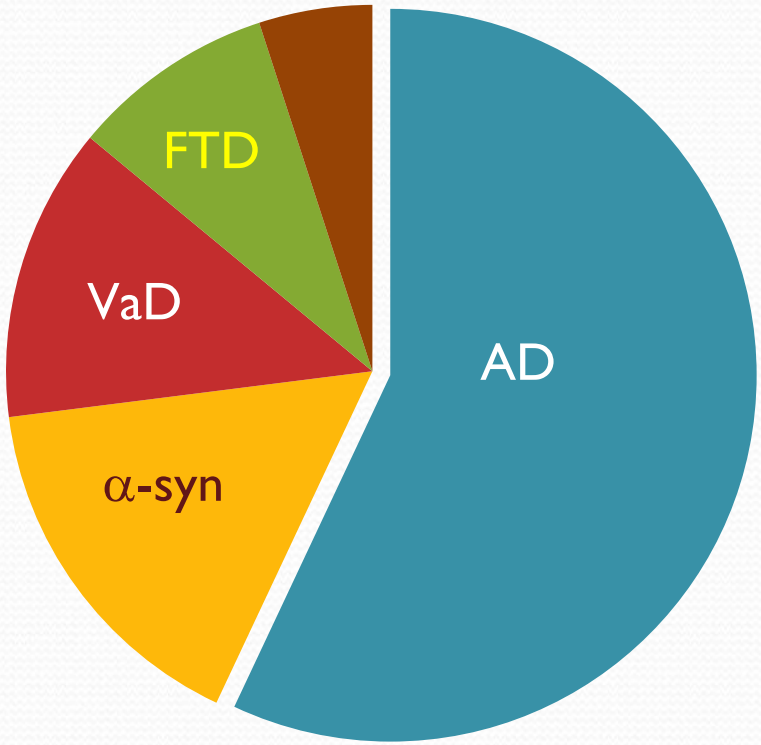
Potential clinical applications of CSF biomarkers in neurodegenerative disease

Dear Graham,
Dear Collegues,

I'm really sorry that I cannot be present!
I wish you a very productive meeting!

Thanks Armand for your kindness!

Dementia



International Work Group terminology

Preclinical AD

"Asymptomatic at risk for AD"
"Presymptomatic AD"

AD patients

"Prodromal AD"
"AD dementia"

Atypical forms of AD

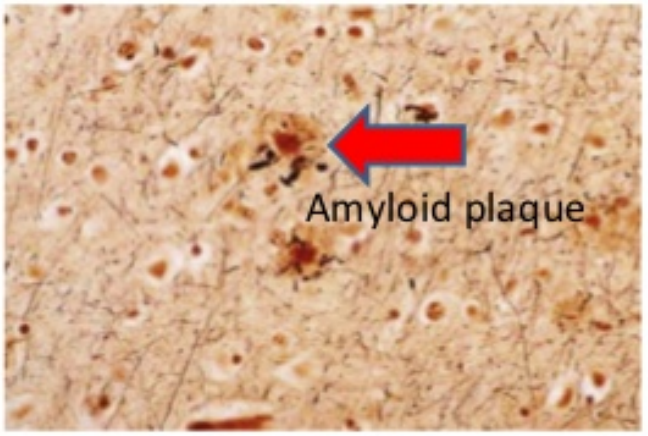
"Progressive aphasia mainly logopenic"
"Posterior atrophy"
"Frontal variant AD"

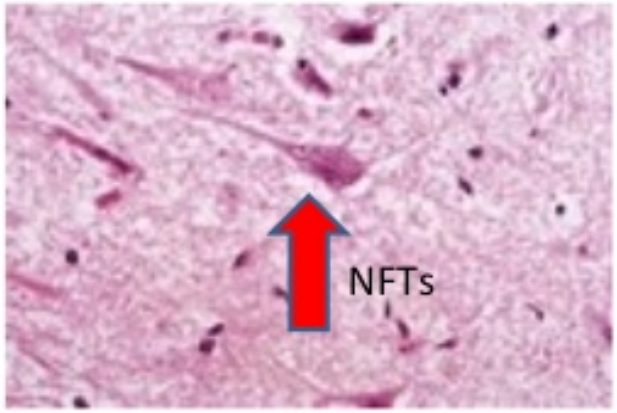
Alzheimer's Disease diagnosis: an open problem for both researchers and clinicians

- In front of merely poor brain pathology, namely the extracellular senile plaques and the intracellular neurofibrillary tangles, in the last 20 years several experimental hypothesis on the pathogenesis of AD have been made, and among them the amyloid metabolism impairment remains the leading one.
- Amyloid represents the main constituent of the senile plaques, while tau and its phosphorylated form represent the main constituents of the neurofibrillary tangles, pathological accumulations of the axonal cytoskeleton.
- The amyloid-based hypothesis provided a pathogenic link between these constituents, and solid background for new advances in both research and clinics

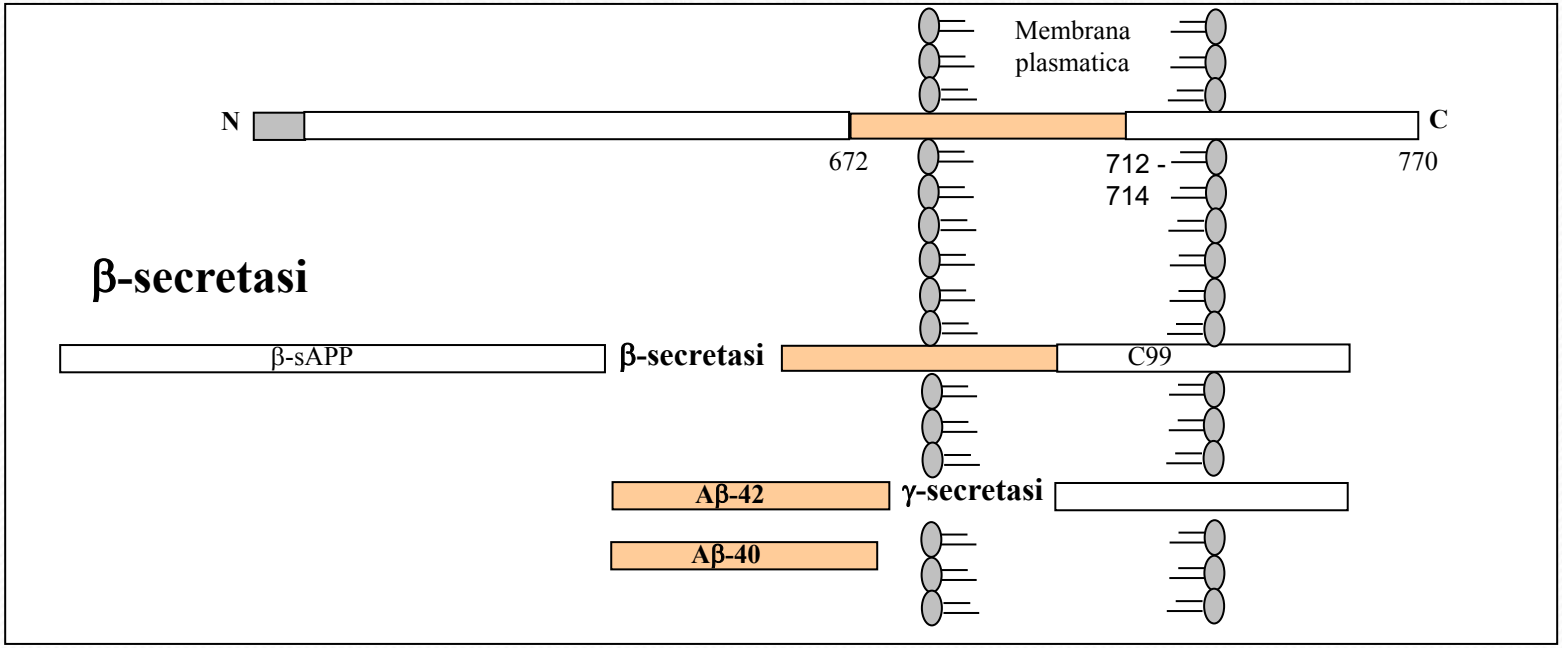
Pathology

- Amyloid plaques,
- Neurofibrillary tangles
- Neuron and Synapse loss
- Neuronal cell death





$A\beta_{42}$


Membrana
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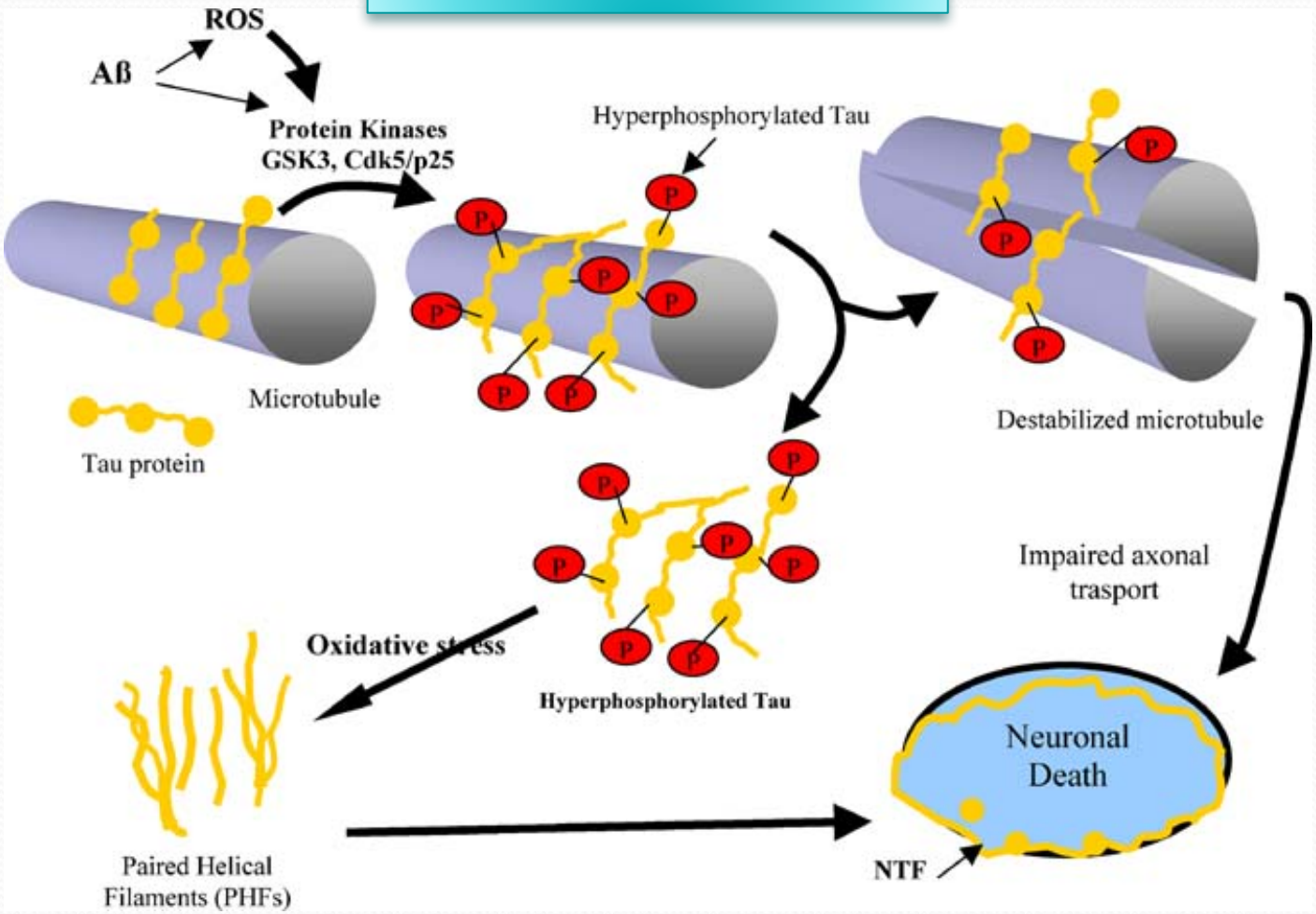
C99
Aβ-42
γ-secretasi
Aβ-40

Tau and p-tau



Formichi 2006

BioMarkers and stages of the disease

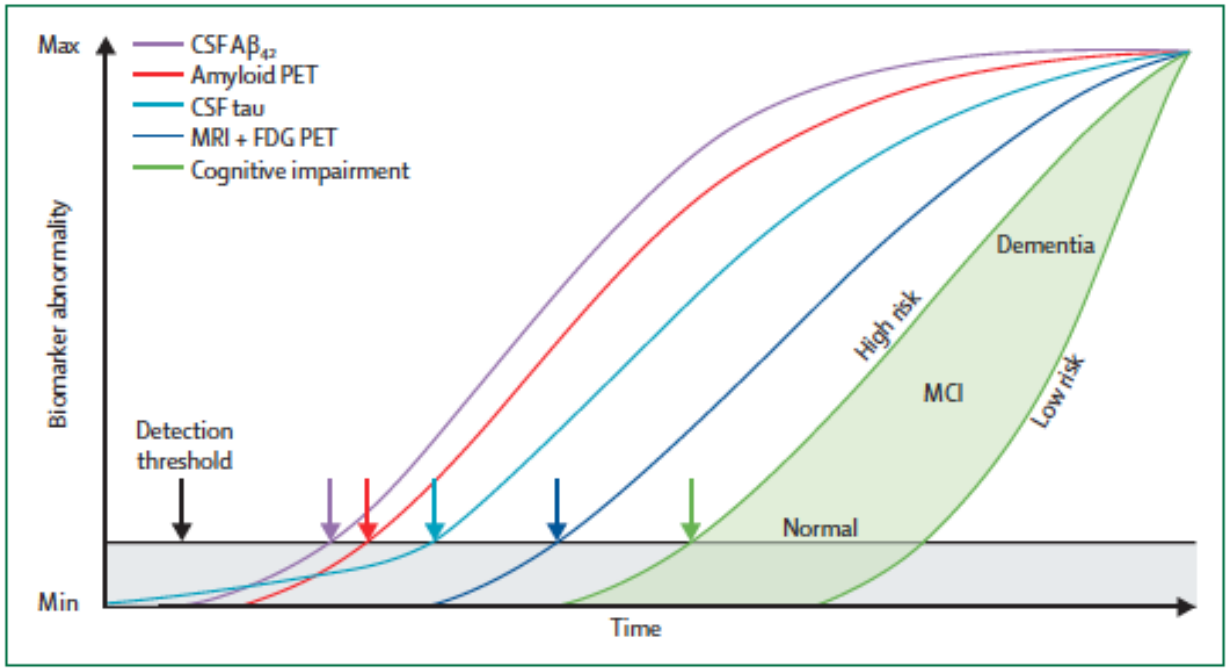


Figure 6: Model integrating Alzheimer's disease immunohistology and biomarkers

- Early modification i pre-clinical and in MCI
- No more significant modification at demential stages

➢ The biomarkers are not staging markers

CSF as potential tool for AD diagnosis

- Several observations indicated that cerebrospinal fluid (CSF), due to its interaction with extracellular space in the brain, may potentially mirror the pathological changes occurring in the brain and represent a valid tool to evaluate the presence of biomarkers .
- To date the most consistent efforts have been made uniquely for Alzheimer's disease (AD), the most common form of dementia among elderly.
- Use of biomarkers changed our diagnostic perspectives giving substantially a clinical diagnosis *in vivo, and at the same* time relevant insights on pathophysiological mechanisms related to AD.
- Amyloid Beta 1-42 (Aβ42), total-Tau (T-Tau) and its phosphorylated form (P-Tau) are the validated biomarkers in use for AD.
- They are used also to diagnose the other forms of dementia, as exclusion criterion

Biomarkers for cognitive decline

Amyloidosis

- low levels of $A\beta_{42}$ in the cerebrospinal fluid (CSF)
- high ligand retention on amyloid-PET

Neurodegeneration

- high levels of tau in the CSF
- hyppocampal/medial temporal atrophy in MRI
- temporal/parietal hypometabolism in FDG-PET

IWG Criteria (FDA and EMA approved)

- Clinical signs and symptoms

(amnestic syndrome of the hippocampal type)

- CSF biomarkers ($A\beta_{1-42}$, Tau, P-tau)
- PET amyloid tracer retention (PiB and others)
- PET bilateral parieto-temporal hypometabolism (FDG)
- MRI medial temporal/hyppocampus atrophy

NIA-AA Diagnostic Criteria

- In the diagnostic criteria for MCI because of AD, developed by the National Institute on Aging and the Alzheimer's Association (NIA-AA), a positive Aβ biomarker (either by amyloid-PET or CSF) together with the presence of a neuronal injury biomarker, such as medial temporal lobe atrophy or elevated levels of tau and p-tau in the CSF, indicates that the MCI syndrome may be because of AD, whereas negative Aβ biomarkers suggest that MCI is unlikely because of AD
- Although brain imaging with MRI, FDG-PET, and amyloid PET often require advanced imaging analyses, which may not be easily accessible everywhere, a lumbar puncture (LP) may be done in many different clinical settings and CSF samples can, if needed, easily be shipped to a central laboratory for analysis

Herukka et al.,Alzheimers Dement. 2017

Tau protein and combined markers in differential diagnosis between AD and Healthy controls			References
	Specificity	Sensitivity	
T-tau	92% 81%	78% 82%	Bibl 2012 Duits 2014
P-tau	91%	81%	Bibl 2012
Aβ42+T-tau	88%	88%	Kang 2013
Tau/Aβ42	90%	85%	Duits 2014
P-tau /Aβ42	88%	85%	Duits 2014
Innotest Amylod/Tau Index	86%	85%	Hulstaert 1999 Riemenschneider 2002
Schoonenboom formula	84%	91%	Schoonenboom 2012
Mattsson formula	90%	80%	Mattsson 2009

Physician's perspectives…..

- These clinical issues are also complicated by laboratory ones, and both raised criticism on the use of CSF biomarkers especially among physicians because:

i) use of CSF biomarkers in clinical practice is often limited because their assessment needs an invasive procedure (lumbar puncture). This is a procedure considered un-necessary since the available pharmacological treatment is independent from biomarker levels.

ii) CSF biomarkers are stable during the entire course of the disease therefore they cannot be used as markers of prognosis or staging of the disease.

iii) Use of PET biomarkers is not readily available and costs are very high.

iv) Laboratory issues reduced the specificity and sensitivity of these markers.

Such skepticism clearly indicates a gap between research progresses, efforts and clinical practice.

Martorana , 2017 CNSNDDT

The Clinicians point of view…..

- Dementia, or major neurocognitive disorder, represents a significant cognitive decline from a previous level of performance in one or more cognitive domains—such as complex attention, executive function, learning, memory, language or perceptual-motor, or social cognition—which interferes with independence in everyday activities.
- To reach a diagnosis of cognitive decline a congruous clinical history, the assessment of cognitive profile (not merely limited to MMSE or MoCA tests), brain imaging remain mandatory.
- Use of biomarkers to support a diagnosis remains strongly suggested in cases where symptoms may occur, especially in the early course of the disease, in other dementias, challenging the diagnosis.

Simonsen et al. Alzheimers Dement. 2017

	Amyloid β1-42	T-tau	Phospho tau $_{181}$		Ref.
Cutoff values	≤ 450 < 550 **< 700**	≥ 350 >375 **> 400**	≥ 62 > 52 **> 60**		Blennow 2001 Mattsson 2013 Mulder 2010 Duits 2014 **Lehmann 2013**
Prodromal AD (predementia AD) (MCI developing AD)	↓	↑	↑	Aβ42/40 ↓ Tau/Aβ42 ↑	Blennow 2003 Dorey 2015 Lewczuk 2015 Mattsson 2009 Brys 2009 Riemenschneider 2002
Preclinical AD (Asymptomatic AD)	↓	**N** ↑	**N** ↑	Tau/Aβ42 ↑ P-tau/Aβ42 ↑	Skoog 2003 Gustafson 2007 Stomrud 2007 Fagan 2007 ….

Aβ-related pathology

- CSF Aβ42 levels recognize uniquely a threshold value indicating either physiological or pathological levels, but never showed a gradient to be related with cognitive decline presentation (typical or atypical), clinical severity, progression rate of the disease or with pharmacological responder's profile.
- Therefore, Aβ is considered a marker of "amyloidosis", referring uniquely to the specific pathophysiological process involving cortical transmission at synaptic level (synaptopathy).
- Amyloidosis is a process that may star even 20 years before any cognitive clinical sign appears.
- Accumulation of amyloid is however not specific for AD, it is inevitable process of aging

Jack et al., 2014 Lancet Neurol

Tau-related pathology

- Currently, T-Tau and p-Tau are considered markers of neurodegeneration
- Tau levels increase are related with cognitive decline degree and with the intensity of neurodegeneration.
- This was observed in patients converting from mild cognitive impairment to AD and in patients presenting with malignant forms of AD.
- The latter condition was characterized by very high levels of T-Tau (> 700 ng/L) or P-Tau (> 90 ng/L) the CSF, and by higher risk of mortality.
- More recently high levels of tau (both t-Tau and p-Tau) were also associated to hippocampal atrophy and with forms of pathologic neural plasticity.

Amyloid peptide pathology

- Aβ or tau pathology alone, are conditions suggested to have an intrinsic potential risk for cognitive symptoms.
- Most of studies on isolated Aβ pathology however, were performed using either Aβ PET or CSF as biomarkers.
- When performed on the same subject, CSF analysis and FDG-PET, thus marker of degeneration, results revealed an unexpected clinico-radiological condition compatible with AD, revealing in that a clear discrepancy with levels of tau protein that remain within the physiological range.
- These cases are currently classified as "potential AD", with unpredictable evolution in terms of progression rate and of type of dementia as well (i.e. Lewy body dementia, that in some cases may presents with same CSF pattern)
- Such classification may reasonably represent a confounding factor for physician in terms of diagnostic and therapeutic approach and for patient's perspectives in terms of life expectancies.

Isolated Tau pathology

- Recent biomarker-based classification individuated a subgroup of individuals (about 20% of cases) with mild cognitive deficits without clear features of AD, with isolated increased levels of markers of neurodegeneration (tau and P-tau), with normal Aβ levels.
- Pathological pattern associated to these cases is the atrophy of the medial temporal-lobe (primary age tauopathy)
- Tau CSF changes when are not associated to low Aβ42 levels, constitute a biomarker pattern of "suspected non-amyloid patient or non-Alzheimer's disease pathophysiology" (SNAP)
- The high tau CSF levels were interpreted as the result of either aging process or white matter lesion burden.
- Alternatively these cases were interpreted an early stage of a condition that might degenerate in AD.
- The latter case could be however unlikely since tau changes temporarily occur later than Aβ, but a decade before clinical onset.

Limits of such recomendations…

- Despite the use of a single biomarker has been considered useful to establish individuals at risk, often it is not sufficient to reach a correct diagnosis, while the combination of them (Aβ42 and P-Tau for example) increases accuracy diagnosis and for the prediction of disease severity.
- In this view, several efforts are underway to combine multiple and new biomarkers to better track the temporally different evolution of each biomarker throughout the disease course.

- **NEW APPROACHES IN BIOMARKERS DISCOVERY**

  - Approch pathogenesis driven

  (amyloid, oxidation, inflammation, dyslipidemia)

  - Approach screening technology driven

  (proteomic, metabolomics)

Proteomics by Mass Spectrometry in CSF

Results from "Explorative" Proteomics

Protein/Peptide	Method	References
Neural Cell Adhesion Molecule 1 Alpha dystroglycan Neuronal Pentraxin Receptor	2-DE LC/MS	Yin GN, Brain Res 2009
Neuronal Cell Adhesion Molecule Chitinase3-like 1,Chromogranin A Carnosinasi 1	2-DE LC-MS	RJ Perrin, PloS One 2011
ApoA1, ApoE, Transthyretin	2-DE LC-MS	Castano E,, Neurol Res 2006 Daviddson P Neuroreport 2002 PurchadesM,Brain Res 2003 Korolainen MA Clin Chem 2007
Pattern of CSF protein in FAD with *PSEN1* and *APP* mutations vs relatives non carrier	LC/MS non labeled	Ringman JM, Arch Neurol 2012
Pattern of Age related changes	LC/MS	Zhang J, Neurobiol Aging 2005

Results from "Targeted" Proteomics

Protein	Method	References
Pattern of Aβ peptides in CSF	Immunoaffinity/MS	E. Portelius, Curr Pharm Des 2011 G Brinkmalm 2012
Pattern of Aβ isoforms and peptides during treatment with disease modifyng drugs	Immunoaffinity/MS	E Portelius, Alzhiemers Res Ther 2010
SNAP 25 in CSF	Immunoaffinity/MS	G Brinkmalm , Mol Neurodegener 2014
Neurogranin Peptides pattern	Immunoaffinity/MS	Kvartsberg H, Alzheimer Dementia 2014
Quantification of ApoE	MRM-MS	Han SH, Mol Cell Prot 2014
Quantitation of aβ42, aβ40, Retinol Binding Protein, Cystatin C	MRM-MS	YS Choi, J Chormatogr. B 2013
Neurogranin	Immunoaffiity/MS	Thorsell A, Brain Res 2010
Quantification of aβ42, aβ40, aβ38	MRM-MS	J Pannee, J Alz Dis 2013

Some Concerns.....

1. Number of partecipants to the studies and stratification of partecipants
2. Different panels of metabolites
3. Low through-put proteomics methods
4. Relative “abundancy” of candidate biomarkers in the analytical samples
5. Harmonization of the preanalytical and analytical steps (different analytical platforms)
6. Limited dynamic range
7. Quality of the study design (STARD)
8. Interpretation of the Big Data

Conclusions

- Use of traditional CSF biomarkers represents a unique opportunity to diagnose individuals with cognitive decline, especially those with early age of onset or those with rapidly progressive rate of decline.
- In this view, their routine use in clinics should be encouraged.
- Our experience and published literature showed that main feature of cognitive decline is heterogeneity.
- The use of biomarkers gives physicians a different perspective in terms of diagnosis, prognosis and treatment .
- They also provide useful insights on the underlying pathophysiological process.
- Eventually, even patients would change their life expectancies, a condition that can reduce the social burden of aging of population.