Marcatori Liquorali nella sindrome Post-polio

Gianluigi Zanusso

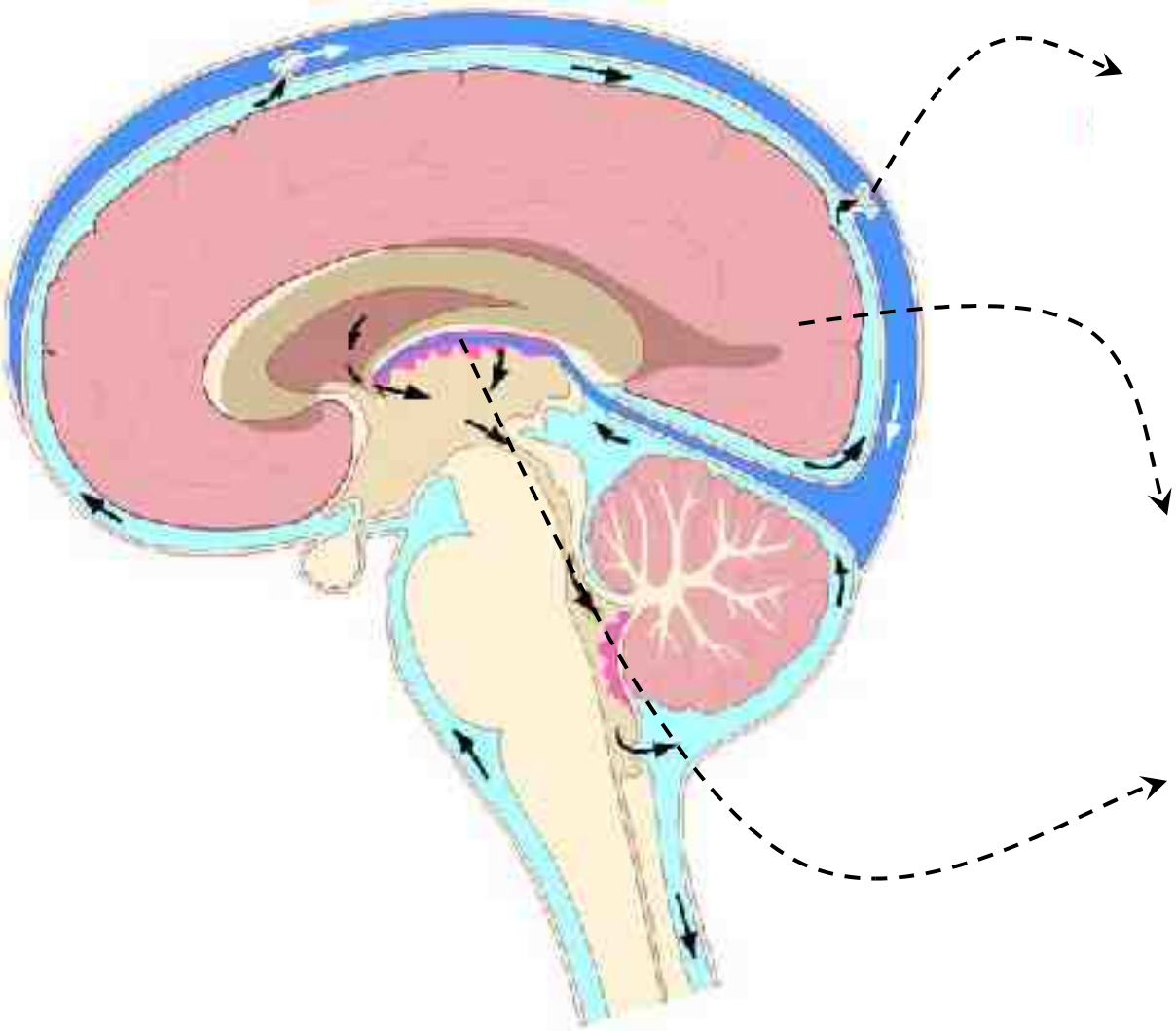
Sezione di Neurologia Clinica,
Dipartimento di Neuroscienze
Università di Verona

Poliomielite e sindrome post-polio: nuove frontiere terapeutiche

Malcesine, Palazzo dei Capitani, 25 Settembre 2010

The Cerebros

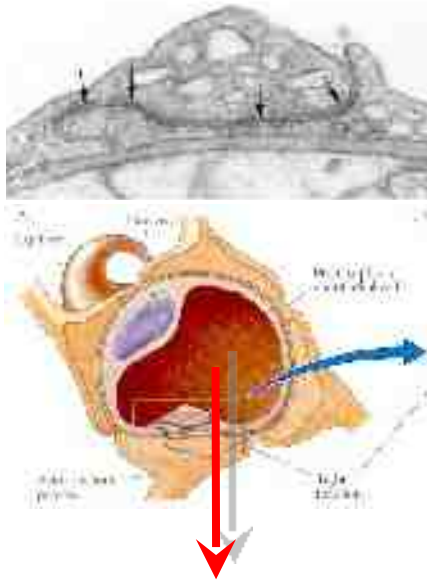
N.J. Abbott / Neurochemistry International 45 (2004) 545–552



Macro-circulation

Micro-circulation

CSF and Nervous Tissue

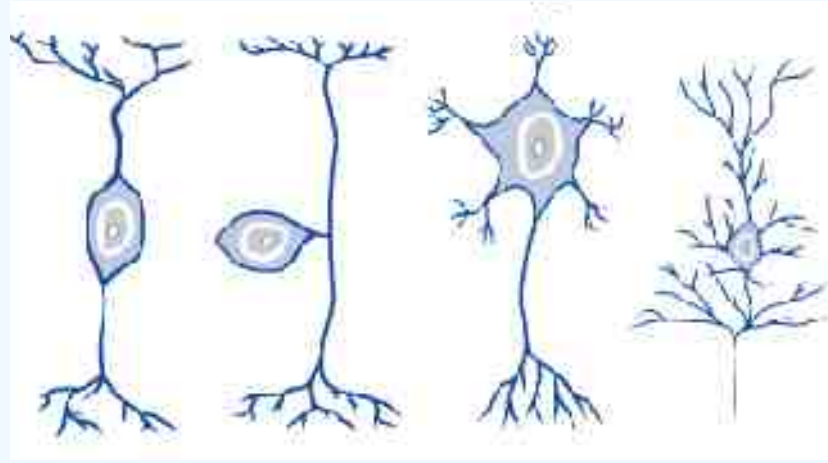


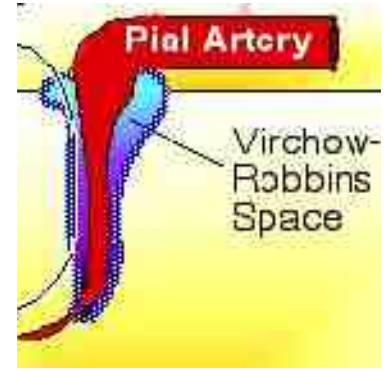
Plasma

High Protein Levels

EEB

Nervous Tissue





CSF

Low Protein Levels

CSF standard

- **Infectious, Inflammatory or Autoimmune Disorders**

  Effect of Blood Brain Barrier damage
  Immune-cells
  Oligoclonal bands

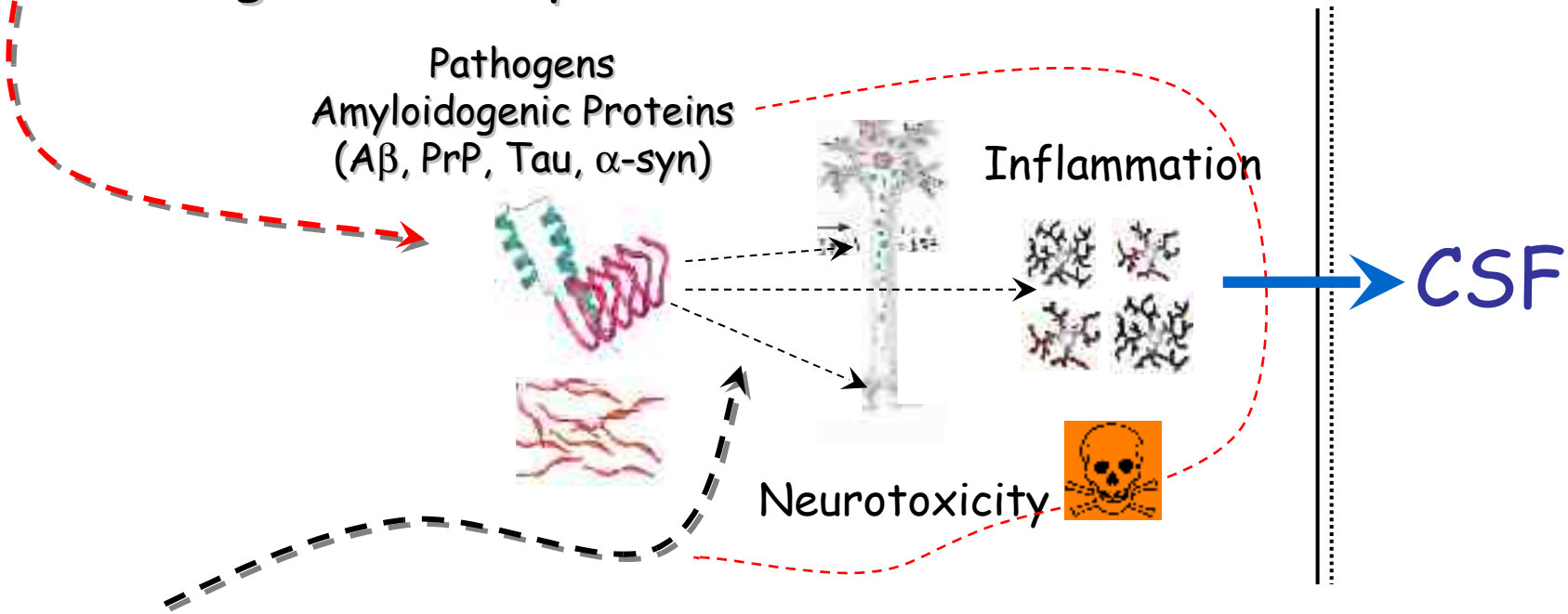
- **Neurodegenerative Disorders**

  No Blood Brain Barrier Damage
  Absence of Oligoclonal bands
  Detection of Neuronal or Glial Dysfunction/Activation
  (S-100, NSE, GFAP, proteina 14-3-3, etc.)

Strategies for Biomarker Detection in the CSF in Neurological Disorders

- **Search for Directly involved Biomarkers**

  Proteins which are directly involved in the neurodegenerative process



- **Search for surrogate biomarkers or Bystanders**

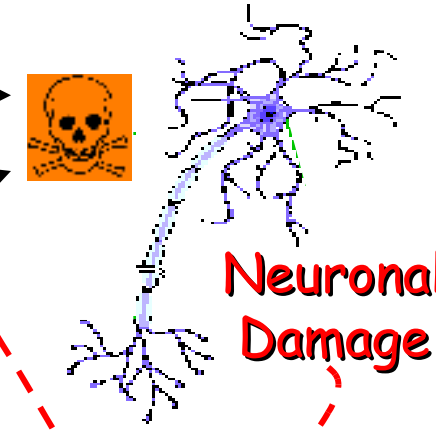
  Proteins which are indirectly involved in the neurodegenerative process

CSF and Post-Polio Syndrome

Hypothesized Mechanisms leading to Motorneuron Dysfunction

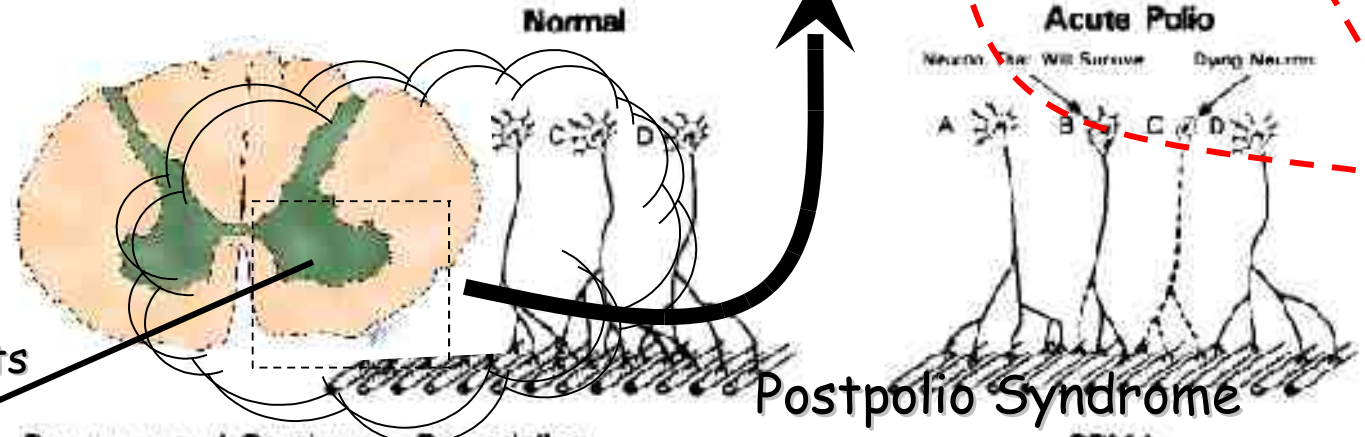
Persistence of Poliovirus → Deregulation of Inflammatory and Immune response

Degenerative process → Alteration of regulatory mechanisms of enlarged motor units



Neuronal Damage

CSF ?



Reinnervation Giant Motor Units

Postpolio Syndrome

Recovery and Continuous Remodeling

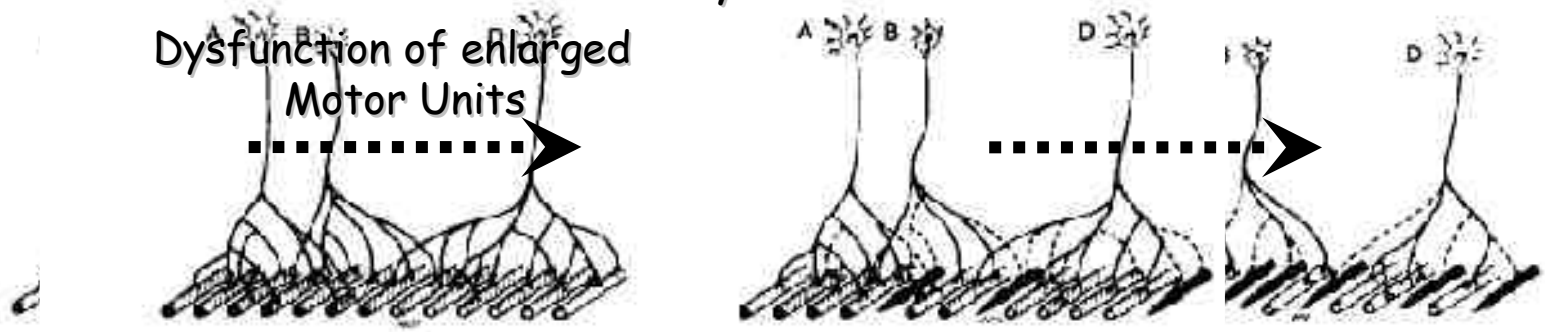
PPMA

Stable Polio

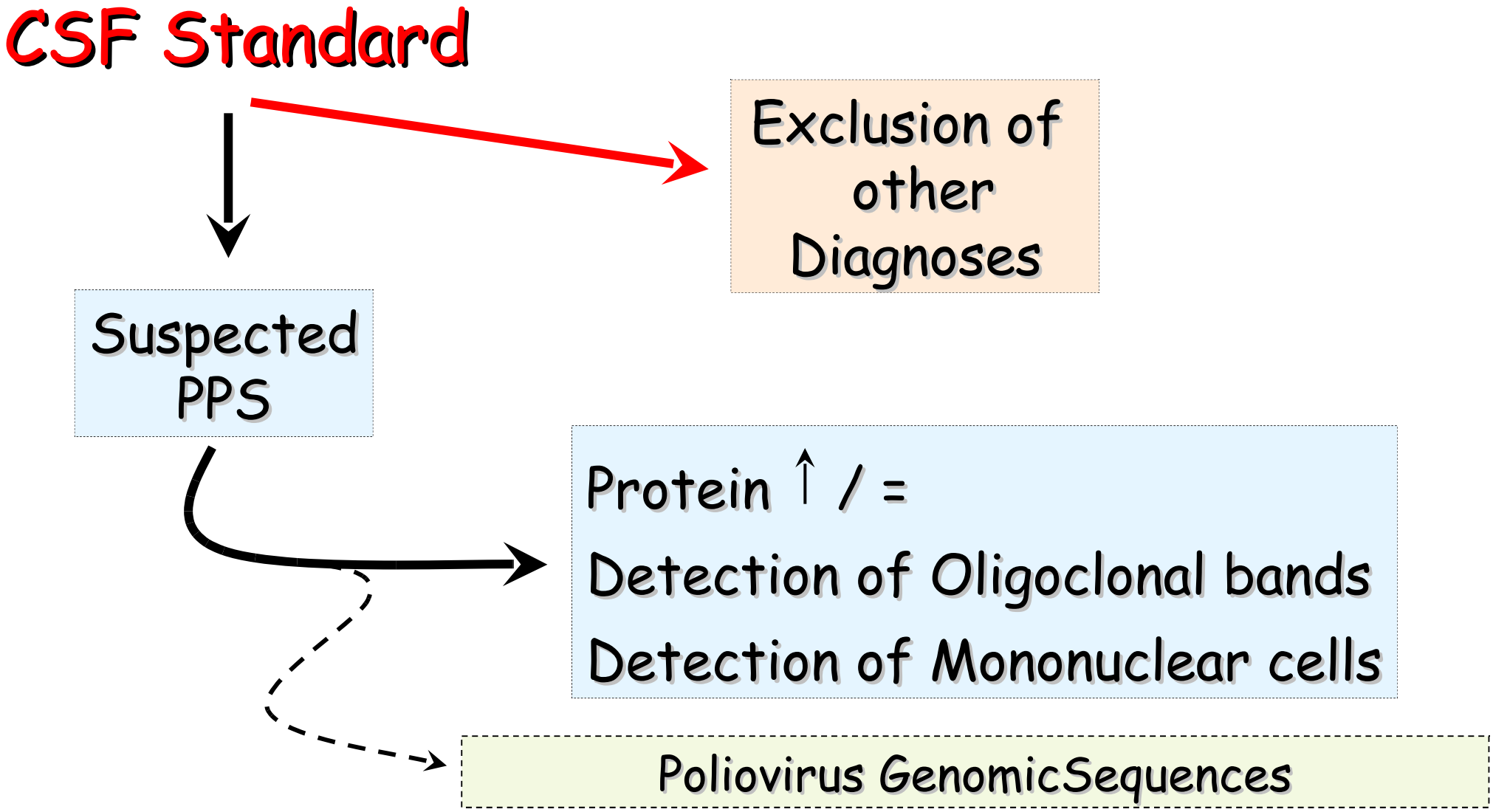
Early Disease

Late Disease

Dysfunction of enlarged Motor Units



Standard CSF in Post-Polio Syndrome



Detection of Oligoclonal Bands

Pros

The New England Journal of Medicine

Volume 325 SEPTEMBER 12, 1991 Number 11

INTRATHECAL IMMUNE RESPONSE IN PATIENTS WITH THE POST-POLIO SYNDROME

MOHAMMAD K. SHARIEF, M.B., CH.B., M.PHIL., ROMAIN HENTGES, M.D., AND MARIA CIARDI, M.D.

Cons

Twenty-One Patients with Strictly Defined Postpoliomyelitis Syndrome: No Poliovirus-specific IgM Antibodies in the Cerebrospinal Fluid

Merja Roivainen, PhD,* Esko Kinnunen, MD, PhD,† and Tapani Hovi, MD, PhD*

Annals of Neurology Vol. 36 No. 1 July 1994 115

ANNALS NEW YORK ACADEMY OF SCIENCES

Antibody Titer to the Poliovirus in Blood and Cerebrospinal Fluid of Patients with Post-Polio Syndrome

BURK JUBELT,[a,b] EDGAR F. SALAZAR-GRUESO,[c] RAYMOND P. ROOS,[c] AND NEIL R. CASHMAN[d]

[a]*Departments of Neurology and Microbiology/Immunology State University of New York Health Science Center Syracuse, New York 13210*

[c]*Department of Neurology University of Chicago Chicago, Illinois 60637*

[d]*Montreal Neurologic Institute McGill University Montreal, Quebec, H3A 2B4 Canada*

Detection of Cytokines and Mononuclear cells

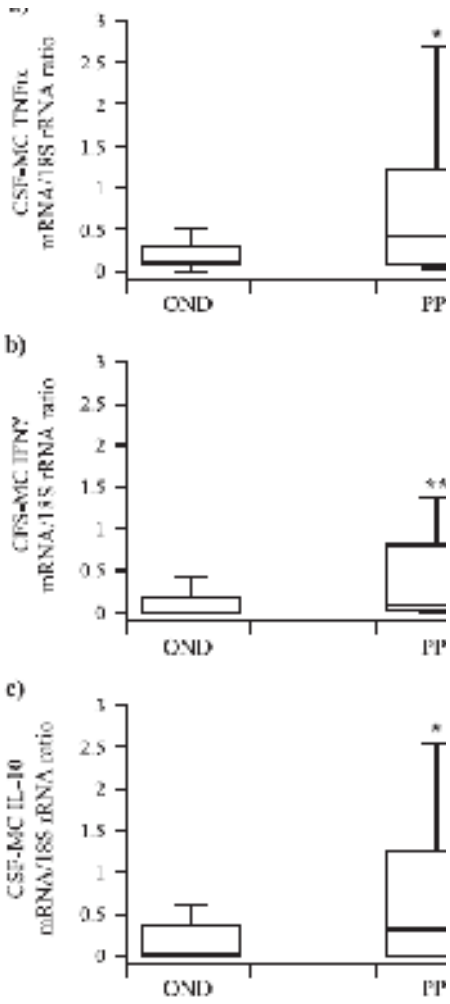
Proinflammatory Cytokine Expression in CSF Mononuclear cells from PPS patients

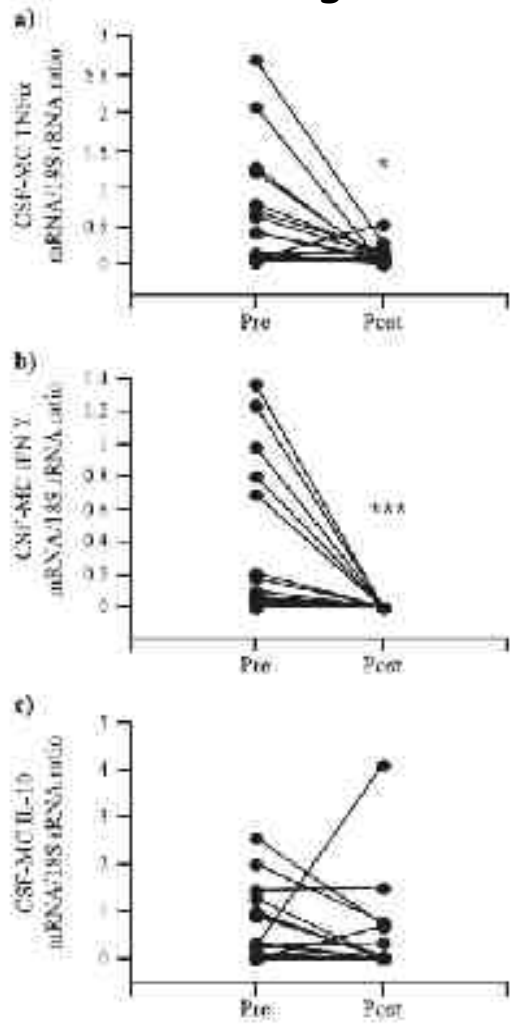
(From Gonzales et al. J Neuroimmunol 2004)

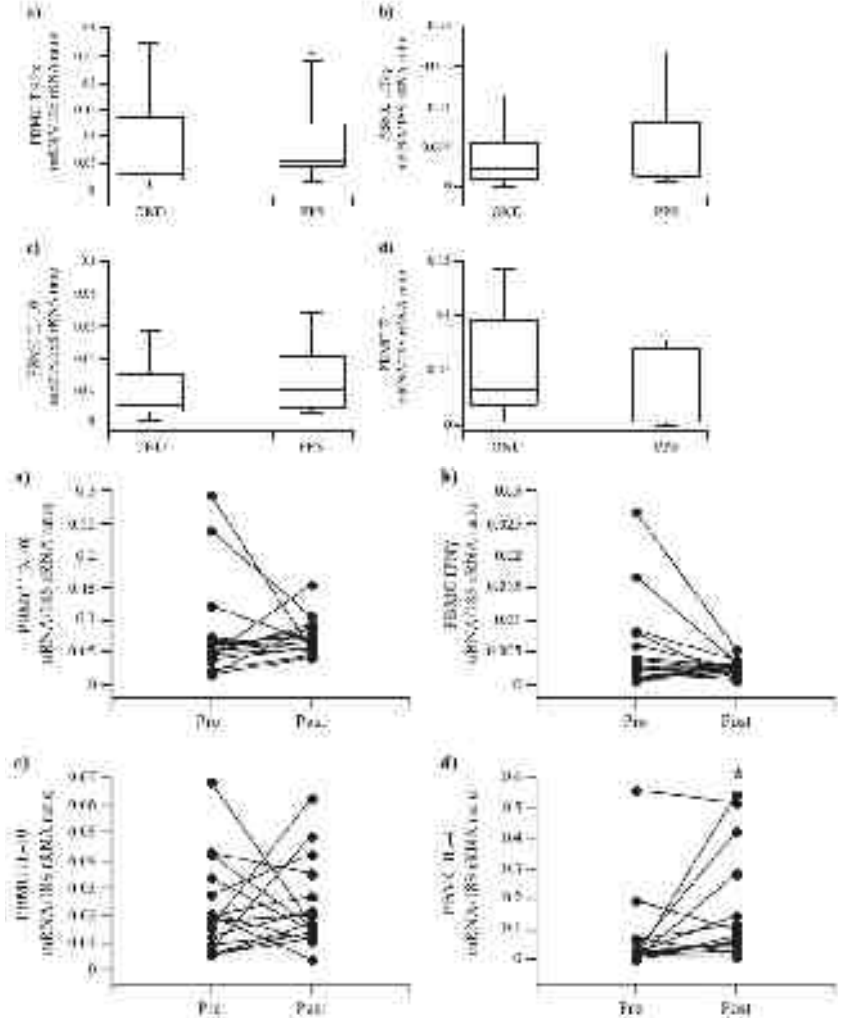
CSF

After IvIg treatment

Blood







Search for Poliovirus Genomic Sequences

Pros

Evidence of Presence of Poliovirus Genomic Sequences in Cerebrospinal Fluid from Patients with Postpolio Syndrome

Virology of the post-polio syndrome

Ambrosius Bij, Salvatore Monaco, Gianluigi Zanusso,

Elena Dalfovo, Lucio Bertolasi & Antonio Toniolo

Evidence for Persistent Enterovirus Infection of the Central Nervous System in Patients with Previous Paralytic Poliomyelitis

P. MUIR, C. NICHOLSON, M. K. SHARIEF, F. J. THOMPSON, N. J. CAIRNS, P. LANTOS, G. T. SPENCER, H. J. KAMINSKI, AND J. B. BANATVALA

Cons

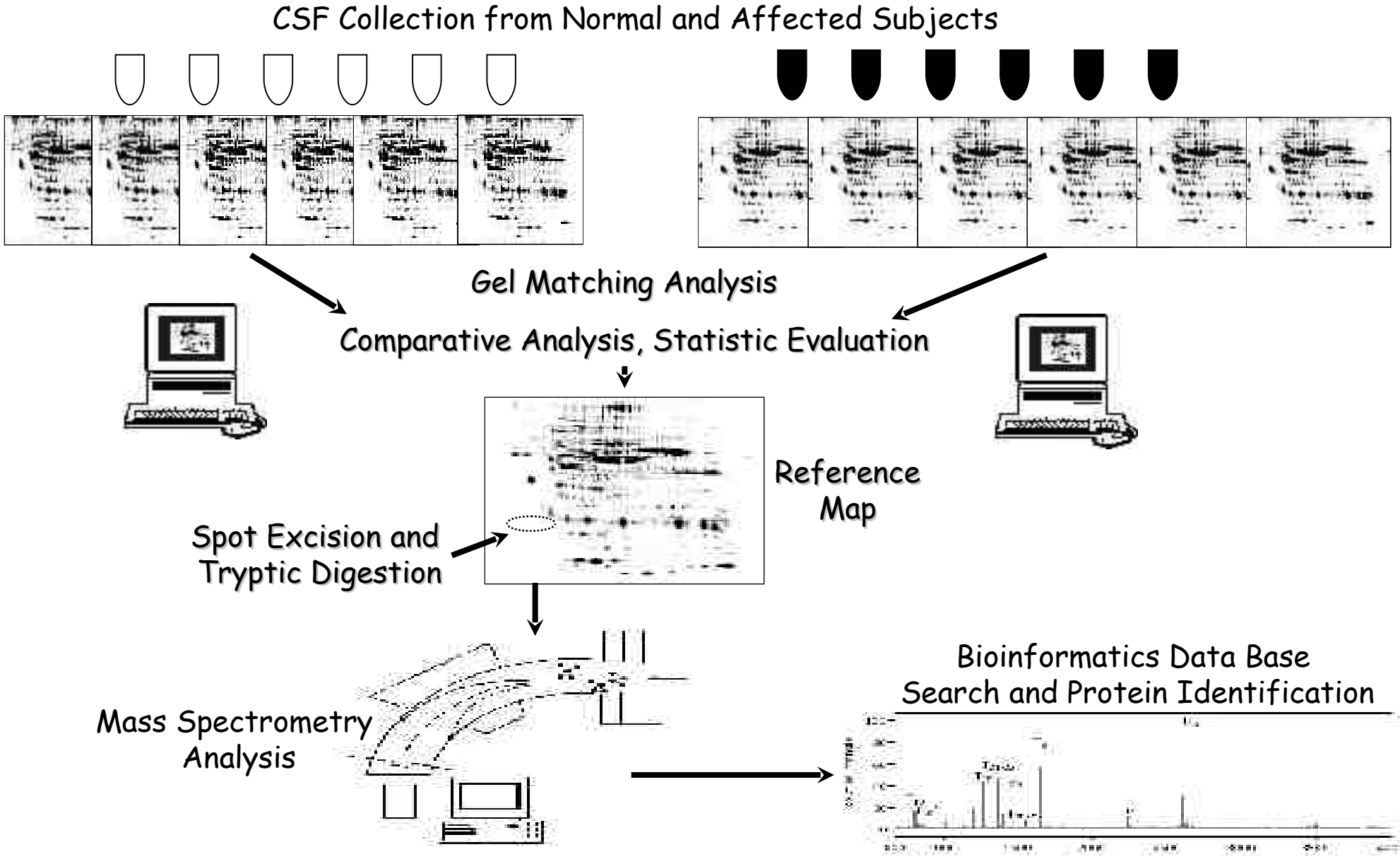
The Postpolio Syndrome: No Evidence for Poliovirus Persistence

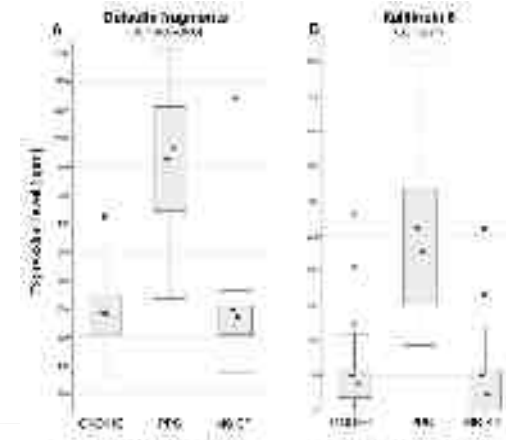
Willem Melchers, PhD,* Marianne de Visser, MD, PhD,‡ Peter Jongen, MD,* Anton van Loon, PhD,§ Ria Nibbeling, BSc,§ Paul Oostvogel, MD,§ Diana Willemse, BSc,* and Jochem Galama, MD, PhD*

To investigate the possibility of poliovirus persistence in patients with the postpolio syndrome, we examined skeletal muscle biopsy specimens, cerebrospinal fluid specimens, and sera for the presence of poliovirus RNA by the polymerase chain reaction, and for IgM antibodies by a poliovirus type-specific IgM antibody-capture enzyme-linked immunosorbent assay. In none of these specimens was poliovirus RNA or a poliovirus type-specific IgM response detected. These results argue against the hypothesis that poliovirus persists in patients with the postpolio syndrome and plays a role in the pathogenesis of the postpolio syndrome.

Melchers W, de Visser M, Jongen P, van Loon A, Nibbeling R, Oostvogel P, Willemse D, Galama J. The postpolio syndrome: no evidence for poliovirus persistence. Ann Neurol 1992;33:728–732

Identification of Disease Specific Protein Biomarkers through Proteomic Profile



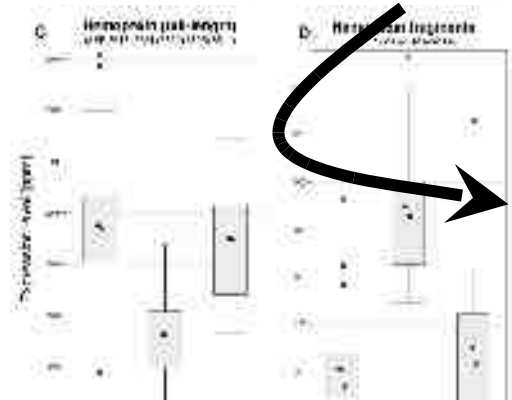


> 2.4

> 5.3

> 4.2

< 2.3



Interpretation

- Kallikrein 6: normally expressed in neurons and oligodendrocyes
up-regulated after inflammatory damages. (Expression of neurite outgrowth or toxic to oligodendrocytes)

- Fragments of Gelsolin: Related to an increase of caspase 3 activity and reduction of antiapoptotic effect

- Hemopexin: Expressed in acute phases of CNS damage

- Expression of a chronic inflammatory CNS damage, possibly related to an autoimmune mechanism or a viral persistence

- These proteins plays a role in the pathophysiology

- Candidate Biomarkers

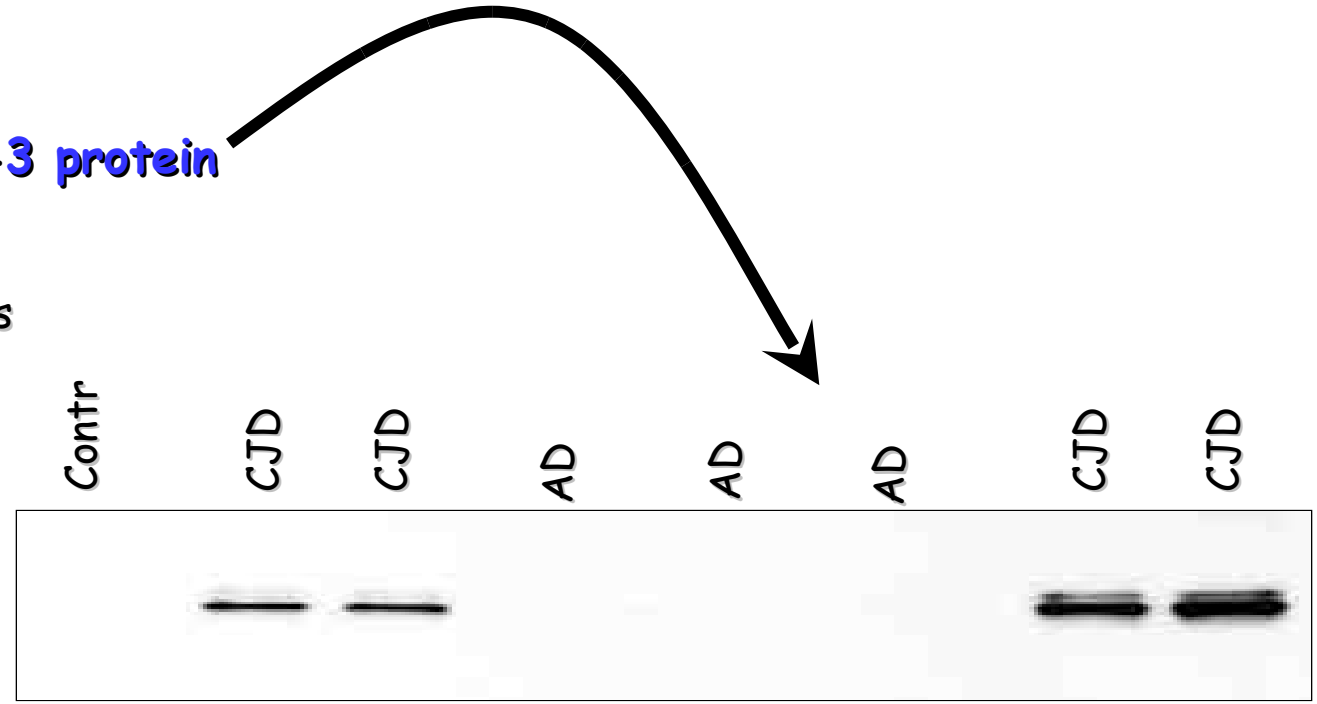
CSF Protein Markers from Neurons and Glial cells in Neurological Disorders

Table 1. Cellular origin of CSF brain-derived proteins

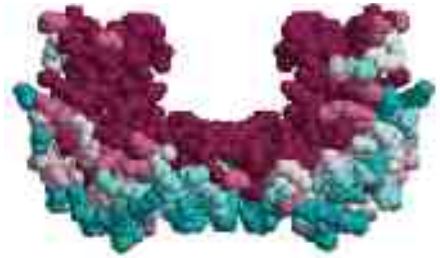
Cellular origin of brain-derived proteins in CSF
Neurones
Neurone-specific enolase (NSE)
14-3-3
Tau protein
Amyloid precursor protein
Amyloid peptides ($A\beta_{40}$, $A\beta_{42}$)
Neurofilament proteins
Chromagranins A + B
Astrocytes
S-100b
Glial acidic fibrillary proteins
Leptomeninges
B-trace protein
Cystatin C
Microglial cells
Ferritin
Oligodendrocytes
Myelin basic protein
Proteolipid protein
Myelin oligodendrocytic glycoprotein
Choroid plexus
Transthyretin

Diagnostic criteria for sporadic CJD

- **Definite**
  - Neuropathologically/immunocytochemically confirmed
- **Probable**
  - Less than 2 years progressive dementia
  - Two of the following clinical signs:
    - Myoclonus
    - Visual or cerebellar problems
    - Pyramidal or Extrapyramidal features
    - Akinetic mutism
  - Typical EEG
  - **OR**
  - Possible and **positive 14-3-3 protein**
- **Possible**
  - Rapidly progressive dementia
  - Two of the above clinical signs
  - Duration < 2 years

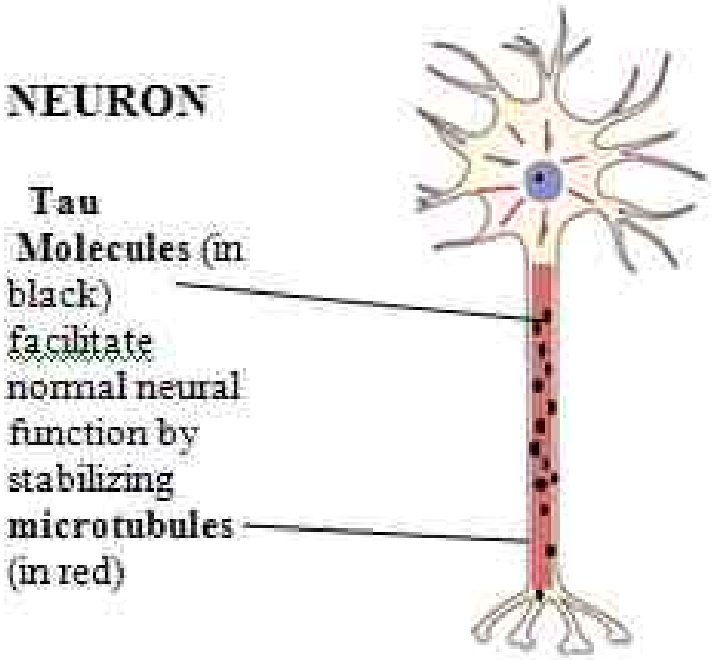


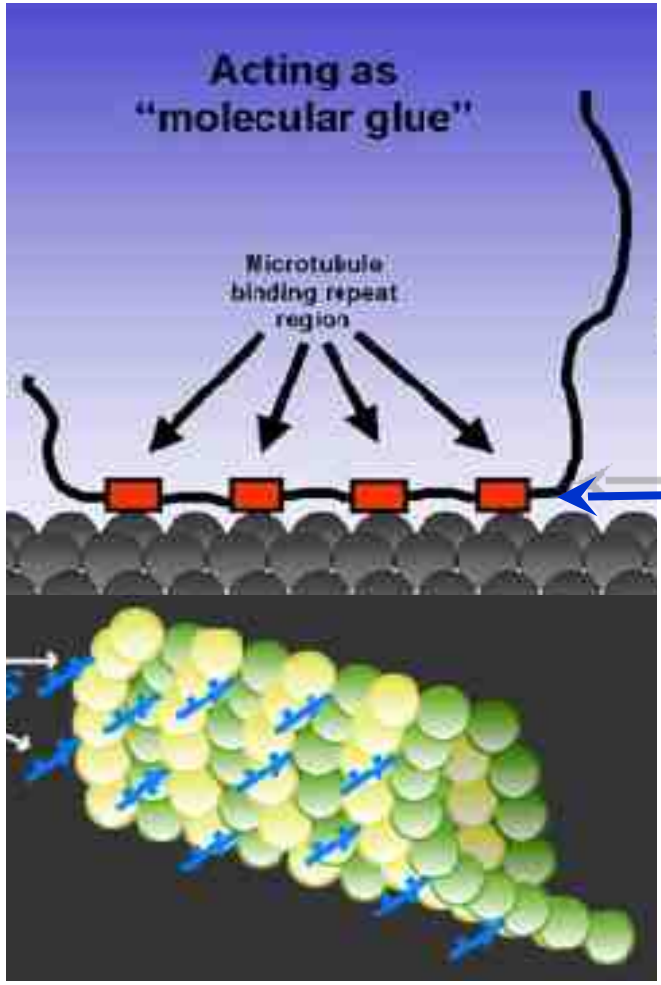
14-3-3 PROTEINS

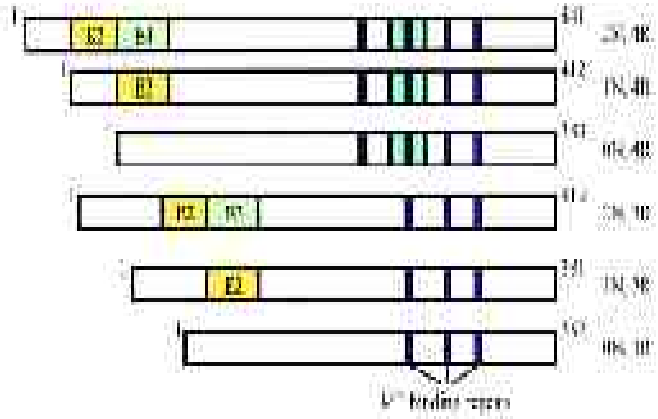


- 7 isoforms of 14-3-3 proteins are recognized chracterized by a high homology and are encoded by distinct genes: β, ε, η, γ, τ, ζ, σ ; α, δ are phosphorylated forms of β, ζ
- The dimeric homo- or hetero- forms of 14-3-3 protein represent the functionally active protein. These are composed by two 30 kDa monomers (acidic P.I. : 4-5)
- Cytosolic localization
- Highly concentrated in neurons (1% of total proteins)
- Modulate functions of several cellular proteins (kinases, phosphatases and transmembrane receptors) and of several biological processes (neuronal development, cellular growth, apoptosis…)
- Antiapoptotic function

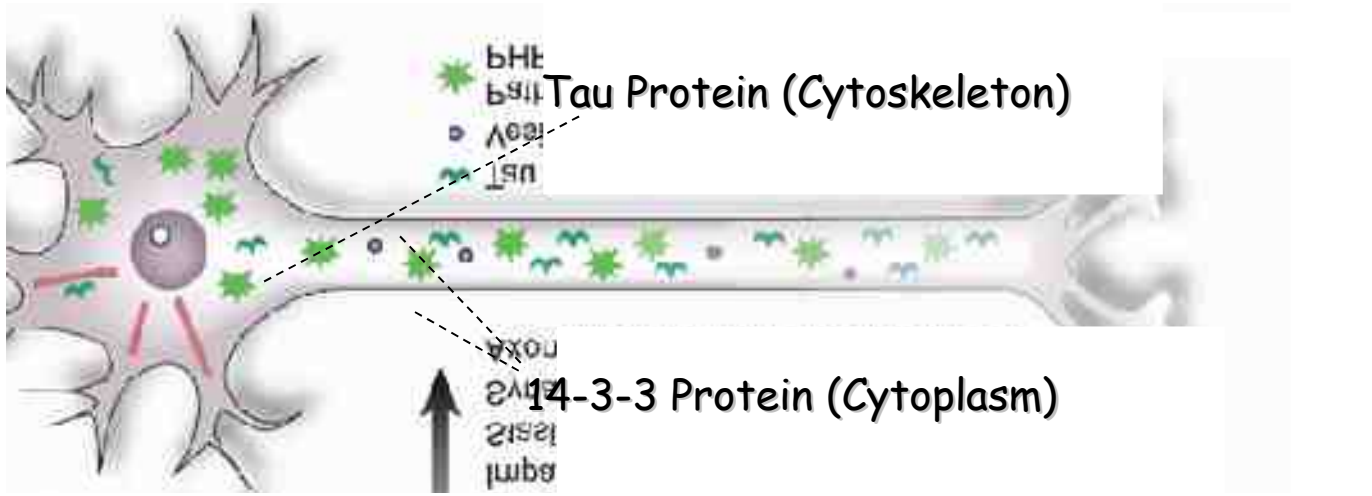
TAU PROTEIN


NEURON
Tau Molecules (in black) facilitate normal neural function by stabilizing microtubules (in red)


Acting as "molecular glue"
Microtubule binding repeat region



The CSF expression of both 14-3-3 protein positivity and Tau Protein levels allow to determine two different cell sites of Neuronal Damage



- Tau protein is detected when occurs a consistent neuronal damage involving cytoskeletal proteins. In contrast, 14-3-3 protein is sensitive to mild toxic events (i.e. iatrogenic, epileptic seizures etc.)

Future Neurol. (2007) 2(4), 451–463

Post-polio syndrome: clinical manifestations and cerebrospinal fluid markers

Michele Fiorini,
Gianluigi Zanusso,
Andreina Baj,
Laura Bernalasi,
Antonio Taniola &
Salvatore Monaco†

†Author for correspondence
University of Verona,
Department of Neurological & Visual Sciences, Policlinico GB Rossi, Piazzale LA Scuro, 10, 37134 Verona, Italy
Tel.: +39 045 812 4461;
Fax: +39 045 585 933;
salvatore.monaco@univr.it

Table 2. Reports of cerebrospinal fluid 14-3-3 protein assay in different neurological disorders.

Disorder	Positive/negative 14-3-3 assay	Ref.
Viral meningoencephalitis	2/7	[68]
	12/21	[69]
Nonviral meningoencephalitis	3/11	[68]
	1/20	[70]
Multiple sclerosis	1/10	[68]
	0/8	[69]
	5/36	[71]
	3/37	[72]
	25/114 (ELISA)	[73]
	24/63	[74]
	14/76	[75]
Alzheimer disease	1/49	[69]
	4/20	[76]
Other dementias	0/5	[68]
	0/14	[69]
	1/31	[70]
Stroke	4/8	[64]
Paraneoplastic diseases	13/70	[77]
Guillain–Barré syndrome	0/5	[68]
	29/98	[78]
Motor neuron disease	0/7	[68]
Noninflammatory neuropathy	0/15 (ELISA)	[73]

Postpolio Syndrome and CSF Markers

Patients

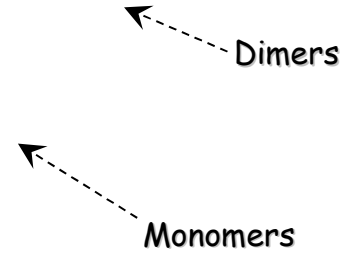
Table 3. Demographic, clinical and laboratory features of patients.

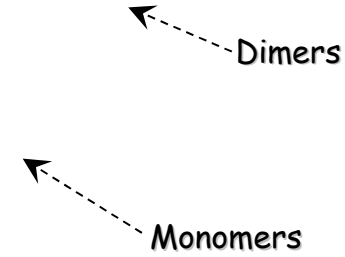
Patient No.	Diagnosis	Age (years)	CSF protein level (mg/dl)	Oligoclonal Bands	Tau (pg/ml)	1D PAGE 14-3-3	2D PAGE high molecular weight 14-3-3	Cystatin C*
1	Post-polio	52	0.24	nd	374	±	+	0.16
2	Post-polio	73	0.85	nd	115	±	+	0.98
3	Post-polio	50	0.41	nd	<60	±	+	0.22
4	Post-polio	58	0.87	nd	199	+	+	4.66
5	Post-polio	81	0.66	nd	210	+	+	nd
6	Post-polio	57	0.25	nd	<60	±	+	nd
7	Post-polio	52	0.35	nd	198	±	+	1.37
8	Post-polio	66	0.37	+	174	-	+	0.91
9	Post-polio	65	0.37	nd	167	±	+	nd
10	Post polio	51	0.37	nd	195	±	+	nd
11	Post-polio	51	0.24	nd	198	-	+	nd
12	Post-polio	73	0.25	nd	160	±	+	0.32
13	Polio	75	0.15	nd	401	±	+	0.56
14	Post-polio	60	0.21	nd	63	±	+	0.36
15	Polio	54	0.20	+	<60	+	+	0.19
16	Post-polio	54	0.27	nd	86	nd	+	1.99
17	Post-polio	52	0.24	nd	331	nd	+	7.5
18	Post-polio	62	0.22	nd	345	nd	+	11.77
19	Polio	62	0.30	nd	390	+	+	0.15

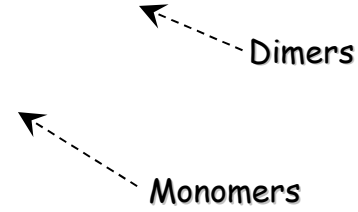
CSF Analysis

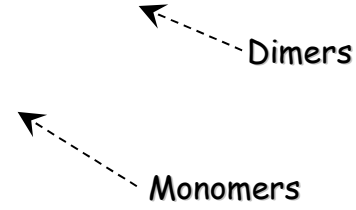
Postpolio Syndrome and CSF Markers

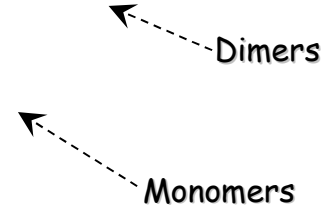
2D-PAGE Analysis











Conclusions: CSF Markers in PPS

- 14-3-3 protein levels are increased in the CSF of patients affected with PPS. This finding is more evident by 2D-PAGE analysis likely related to the presence of dimeric forms of 14-3-3 protein.

- 2D-PAGE analysis of 14-3-3 protein shows a pattern similar to that observed in neurological inflammatory disorders but different from ALS

- To provide insights about the inflammatory events occuring in PPS a detailed characterization of distinct 14-3-3 protein isoforms is ongoing.

- However, the low Tau protein levels detectd in PPS exclude an acute or widespread neuronal damage.

Ringraziamo i Colleghi di Malcesine Drs M. Martini e B. Danzi che attraverso un' attiva collaborazione, hanno permesso di perseguire questo studio