Keynote 2: Integration of Biomarkers and Quantitative Modeling – Cerebrospinal Fluid

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Kaj Blennow, Professor
University of Gothenburg

CONFIDENTIAL
Integration of Biomarkers and Quantitative Modeling – Cerebrospinal Fluid

Kaj Blennow, Professor

Academic Chair in Neurochemistry, University of Gothenburg
Use of CSF biomarkers for Alzheimer’s disease

• Diagnostics
  - Select true AD cases for inclusion in clinical trials
  - Make a correct diagnosis for initiation of treatment, especially DMTs in the future

• Theragnostics
  - Identify target engagement of a drug candidate
  - Identify downstream effects of drug candidates on AD pathophysiology (e.g. on synaptic and neuronal degeneration)

• Research: clinical and epidemiology
  - Study AD pathophysiology directly in patients and elderly
  - Identify genetic and environmental / life-style risk factors for specific AD pathophysiology (amyloid, tau, neurodegeneration)
The core fluid biomarkers for Alzheimer’s disease

- **Phospho tau**: Phosphorylation state of tau / development of tangles
  - Axonal degeneration

- **Neurofilament light (NFL)**: Neuronal degeneration

- **Total tau**: Neuronal degeneration

- **Aβ 42+40**: β-amyloid metabolism / development of senile plaques
The three Innotest ELISA methods for AD CSF biomarkers

**tau Protein in Cerebrospinal Fluid**
A Biochemical Marker for Axonal Degeneration in Alzheimer Disease?

K. Blennow, A. Wallin, H. Ågren, C. Spenger, J. Siegfried, and E. Vanmechelen

*Mol Chem Neuropathol 1995*

**Cerebrospinal Fluid β-Amyloid (1-42) in Alzheimer Disease**
Differences Between Early- and Late-Onset Alzheimer Disease and Stability During the Course of Disease

Niels Andreasen, MD; Camilla Hesse, Pia Davidsson, PhD; Lenart Mithoev, MD, PhD; Anders Wallin, MD, PhD; Bengt Winblad, MD, PhD; Hugo Vanderstichele, PhD; Eugeen Vanmechelen, PhD; Kaj Blennow, MD, PhD

*Arch Neurol 1999*

**Quantification of tau phosphorylated at threonine 181 in human cerebrospinal fluid: a sandwich ELISA with a synthetic phosphopeptide for standardization**


*Neuroscience Letters 2000* 30-35

**3D6**

3D6

1 β-amyloid 42

**21F12**

21F12

**AT270**

AT270

**HT7**

HT7

**BT2**

BT2

**AT120**

AT120

300% increase in AD

Intensity of neurodegeneration

50% decrease in AD

β-amyloid aggregation / deposition

200% increase in AD

tau phosphorylation / pathology?
The AD core CSF biomarkers reflect key pathogenic events and are highly clinically validated.

**CSF T-tau**
- Intensity of neurodegeneration
- 250% increase in AD

**CSF Aβ42**
- Brain amyloid deposition
- Reduction to 50% in AD

**CSF P-τau**
- Phosphorylation state of tau and tau pathology?
- 200% increase in AD

- 188 studies
- 20,600 AD patients and controls

- 168 studies
- 19,600 AD patients and controls

- 116 studies
- 14,300 AD patients and controls

CSF and blood biomarkers for the diagnosis of Alzheimer’s disease: a systematic review and meta-analysis

Bob Olson, Ronald Launer, Ulf Andresson, Anouk Oyefjell, Erik Perskog, Maria Björke, Mikko Helppo, Christoffer Rön, Caroline Olsson, Gabrielle Sroebel, Elizabeth Wu, Kelly Dakin, Max Petsch, Kaj Blennow, Henrik Zetterberg
The core AD CSF biomarkers – performance to identify prodromal AD

Large multi-center studies confirm high predictive value of the AD core biomarker profile for prodromal AD

The core AD CSF biomarkers show high diagnostic performance also in the MCI stage, when disease-modifying compounds have a chance to be effective

Prevalence and prognostic value of CSF markers of Alzheimer’s disease pathology in patients with subjective cognitive impairment or mild cognitive impairment in the DESCRIPA study: a prospective cohort study

Cerebrospinal Fluid Biomarker Signature in Alzheimer’s Disease Neuroimaging Initiative Subjects

Article

Association between CSF biomarkers and incipient Alzheimer’s disease in patients with mild cognitive impairment: a follow-up study

Sensitivity for MCI-AD 95%
Specificity for stable MCI and MCI-other 87%
Cerebrospinal fluid analysis detects cerebral amyloid-β accumulation earlier than positron emission tomography

437 non-demented subjects from ADNI
- Baseline CSF Aβ42 and amyloid PET
- Follow-up amyloid PET after 2 years

CSF Aβ42 is an earlier biomarker than amyloid PET

CSF +/ PET - subjects show future amyloid accumulation (similar to CSF and PET +) but not yet evidence of neurodegeneration

Table 2 Longitudinal comparisons of amyloid-β accumulation

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>160</td>
<td>26</td>
<td>167</td>
<td></td>
</tr>
<tr>
<td>Global amyloid-β PET (% SUVR change/year)</td>
<td>0.35% (0.14–0.56)</td>
<td>1.2% (0.49–1.8)</td>
<td>1.2% (0.90–1.4)</td>
<td></td>
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</table>

A-B = 0.018 B-C = 0.86
The CSF Aβ 42/40 ratio compensates for low/high "total" Aβ production? 
biomarker dependence on CSF dynamics? 
pre-analytical loss of Aβ42 (and Aβ40)?
What is needed to get the core AD CSF biomarkers into the clinic?

**Reference Measurement Procedure (RMP)**

- Mass spectrometry gold standard method

**Certified Reference Material (CRM)**

- Gold standard CSF pool with exact levels
- ✔ Certified Aβ42 CRMs available
- ✔ Re-calibration of assays successful

**Fully automated instruments**

- High precision methods without manual steps

- Roche – Cobas Elecsys
- Fujirebio – Lumipulse
- Euroimmune – RA Analyzer
The core AD CSF biomarkers – performance compared with amyloid PET

Study cohorts:
- BioFINDER (n= 277)
- ADNI (n= 646)

Cobas Elecsys assays:
- Aβ1-42, tTau and Ptau

Amyloid PET:
- Visual read (3 raters)

Concordance vs. visual amyloid PET:
- CSF pTau/Aβ42: OPA = 89.9 - 90.3 %
- CSF tTau/Aβ42: OPA = 89.2 – 89.9 %
- Inter-rater PET agreement: OPA = 90%

CSF biomarkers and amyloid PET can be used interchangeably
Tau protein is cleaved to fragments before being secreted to the CSF (and blood?)

- No full length tau
- Several shorter tau fragments with different MW
- Two identical tau fragments as with HT7
- Other HT7 tau fragments missing
- Weak Tau12 unique tau fragment

CSF tau is truncated at the end of the mid-domain

**RP-HPLC separation of CSF proteins** ➔ **SDS-PAGE + Western**

**Tau Kinetics in Neurons and the Human Central Nervous System**

Chihiro Sato,1,2 Nicolas R. Barthélemy,1,3 Kwasi G. Mawuenyega,1 Bruce W. Patterson,1 Brian A. Gordon,1 Jennifer Jocket-Balsarotti,1 Melissa Sullivan,1 Matthew J. Crisp,1 Tom Kasten,1 Kristopher M. Kimness,2 Nicholas M. Karaan,1 Kevin E. Yarasheski,1 Alaina Baker-Nigh,1 Tammy L.S. Benzinger,1 Timothy M. Miller,1,4 Celeste M. Kirch,1,4 and Randall J. Bateman1,4,5

**A HT7 (aa 159-163)**

- Tau MW: 1-6
- 1N/2N 2N

**B Tau12 (aa 9-18)**

- Tau MW: 1-6
- No full length tau
- Several shorter tau fragments with different MW

**C**

- CSF tau (ng/mL)
- N term
- Mid domain
- MTBR
- C term

**Figure B**

- Brain Tau (ng/mL tissue)
- 1N/2N 2N
- 4R

**Figure C**

- CSF tau (ng/mL)
- Tau13
- HJ8.5
- HJ8.7
- Tau1
- Tau5
- HJ9.3
- HJ9.1
- Tau7
Asparagine endopeptidase (AEP) cleaved Tau368 may be a biomarker for tau pathology

- AEP cleaves tau at position 368
- AEP cleavage impairs microtubule assembly function
- AEP cleavage induces tau aggregation, with truncated tau in NFTs

**Immunohistochemistry (tau368 antibody)**

| Control | AD |

**IP of CSF (ab KJ9A) followed by MS/MS**

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SLGNIHKPGGGQVEKSEKLDFKDRVQSKIGSLDNITHVPGGGGN
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324
368

**CSF contains semi-tryptic AEP-cleaved tau (ending at tau368)**
CSF Tau368 is a novel candidate biomarker for tau pathology

- Clinical cohort:
  37 IWG-2 pos AD; 45 controls

GTP1 tau PET cohort, n= 49
Classification by amyloid PET

- Simoa method for tau 368 KJ9A and tau368 Abs

→ CSF tau368/T-tau ratio may compensate for basic tau secretion to CSF – in analogy with the Aβ 42/40 ratio
→ C-terminal tau fragments are retained in tau aggregates, while N-mid domain fragments are secreted?
Neurofilament light (NFL) protein in different neurodegenerative disorders

Cytoskeleton proteins in CSF distinguish frontotemporal dementia from AD

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>NFL (pg/mL)</th>
<th>Tau (pg/mL)</th>
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<tbody>
<tr>
<td>FTD</td>
<td>1442 ± 1183†</td>
<td>354 ± 140</td>
</tr>
<tr>
<td>EAD</td>
<td>498 ± 236</td>
<td>751 ± 394‡</td>
</tr>
<tr>
<td>LAD</td>
<td>1006 ± 727§</td>
<td>699 ± 319‡</td>
</tr>
<tr>
<td>Controls</td>
<td>241 ± 166</td>
<td>375 ± 176</td>
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CSF neurofilament light differs in neurodegenerative diseases and predicts severity and survival

• Patients with dementia (n= 3,356)
Higher CSF NFL in AD dementia and progressive MCI
Intermediate levels in stable MCI

Within the MCI group – higher CSF NFL predicts faster rate of cognitive decline

ADNI cohort: 110 Controls
91 stable MCI
101 progressive MCI
95 AD dementia
The synaptic protein neurogranin:
- Abundant in cortex, hippocampus, amygdala
- Concentrated in dendritic spines
- Important for memory consolidation and LTP induction

Marked increase in CSF neurogranin in AD and prodromal AD (using several different assays and mass spec)
High CSF neurogranin predict future rate of cognitive decline

Cerebrospinal fluid neurogranin concentration in neurodegeneration: relation to clinical phenotypes and neuropathology

Erik Portellius\(^1\), Bob Olsson\(^1\), Kina Höglund\(^1\), Nicholas C. Cullen\(^1\), Hilin Kvartsberg\(^1\), Ulf Andreasson\(^1\), Henrik Zetterberg\(^1\), Åsa Sandellius\(^1\), Leslie M. Shaw\(^1\), Virginia M. Y. Lee\(^5\), David J. Irwin\(^8\), Murray Grossman\(^8\), Daniel Weintraub\(^7\), Alice Chen-Plotkin\(^7\), David A. Wolk\(^9\), Leo McCluskey\(^9\), Lauren Elman\(^9\), Jennifer McBride\(^9\), Jon B. Toledo\(^9\), John Q. Trojanowski\(^9\), Kaj Blennow\(^1\)

+ AD, PD, PDD, PSP, CBD, ALS, FTD, PCA

>900 cases in total
CSF biomarkers to identify target engagement in man

**SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE**

**ALZHEIMER’S DISEASE**

The BACE1 inhibitor verubecestat (MK-8931) reduces CNS β-amyloid in animal models and in Alzheimer’s disease patients

Matthew E. Kennedy,1* Andrew W. Stamford,2* Xia Chen,1 Kathleen Cox,3 Jared N. Cumming,2 Marissa F. Dockendorf,2 Michael Egan,6 Larry Ereshefsky,2 Robert A. Hodgson,1 Lynn A. Hyde,1 Stanford Jhee,1 Huub J. Kleijn,6 Reshma Kovelkar,1 Wei Li,1 Britta A. Mattson,6 Hong Mei,3 John Palcza,1 Jack D. Scott,7 Michael Tanen,2 Matthew D. Troyer,7 Jack L. Tseng,7 Julie A. Stone,3 Eric M. Parker,1* Mark S. Forman2*

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**Figure A**

- CSF Aβ40 % of baseline over time for different dose groups.
- Placebo (n = 8 to 10), 150 mg (n = 6 to 9), 10 mg (n = 3 to 5), 250 mg (n = 7 to 9), 40 mg (n = 4 to 6).

**Figure B**

- CSF Aβ42 % of baseline over time for different dose groups.

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**CSF biomarkers may be used to identify target engagement (and for dose finding)**
Target engagement on β-amyloid may not translate to disease-modifying effect / clinical benefit

- Downstream biomarker effects on neurodegeneration may be important indicators of disease-modification

Verubecestat BACE inhibitor trial
Phase I showed 80% reduction in Aβ production

78 weeks: placebo, 12 and 40 mg
Terminated early for futility
Can CSF NFL be used to monitor drug effects on neurodegeneration?

- Spinal muscular atrophy (SMA) 12 children
- Intrathecal nusinersen (Spinraza)
  - Antisense oligonucleotide increasing CNS levels of survival motor neuron (SMN) protein
- CSF samples before each treatment

Reduction in serum NFL with effective DMTs – correlates with clinical benefit
Serum NFL may be useful to monitor downstream drug effects on intensity of neurodegeneration
Thanks for listening!