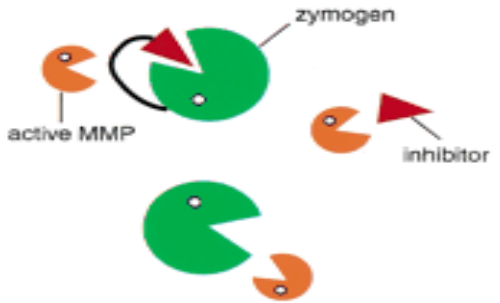
Regolare la migrazione cellulare



Regolare la proliferazione, l'apoptosi e la morfogenesi cellulare (sviluppo e morfogenesi SNC)

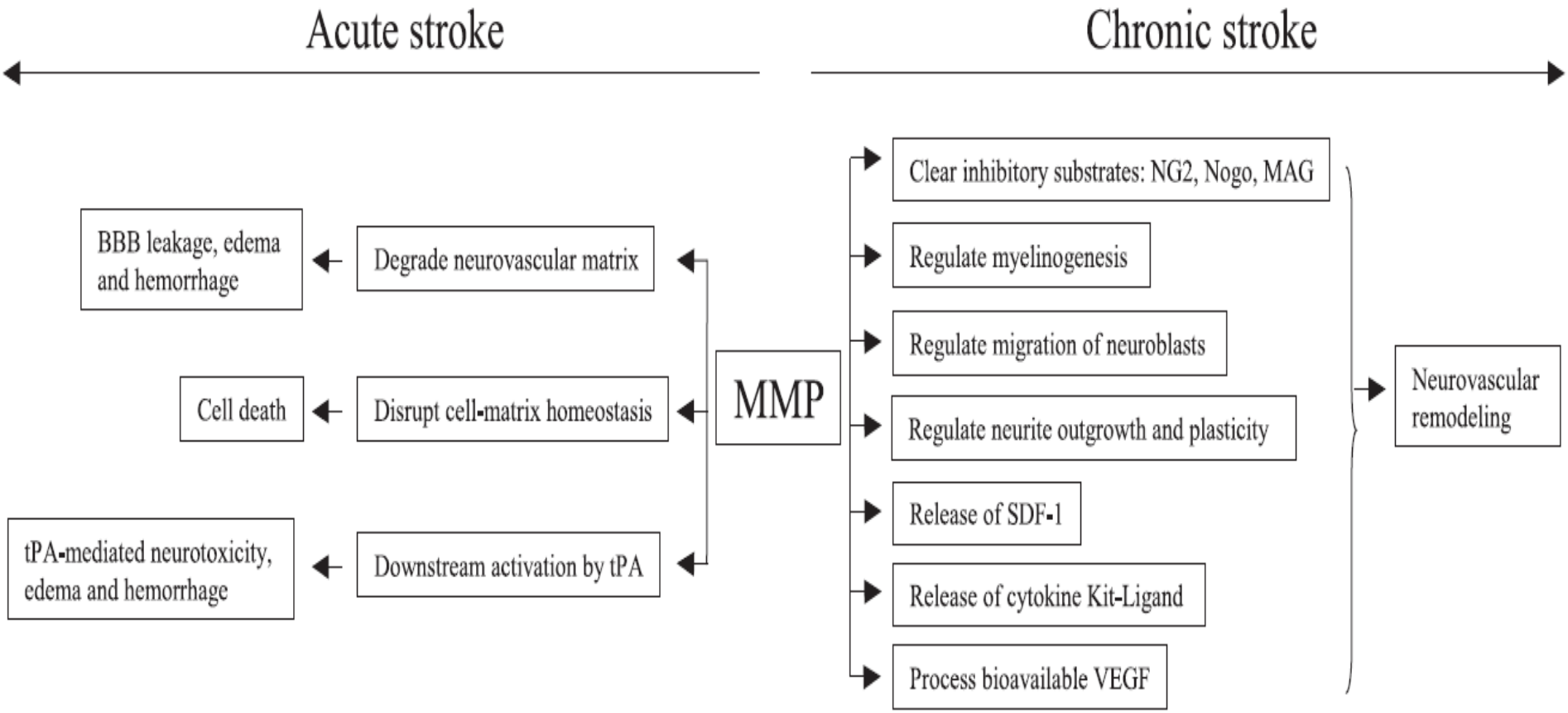


Modulare l'attività di fattori di crescita e loro recettori

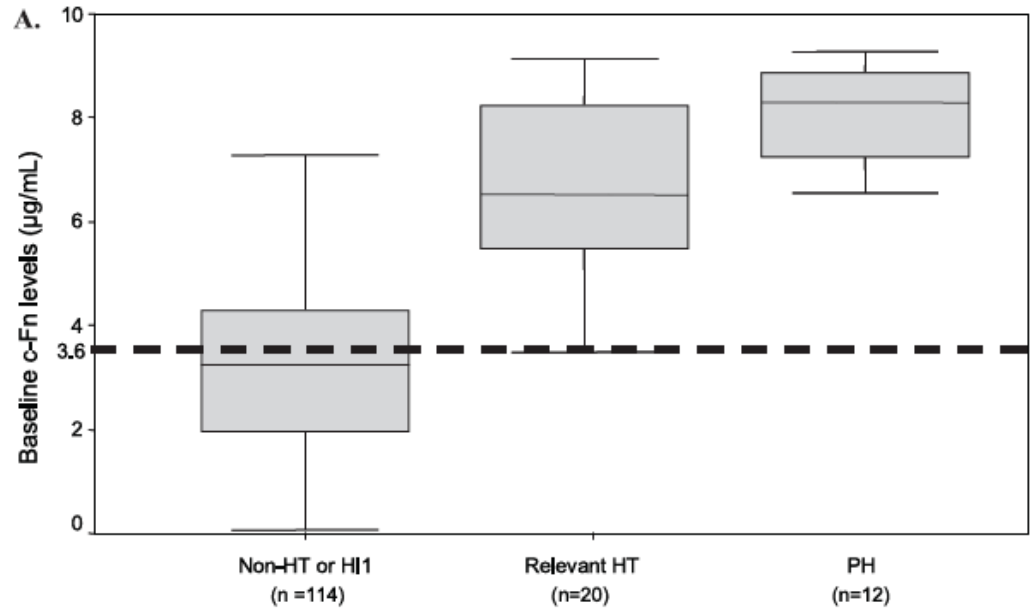


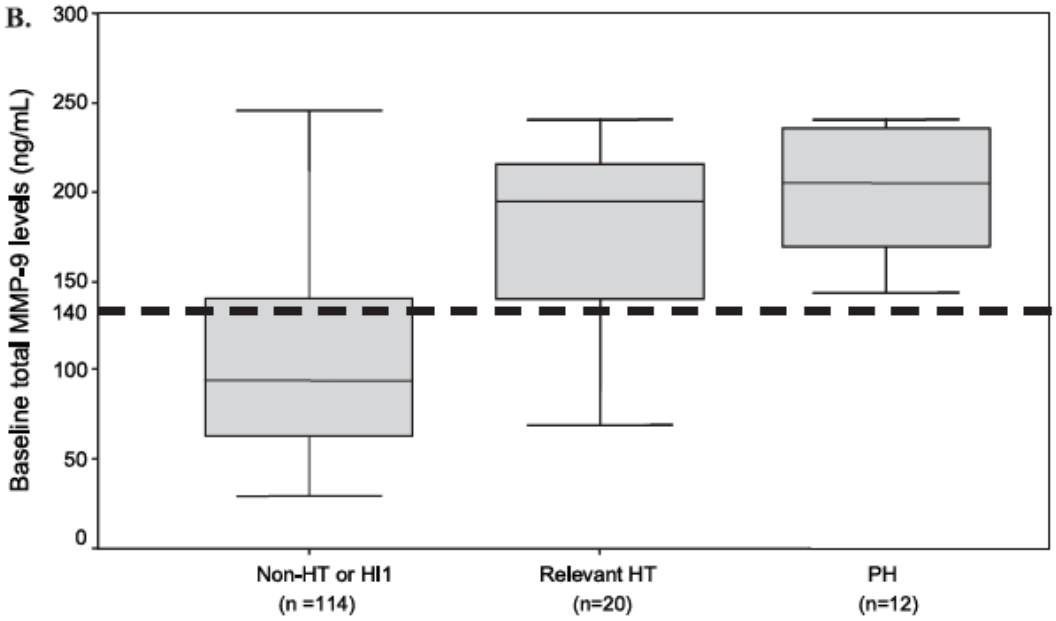
Regolare il bilancio dell'attività proteolitica della matrice

MMPs NELL'ISCHEMIA CEREBRALE: FUNZIONE TEMPO DIPENDENTE



Type of hemorrhagic transformation





134 pazienti

prelievo in urgenza (< 3 h)

ELISA assay

(PH sintomatica in 4 pz, 0.2%)

Accuratezza diagnostica combinata:

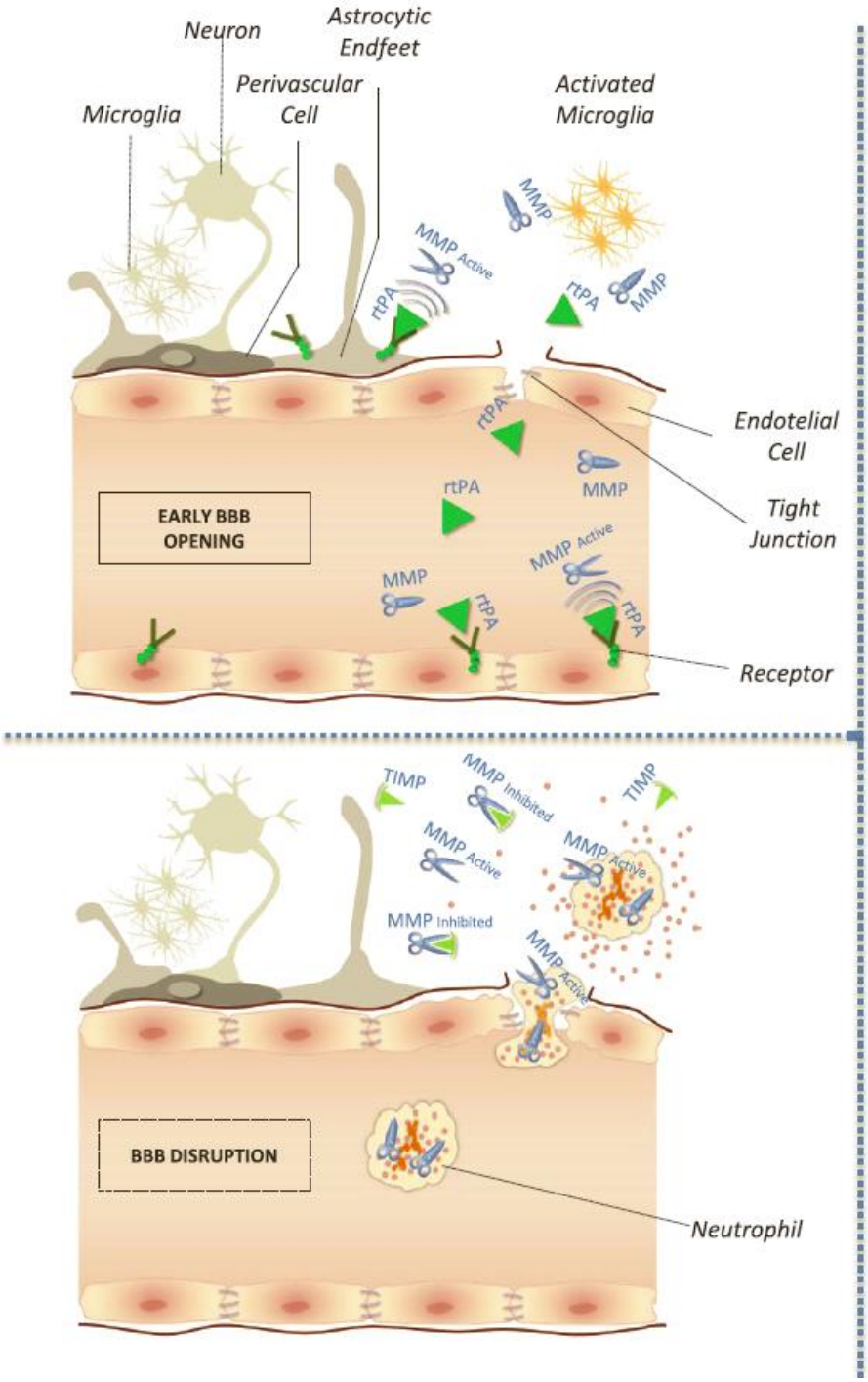
sensibilità 92%

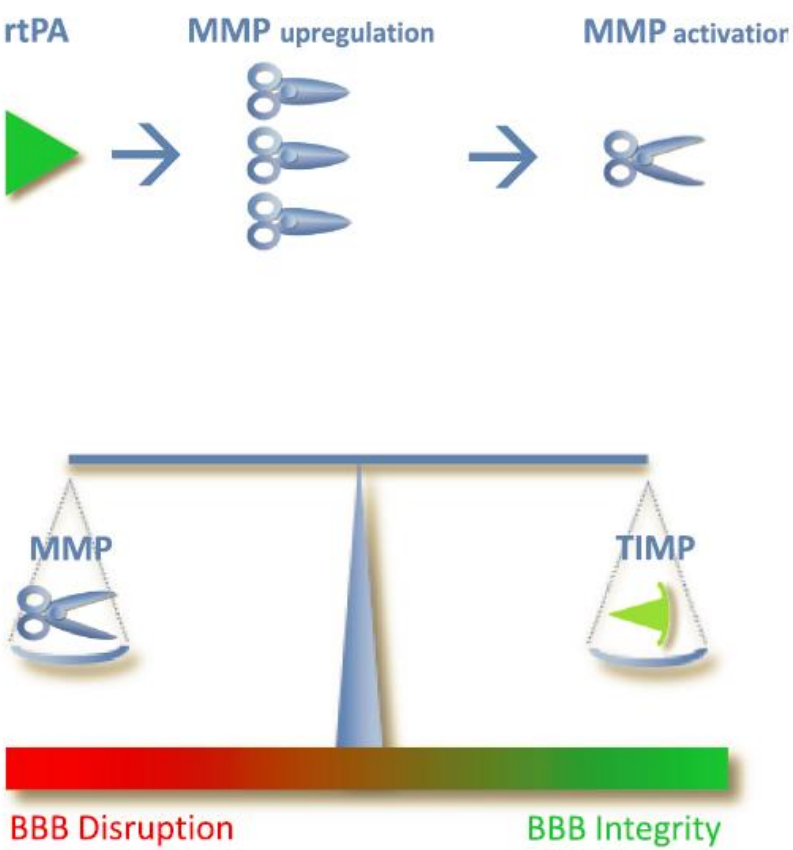
specificità 87%

Castellanos, Stroke 2007

Studio clinico **MAGIC** multi-centrico italiano
Centro coordinatore AOUC Careggi Firenze
Prof. D. Inzitari

-MMP 1, 2, 3, 8, 9,14 Activity
-MMP 1,2, 3, 8, 9,12, 13 Antigene
-TIMP-1, TIMP-2
-VON WILLERBRAND, D-Dimero, PAI
-TFPI
-TF
-TAFI
- P, E-SELECTINA
-CITOCHINE (IL-4, IL-10, IL- 1Beta, IL-6, IL-8, IL-12, TNF-alpha, VEGF, MIP-1 alpha / Beta, IP-10, MCP-1)
-Mieloperossidasi
-ICAM-1





Piccardi et al 2015

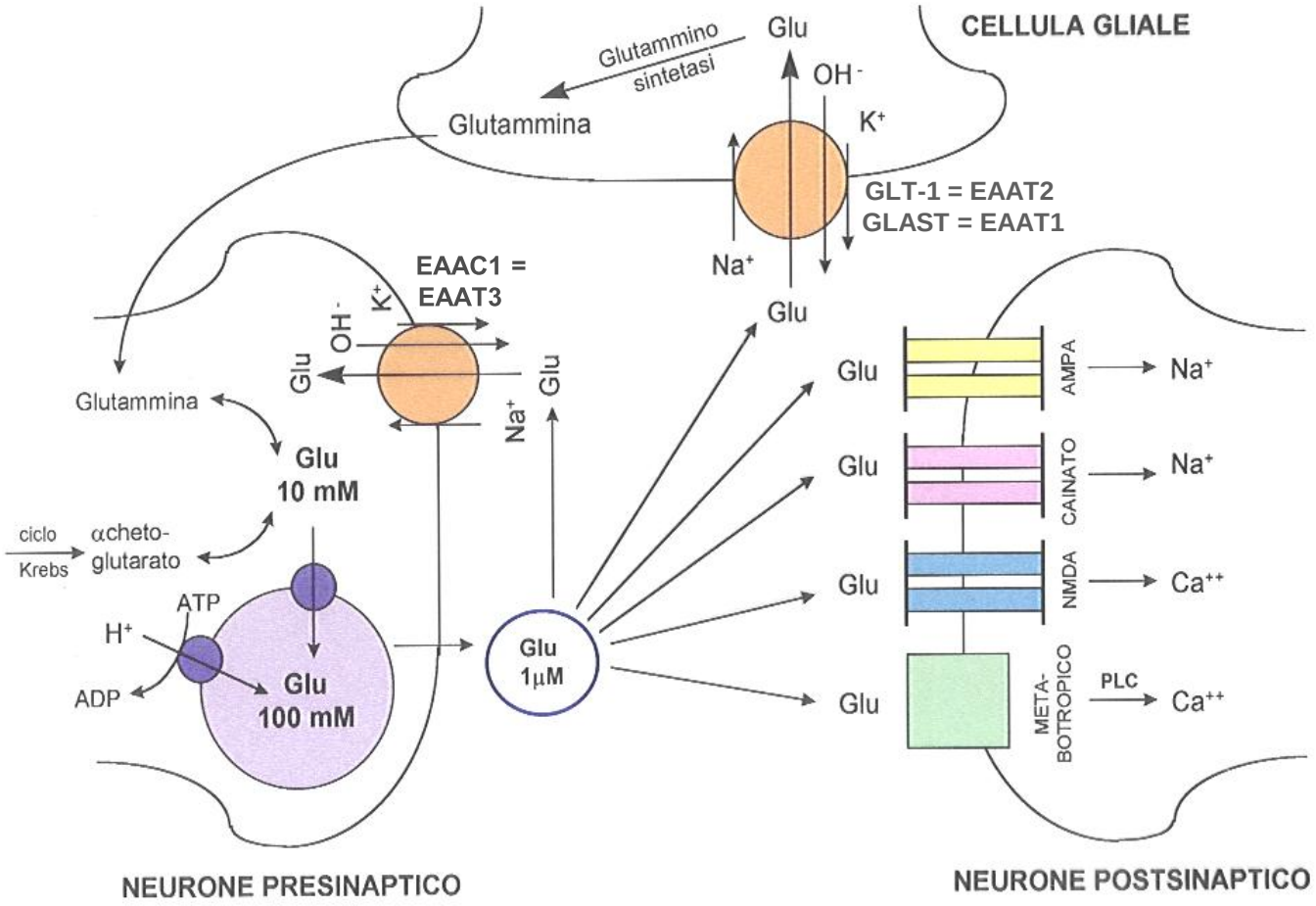
Unbalanced metalloproteinase-9 and tissue inhibitors of metalloproteinases ratios predict hemorrhagic transformation of lesion in ischemic stroke patients treated with thrombolysis: results from the MAGIC study

Benedetta Piccardi[1], Vanessa Palumbo[2], Mascia Nesi[2], Patrizia Nencini[2], Anna Maria Gori[3], Betti Giusti[3], Giovanni Pracucci[1], Paolina Tonelli[1], Eleonora Innocenti[1], Alice Sereni[3], Elena Sticchi[3], Danilo Toni[4], Paolo Bovi[5], Mario Guidotti[6], Maria Rosaria Tola[7], Domenico Consoli[8], Giuseppe Micieli[9], Rossana Tassi[10], Giovanni Orlandi[11], Francesco Perini[12], Norina Marcello[13], Antonia Nucera[14], Francesca Massaro[15], Maria Luisa DeLodovici[16], Giorgio Bono[16], Maria Sessa[17], Rosanna Abbate[3] and Domenico Inzitari[1,18], On behalf of the MAGIC Study Group*

Results: Adjusting for major clinical determinants, only increase in MMP9/TIMP1 and MMP9/TIMP2 ratios remained significantly associated with sICH (odds ratio [95% confidence interval], 1.67 [1.17–2.38], $p = 0.005$; 1.74 [1.21–2.49], $p = 0.003$, respectively). Only relative increase in MMP9/TIMP1 ratio proved significantly associated with relevant HT (odds ratio [95% confidence interval], 1.74 [1.17–2.57], $p = 0.006$) with a trend toward significance for MMP9/TIMP2 ratio ($p = 0.007$).

Discussion: Our data add substantial clinical evidence about the role of MMPs/TIMPs balance in rtPA-treated stroke patients. These results may serve to generate hypotheses on MMPs inhibitors to be administered together with rtPA in order to counteract its deleterious effect.

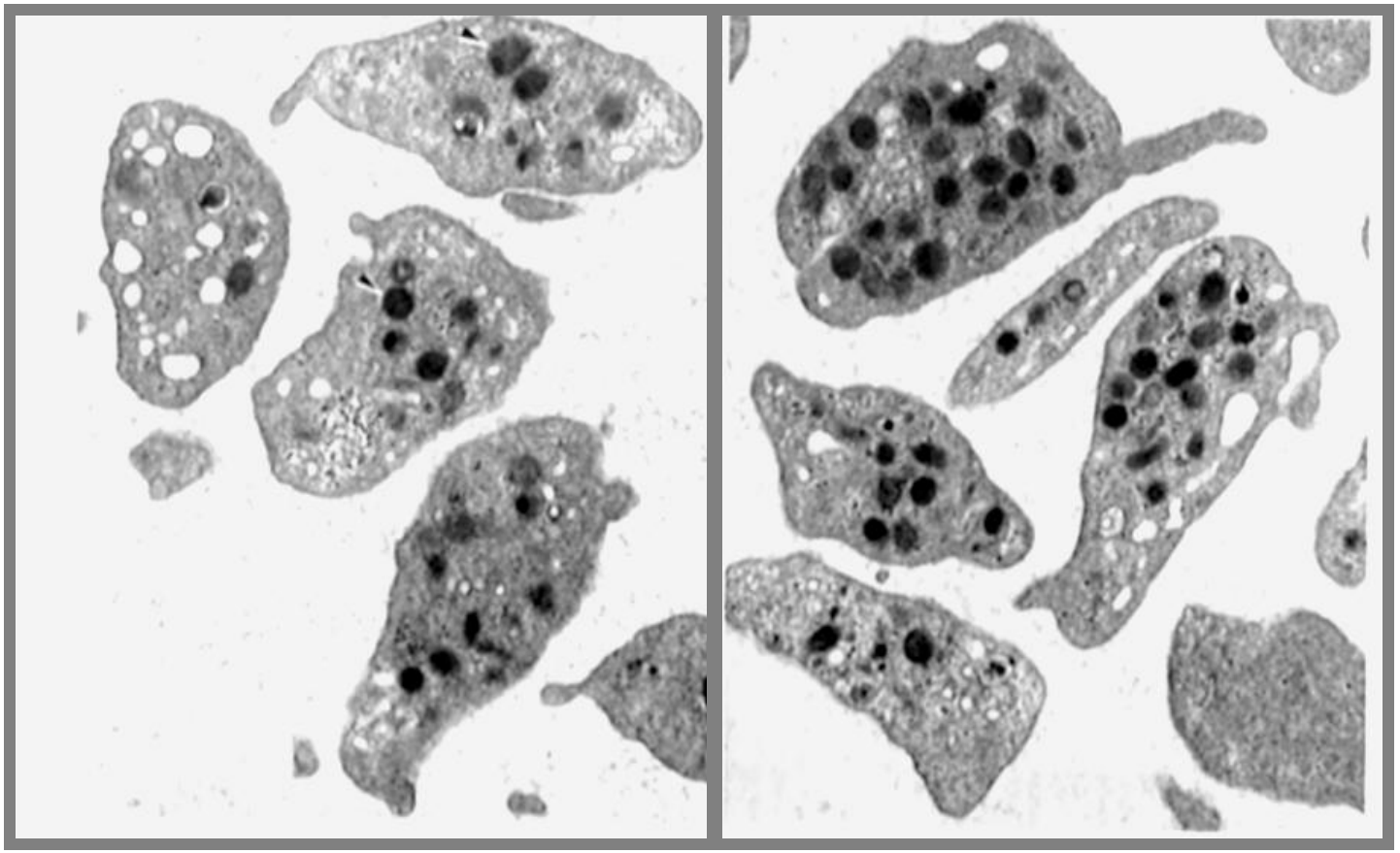
Neurotrasmissione glutammatergica



ANALISI DEL GLUTAMMATO NEI PAZIENTI

- Spettroscopia RMN
- Microdialisi in vivo
- Livelli liquorali
- Livelli plasmatici
- Uptake e trasportatori in tessuti periferici

Immagini in microscopia elettronica di piastrine di pazienti con ictus



PAZIENTI ICTUS CONTROLLI

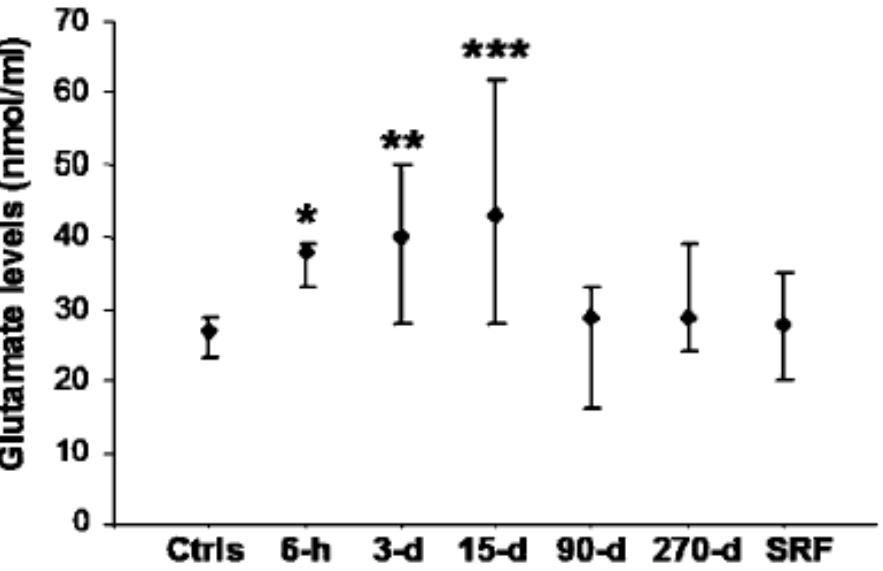
Journal of Cerebral Blood Flow & Metabolism (2005), 1–7

www.jcbfm.com

Increased plasma glutamate in stroke patients might be linked to altered platelet release and uptake

Angelo Aliprandi[1,2], Marco Longoni[1,2], Lorenzo Stanzani[1,2], Lucio Tremolizzo[1,2], Manuela Vaccaro[1,2], Barbara Begni[1], Gloria Galimberti[1], Rosanna Garofolo[1] and Carlo Ferrarese[1,2]

[1]*Department of Neuroscience and Biomedical Technologies, University of Milano-Bicocca, Monza, Italy;*
[2]*Department of Neurology, San Gerardo Hospital, Monza, Italv*



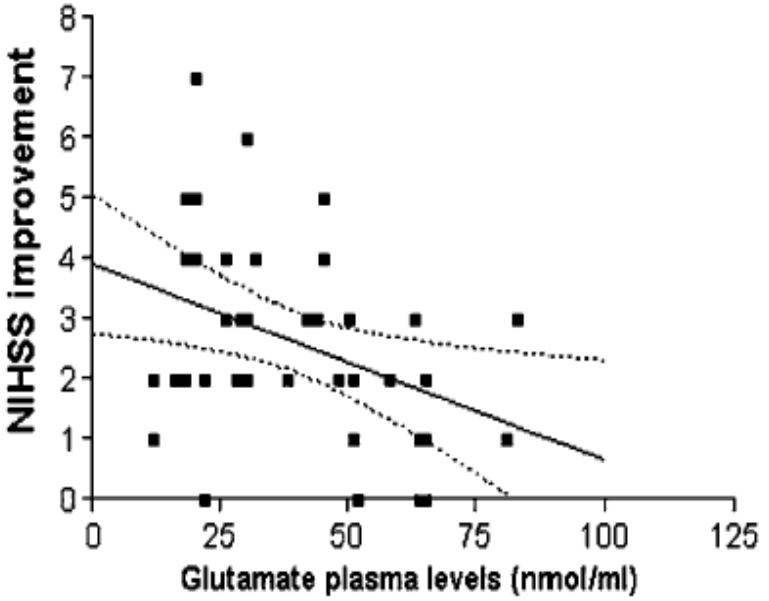
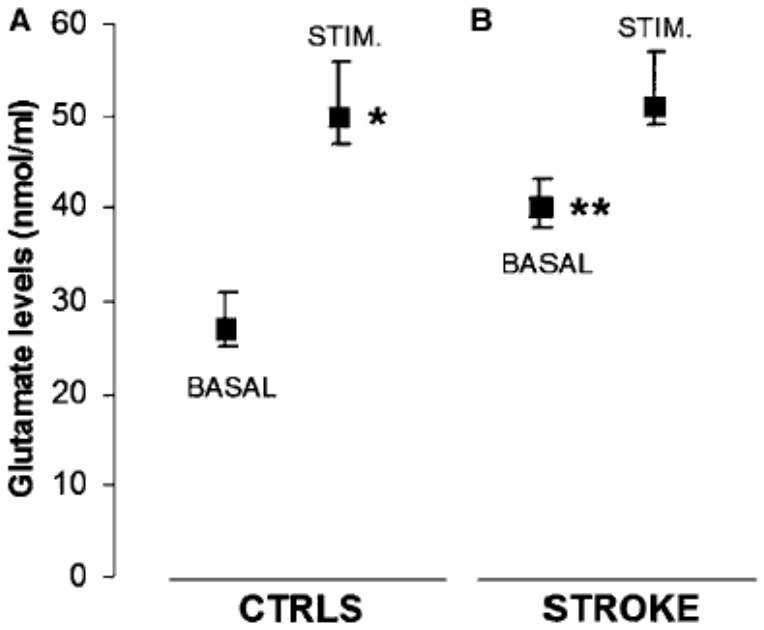


Figure 2 Correlation between glutamate plasma levels (day 3) and NIHSS improvement between days 3 and 15. $r = -0.36$, $P < 0.05$. 95% confidence interval is shown.

Increased glutamate release from platelets

Decreased glutamate uptake from platelets



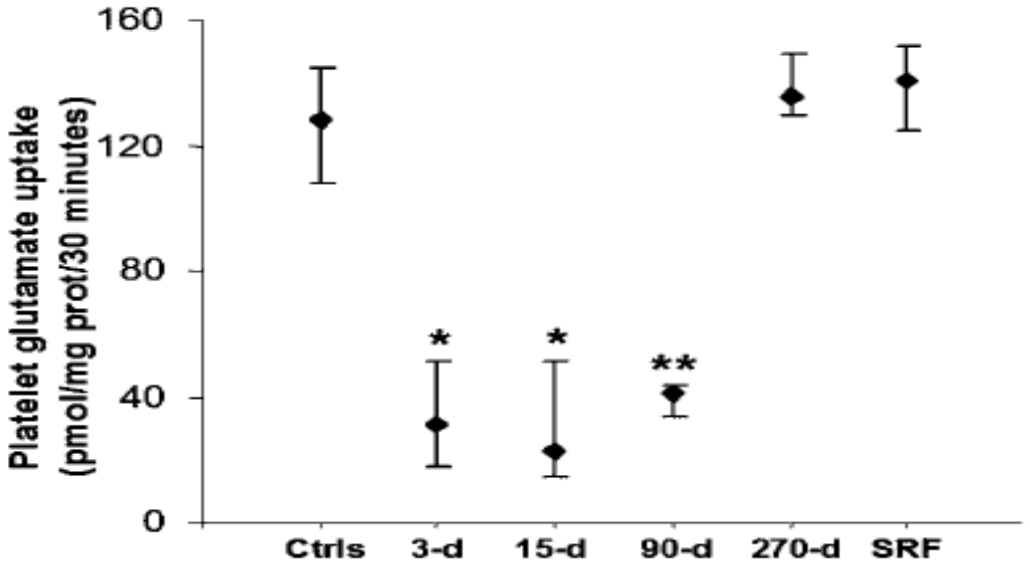
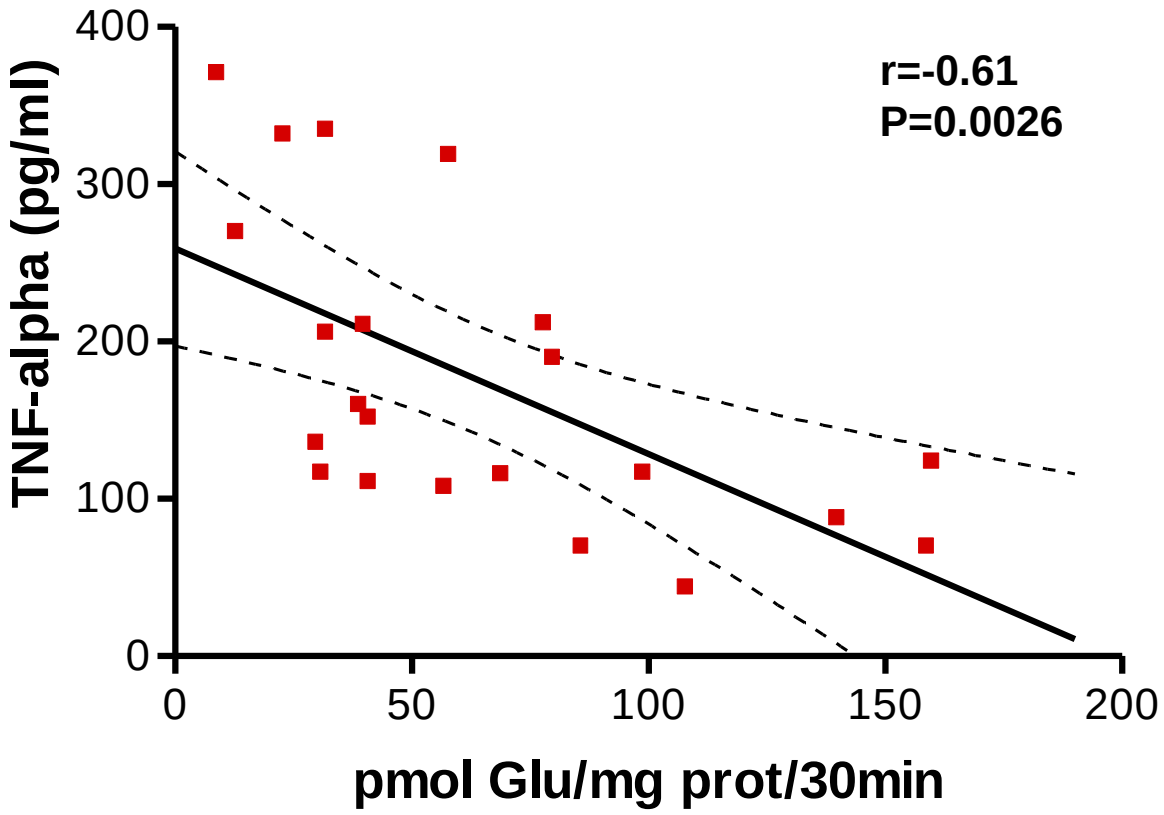
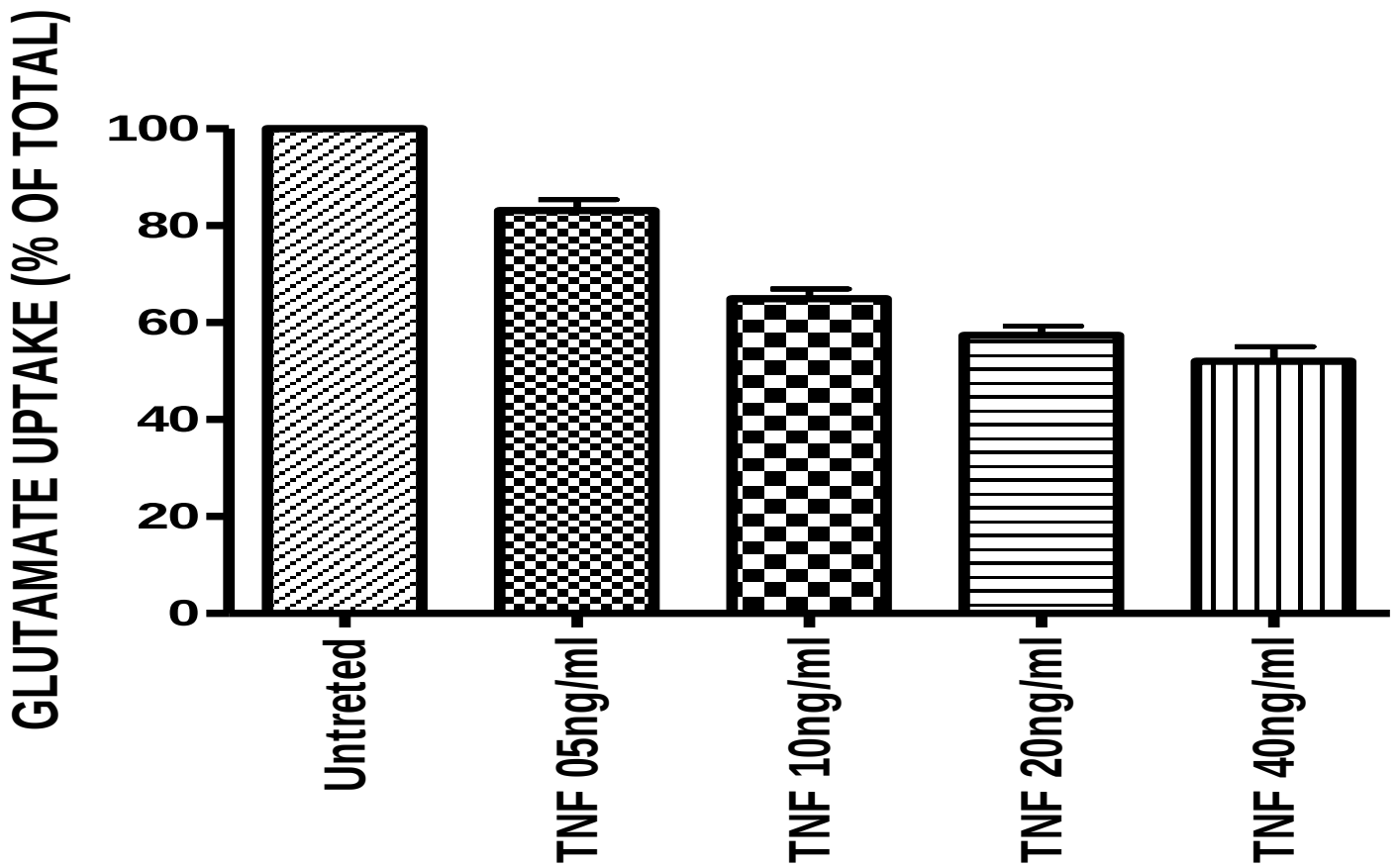


Figure 3 Glutamate release from platelets of stroke patients and healthy controls. *Ex vivo* platelets from healthy subjects (**A**, Ctrls) release glutamate after aggregation (*$P<0.0001$ stimulated Ctrls versus basal Ctrls). In stroke patients (**B**), plasma glutamate levels are increased compared with Ctrls (**$P<0.01$ basal Stroke versus basal Ctrls), but almost no release is shown on stimulation. Values are expressed as median and quartiles (25% and 75%).

CORRELAZIONE TRA TNF-A PLASMATICO E TRASPORTO PIASTRINICO DI GLUTAMMATO DOPO ICTUS



Effect of TNF alpha on platelet glutamate uptake



TNF-alfa ⟶ PLT Uptake GLUT ⟶ Plasma GLUT

DANNO NEUROLOGICO CORRELATO

Medical Hypotheses 73 (2009) 553–554

Contents lists available at ScienceDirect

Medical Hypotheses

journal homepage: www.elsevier.com/locate/mehy

Platelets might mediate the increase of plasma glutamate in acute ischemic stroke: Relevance for early neurological deterioration

Lucio Tremolizzo [a,b,*], Fabrizio Piazza [b], Marco Longoni [c], Carlo Ferrarese [a,b]

[a] Department of Neurology, "San Gerardo" Hospital, Monza, Italy
[b] Department of Neuroscience and Biomedical Technologies, University of Milano-Bicocca, U8 – via Cadore 48, 20052 Monza, MI, Italy
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ARTICLE INFO

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SUMMARY

In this brief paper we would like to hypothesize that platelets might represent a "peripheral" contributory source for the elevation of plasma glutamate levels in the setting of acute ischemic stroke: available evidence and possible mechanisms will be discussed, especially drawing attention to the possible relevance for the pathophysiology of early neurological deterioration.

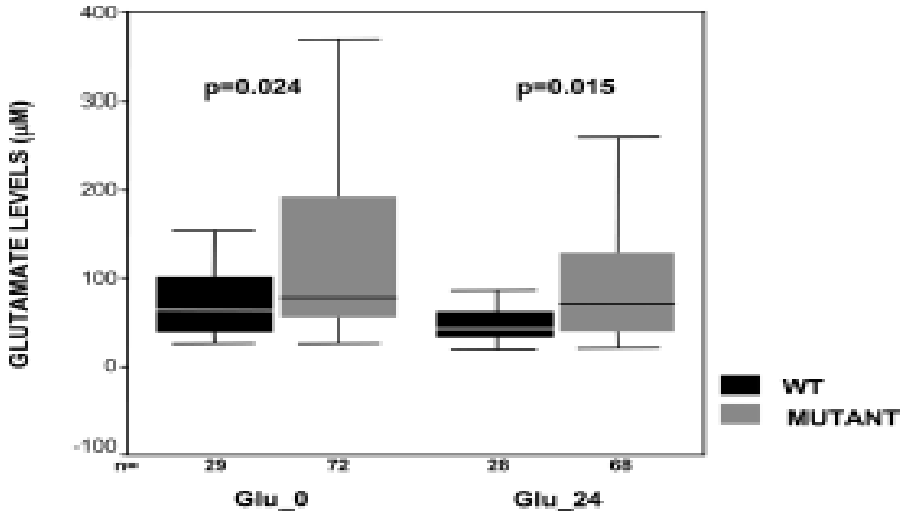
- Correlazione inversa tra uptake glutammatergico piastrinico e TNF-alfa plasmatico in pazienti con ictus ischemico
- Inibizione ex vivo dell'uptake di glutammato piastrinico da TNF-alfa: relazione dose-risposta

A polymorphism in the *EAAT2* promoter is associated with higher glutamate concentrations and higher frequency of progressing stroke

Judith Mallolas,[1] Olivia Hurtado,[2] Mar Castellanos,[1] Miguel Blanco,[4] Tomás Sobrino,[4] Joaquin Serena,[1] José Vivancos,[3] José Castillo,[4] Ignacio Lizasoain,[2] Maria A. Moro,[2] and Antoni Dávalos[5]

AP-2–cotransfected wild-type promoter. We also show that GCF2 is expressed in ischemic rat brain, suggesting that decreased glutamate uptake occurs in individuals carrying the mutation after stroke. These findings may explain individual susceptibility to excitotoxic damage after stroke as well as the failure of glutamate antagonists in those patients without this polymorphism.

J Exp Med, 2006



OPEN ACCESS Freely available online

PLoS one

Expression Profile of MicroRNAs in Young Stroke Patients

Kay Sin Tan[1], Arunmozhiarasi Armugam[2], Sugunavathi Sepramaniam[2], Kai Ying Lim[2], Karolina Dwi Setyowati[2], Chee Woon Wang[1], Kandiah Jeyaseelan[2]*

1 Departments of Medicine and Molecular Medicine, University of Malaya, Kuala Lumpur, Malaysia, 2 Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore

Received April 20, 2009; **Accepted** October 5, 2009; **Published** November 2, 2009

Table 4. microRNAs and their target biological processes.

Process/Targets	microRNA/paralogs	
	Up regulated in this stroke study	Down regulated in this stroke study
Angiogenesis[18]	miR-19b, -130a, -15a,-16, -222, -320	
Hematopoietic regulation[5]	miR-16, -24, -30c, -106b, -223,	
Immune response[7]	miR-15a, -16, -24, -29a, -93, -181a, -223	
Lymphocyte regulation (differentiation & proliferation)[7]	miR-101, -181a, -16, -24, -22, -103, -30c, let-7c	
Cardiac/vascular function[20]	miR-23a, -23b, -24, -29a, -29c, -30a,-30c, -30d -103, -222	miR-126
Endothelial cell migration, differentiation and survival[18]	miR-126, -222	
Metabolic process regulation[6]	miR-130a, -29a, -29c, -320	
Hypoxia regulation[19]	miR-23a, -23b, -24, -103, -93, -181a, -15a, -16, -101, -126, -320, let-7c, let-7e,	miR-7, let-7a
Traumatic brain Injury (mouse brain)[12]	miR-103, -130a, -185, -191, -19b, -22, -222, -223, -23a, -23b, -30c, -320, -652, -744	
Stroke model (blood of rat-MCAo model)[11]	let-7c, miR-103, -106b, -16, -185, -191, -19b, -23a, 320,	let-7a
Stroke model (brain of rat MCAo model)[11,13]	let-7c, let-7d*, let-7e, miR-103, -126, -130a, -16, -181a, -185, -191, -222, -223, -23a, -23b, -24, -30c, -324-5p, -320,-29a, -29c, -7	let-7a

Clinical Chemistry 49:10
1733–1739 (2003)

Oak Ridge Conference

Early Biomarkers of Stroke

MARK A. REYNOLDS,[1] HOWARD J. KIRCHICK,[1] JEFFREY R. DAHLEN,[1] JOSEPH M. ANDERBERG,[1] PAUL H. MCPHERSON,[1] KEVIN K. NAKAMURA,[1] DANIEL T. LASKOWITZ,[2] GUNARS E. VALKIRS,[1] and KENNETH F. BUECHLER[1*]

Methods: ELISAs for >50 protein biomarkers were developed for use on a high-throughput robotic workstation. These assays were used to screen plasma samples from 214 healthy donors and 223 patients diagnosed with stroke, including 82 patients diagnosed with acute ischemic stroke. Marker assay values were first compared by univariate analysis, and then the top markers were subjected to multivariate analysis to derive a marker panel algorithm for the prediction of stroke.

Results: The top markers from this analysis were S-100b (a marker of astrocytic activation), B-type neurotrophic growth factor, von Willebrand factor, matrix metalloproteinase-9, and monocyte chemotactic protein-1. In a panel algorithm in which three or more marker values above their respective cutoffs were scored as positive, these five markers provided a sensitivity of 92% at 93% specificity for ischemic stroke samples taken within 6 h from symptom onset.

Conclusion: A marker panel approach to the diagnosis of stroke may provide a useful adjunct to CT scanning in the emergency setting.

Invited Article: Searching for oracles?

Blood biomarkers in acute stroke

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Neurology® 2009;73:393–399

Table Overview of diagnostic accuracy measures for biomarkers and biomarker panels in potential fields of application

Biomarker	Protocol	No.	Sensitivity	Specificity
For diagnosing ischemic stroke				
NMDA-R Ab[7]	IS vs controls, <3 h	304	97	98
4-marker panel[9]	IS vs controls, <24 h	222	90	90
5-marker panel[10]	IS vs mimics, <6 h	130	81	70
4-marker panel[11]	IS vs mimics, <24 h	654	85	34
PARK7[19]	IS vs other patients	787	58	90
NDKA[19]	IS vs other patients	787	67	90
For diagnosing intracerebral hemorrhage				
GFAP[22]	ICH vs IS, <6 h	135	79	98
APO C-III[27]	ICH vs IS, <6 h	42	94	87
For predicting hemorrhagic complications after thrombolysis				
MMP-9[31]	PH vs others	41	100	78
MMP-9[34]	HI-2, PH vs others	134	81	88
c-Fn[34]	HI-2, PH vs others	134	100	96
S100B[35]	PH-2 vs others	275	46	82
PAI-1, TAFI[36]	Symptomatic ICH vs others	77	75	98
For predicting malignant infarction				
S100B after 24 h[40]	Malignant vs nonmalignant	51	94	83
c-Fn[41]	Malignant vs nonmalignant	75	90	100

NMDA-R = *N*-methyl-D-aspartate receptor; IS = ischemic stroke; NDKA = nucleoside diphosphate kinase A; GFAP = glial fibrillary acidic protein; ICH = intracerebral hemorrhage; MMP-9 = matrix metalloproteinase-9; PH-1, PH-2 = parenchymal hemorrhage; HI-1, HI-2 = hemorrhagic infarction.

Take home massage…per il clinico

LIMITI ATTUALI:

- **I risultati vengono da studi su popolazioni molto selezionate, di piccole dimensioni e con intervalli temporali variabili**
- **Non sempre i risultati sono riproducibili nei diversi studi**
- **Alcuni test sono ancora molto costosi e richiedono tempi di laboratorio "lunghi"**

PROSPETTIVE FUTURE:

- **Sviluppo di pannelli di test diagnostici di ischemia acuta**
- **Utilizzo come possibili indicatori precoci di prognosi**
- **Possibili indicatori di efficacia e sicurezza di farmaci trombolitici/neuroprotettivi nell'ictus acuto**
- **Utilizzo di neuroprotezione per aumentare la finestra terapeutica di riperfusione**

Potenziali target terapeutici (neuroprotezione)

TARGET DI NEUROPROTEZIONE

- Antagonisti del glutammato
- Calcio antagonisti
- Inibitori dell’Ossido Nitrico
- Radical Scavengers
- Antiapoptotici
- Antiadesione
- Anticitochine

1,026 Experimental Treatments in Acute Stroke

Victoria E. O'Collins, B.Sci,[1] Malcolm R. Macleod, MRCP, PhD,[3] Geoffrey A. Donnan, MD, FRACP,[2] Laura L. Horky, MD, PhD,[2] Bart H. van der Worp, MD, PhD,[4] and David W. Howells, PhD[1]

Objective: Preclinical evaluation of neuroprotectants fostered high expectations of clinical efficacy. When not matched, the question arises whether experiments are poor indicators of clinical outcome or whether the best drugs were not taken forward to clinical trial. Therefore, we endeavored to contrast experimental efficacy and scope of testing of drugs used clinically and those tested only experimentally. *Methods:* We identified neuroprotectants and reports of experimental efficacy via a systematic search. Controlled in vivo and in vitro experiments using functional or histological end points were selected for analysis. Relationships between outcome, drug mechanism, scope of testing, and clinical trial status were assessed statistically. *Results:* There was no evidence that drugs used clinically (114 drugs) were more effective experimentally than those tested only in animal models (912 drugs), for example, improvement in focal models averaged 31.3 ± 16.7% versus 24.4 ± 32.9%, $p > 0.05$, respectively. Scope of testing using Stroke Therapy Academic Industry Roundtable (STAIR) criteria was highly variable, and no relationship was found between mechanism and efficacy. *Interpretation:* The results question whether the most efficacious drugs are being selected for stroke clinical trials. This may partially explain the slow progress in developing treatments. Greater rigor in the conduct, reporting, and analysis of animal data will improve the transition of scientific advances from bench to bedside.

Ann Neurol 2006;59:467–477

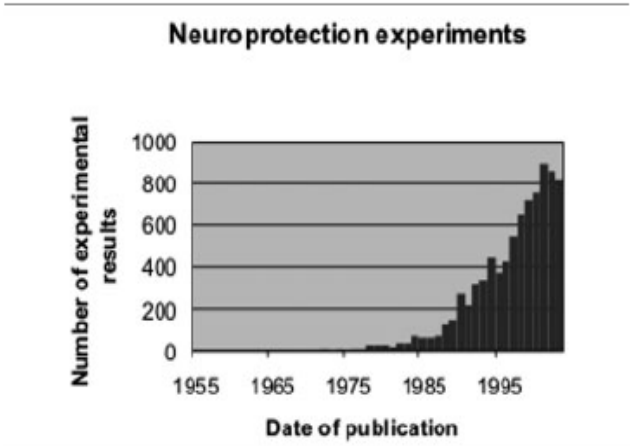


Fig 1. Neuroprotection experiments identified from published reports (1955–2003).

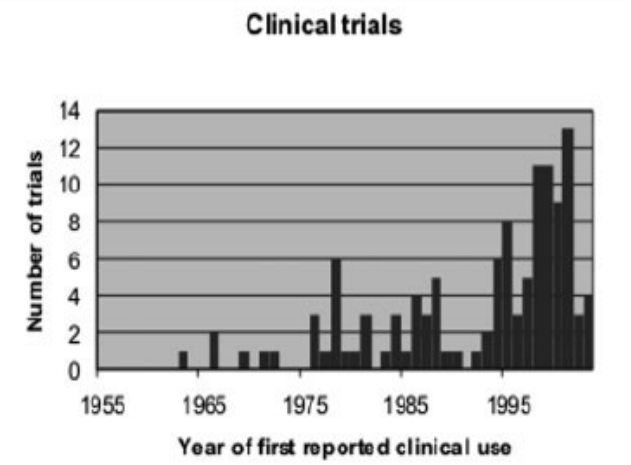
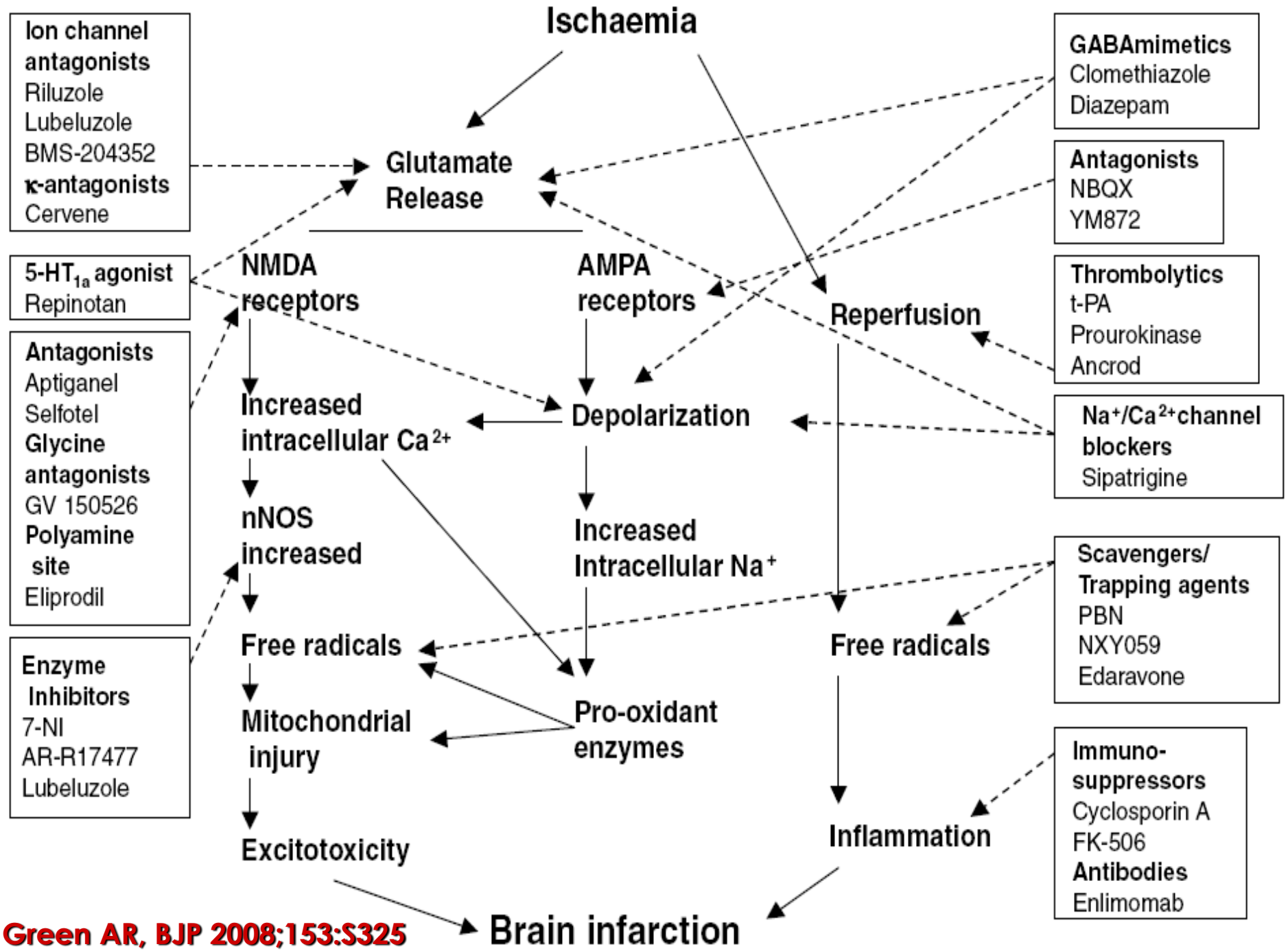


Fig 2. First reported clinical trials of inventions in acute stroke patients (1955–2003).

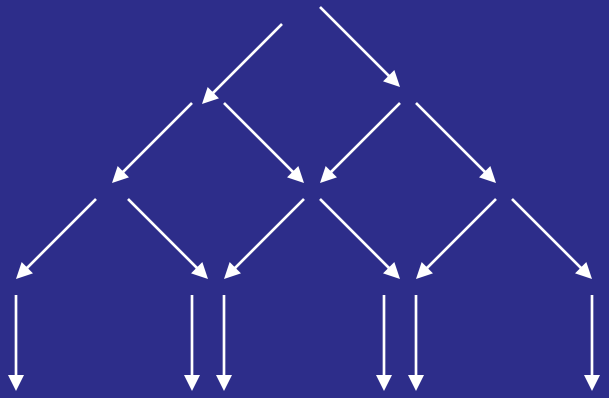


Green AR, BJP 2008;153:S325

Three possible models to explain cell death

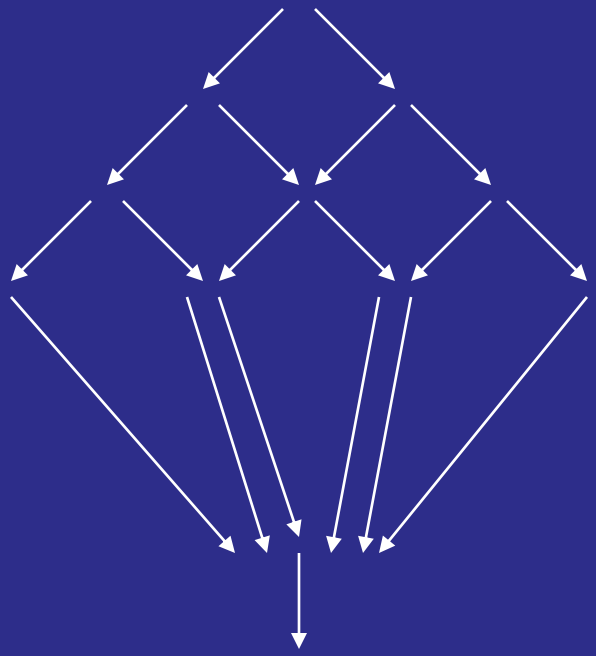
1.Ischaemia → → → Cell death

2.Ischaemia



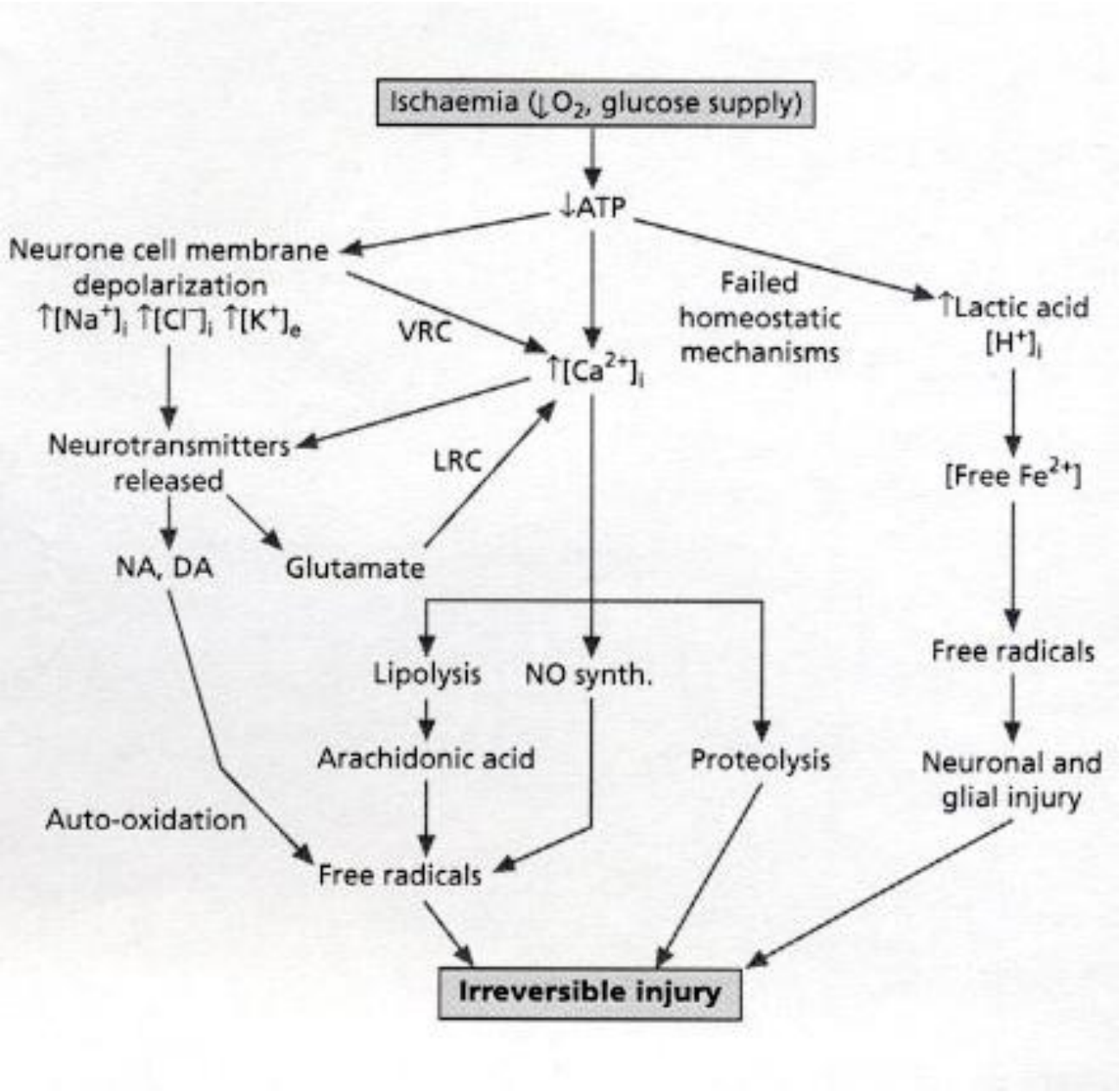
Cell death

3.Ischaemia



Cell death

Potential mechanisms of ischaemic brain damage *(from Pulsinelli et al 1992)*



Toward Wisdom From Failure

Lessons From Neuroprotective Stroke Trials and New Therapeutic Directions **(*Stroke*. 2002;33:2123-2136.)**

- Neuroprotezione “mirata”, basata su marcatori biologici e imaging
- Scelta dei farmaci più attivi nei modelli sperimentali
- Cocktail di vari neuroprotettori associati a trombolisi
- Neuroprotezione ➔ Neuroriparazione
- Riabilitazione associata a terapie farmacologiche e fattori neurotrofici

Italian Stroke Organization (ISO) Basic Science

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Carlo Ferrarese
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ISO
ITALIAN STROKE ORGANIZATION

Vincere l'Ictus Cerebrale?
Insieme, si può fare!!

SPREAD
Stroke Prevention And Educational Awareness Diffusion

22 Laboratori attivi nella ricerca sullo Stroke Sperimentale sul territorio Nazionale

Linee di Ricerca Principali

Neuroinfiammazione o microglia	6
Target molecolari vari (Na-Ca exchanger, mGlu1, Endocannabinoidi, PARP, GRP17, purinergici)	4
Apoptosi o morte neuronale	3
Cellule Staminali o terapia cellulare	3
Neurorepair o Neuroplasticità	3
Oligodendrociti	2
Brain conditioning	1
Collaterali	1

Totale 23

Italian Stroke Organization (ISO) Basic Science

1° Workshop
Roma 26 Gennaio 2016

Discussione

- organizzazione di un trial preclinico multicentrico italiano
- scelta dei targets
- scelta dei bandi di finanziamento

ISO
ITALIAN STROKE ORGANIZATION

Questioni aperte…

- ridurre la distanza tra i modelli animali e gli studi clinici (***ricerca traslazionale***)

- standardizzazione dei metodi di laboratorio (ELISA assay)
- accurata selezione e caratterizzazione dei pazienti (in particolare riguardo ai tempi dall'esordio dei sintomi)

- necessità di studi multi-centrici prospettici
- quali biomarcatori utilizzare come end-point surrogato nei trial clinici