Challenges in Development of Biomarkers in Neurodegeneration

“An Industry Perspective”

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• Dr. Iswariya Venkataraman is a full-time employee of EUROIMMUN USA
EUROIMMUN – Corporate Introduction

- Founded in Lubeck, Germany in 1987
- 14 Subsidiaries Worldwide
- 3000+ Employees Worldwide
- 200+ Employees with Doctoral Degrees
- 400M+ EUROIMMUN Revenue in 2019
- 3500+ Products in Comprehensive Catalog
- 350+ Patents, Applications & Licenses
- In 2017 we were acquired by PerkinElmer for 1.3BIL
Agenda

Biomarkers in Neurodegeneration

EUROIMMUN Biofluid Biomarkers

Challenges in Development of Biofluid Biomarkers

In vitro Diagnostic Industry Goals
Biomarkers in Alzheimer’s Disease

1990s
Amyloid cascade hypothesis

1990s–2000s
Reduced Aβ$_{42}$ levels in Alzheimer’s Disease

2010
Biomarkers before clinical symptoms
Guidelines for in Alzheimer’s Disease

1984: NINCDS-ADRDA Criteria
Clinical-pathological definition

2011: NIA-AA Criteria
Clinical Syndrome with biomarkers for amyloid and neurodegeneration

2018: NIA-AA Research Framework
Alzheimer’s Disease as biological entity defined by positive biomarkers for amyloid and tau
“The term “Alzheimer’s disease” refers to an aggregate of neuropathologic changes & thus is defined in vivo by **biomarkers** and by **postmortem**
NIA-AA Research Framework

| A|T|(N) profiles | Biomarker category |
|---|---|---|
| A-T-(N)- | Normal AD biomarkers |
| A+T-(N)- | Alzheimer’s pathologic change |
| A+T+(N)- | Alzheimer’s disease |
| A+T+(N)+ | Alzheimer’s disease |
| A+T-(N)+ | Alzheimer’s and concomitant suspected non Alzheimer’s pathologic change |
| A-T+(N)- | Non-AD pathologic change |
| A-T-(N)+ | Non-AD pathologic change |
| A-T+(N)+ | Non-AD pathologic change |

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**ATN**

- **Amyloid Pathology**
  - Amyloid plaques
    - Amyloid PET
    - CSF Aβ42 or Aβ42/Aβ40

- **Tauopathy**
  - Tangles
    - Tau PET
    - CSF pTau(181)

- **Neurodegeneration**
  - Unspecific to AD
    - Anatomic MRI
    - FDG PET
    - CSF total Tau

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Jack et al Alzheimer’s & Dementia (2018)
Biofluid Biomarkers from EUROIMMUN
## EUROMMUN Neurodegeneration ELISA Portfolio

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>CSF</th>
<th>Plasma</th>
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</thead>
<tbody>
<tr>
<td><strong>Alzheimer's Disease</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aβ (40, 42)</td>
<td>✔</td>
<td>✔</td>
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<tr>
<td>Tau (Total &amp; Phospho)</td>
<td>✔</td>
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<td><strong>Parkinson's Brain</strong></td>
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<tr>
<td>Alpha-Synuclein</td>
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<tr>
<td><strong>ALS</strong></td>
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<tr>
<td>Neurofilaments</td>
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<td>✔</td>
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<tr>
<td>pNFH</td>
<td>✔</td>
<td>✔</td>
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<tr>
<td>NFL*</td>
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<tr>
<td><strong>Synaptic Markers</strong></td>
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<tr>
<td>Neurogranin</td>
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<td>✔</td>
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<tr>
<td>BACE 1</td>
<td>✔</td>
<td>✔</td>
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</tbody>
</table>

*: under development

### Biomarkers for neurodegeneration

#### Biomarkers for synaptic integrity
- Pre/post-synaptic Neurogranin (trunc P75)
- BACE1

#### Biomarkers for brain pathology
- Tau pathology
  - P-tau (181)
- Lewy body pathology
  - Alpha-Synuclein
- Amyloid pathology
  - Aβ (1-42)
  - Aβ (1-40)
  - Aβ (1-38)
- Neuronal degeneration
  - Total-tau
  - P-neurofilament H

#### Risk factors for neurodegenerative diseases
- Apolipoprotein E
  - Phenotype: ApoE 4, pan-ApoE
  - Genotype: ApoE 2, 3, 4
EUROIMMUN *Chemiluminescence* Platform

Random Access Analyzer 10

- Time to First Result: **25 minutes**
- 60 samples & 10 parameters
- Throughput: 85 tests/hour

- CSF Aβ40 and Aβ42
- CSF Tau and P-Tau
- Blood Aβ40 and Aβ42
- Neurofilaments
EUROIMMUN: CSF Aβ 42/40 ratio Concordance to PET

83% Concordance

93% Concordance

Aβ42/40 ratio shows good concordance (93%) with PET results

Janelidze et al. 2015
Plasma Aβ 42/40 ratio significantly differentiates AD from control groups

Unpublished data
AUC plasma Aβ 42/40 ratio measured with ELISA = 86%
EUROIMMUN: Neurogranin/BACE1 Ratio Correlation to MMSE

- Neuogranin or BACE1 alone are not a useful tool to identify AD or MCI
- Neurogranin/BACE1 ratio demonstrated significant correlation with rate of cognitive decline
- None of the single analytes or other combinations demonstrated the same prognostic value

A higher ratio indicated a more rapid decline in MMSE scores

De Vos A et al 2016
Serum pNfH concentrations were elevated up to 18 months prior to ALS diagnosis vs healthy controls.

- 62% of samples had a concentration beyond the cutoff of 81.9 pg/mL.
- Prognostic marker: concentration >226 pg/mL corresponded to a 2x increased risk of death.

**Serum pNf-H: a candidate diagnostic and prognostic biomarker for ALS**

*De Schaepdryver et al., 2019*
Challenges Associated in Biomarker Assay Development
Assay Development in a Snapshot

**Research & Development**

**Assay Design**
- Biomarker identification / IP & licensing
- Assay platform

**(Analytical) Validation**
- Precision
- Reproducibility
- Calibration using reference materials

**Design Transfer**
- Transfer to production department
- Establishment of RUO test

**Assay Adaptation**
- Optimization of design variables (antibody concentrations, blocking)
- Standardization of production (manufacturing instructions, quality control)

**(Clinical) Qualification**
- Clinical performance
- Method comparison (reference test)

**Regulatory Process**
- Appropriate IVD approval

**Commercial Production**

**Market Launch**

RUO
Challenges Associated in Biomarker Measurement

- Increasing biomarkers identified
- Establishment of a **Global Cut-off Value**
- **Non-commutable** assays
- **Invasive** sample collection methods
- **SOPs** for sample collection

Interpreting radiotracer PET evidence of amyloid plaques or CSF levels of Aβ42 as binary constructs for disease state requires the establishment of cut points, which vary by the method of quantification [19]. Typically, the goal of...
Pathological mechanisms & Associated Biofluid Biomarkers in Neurodegeneration

Molinuevo, J.L. et al 2018
Large Variabilities in Measurement of Aβ42

Day 1: 500 pg/mL
Day 2: 510 pg/mL
Day 3: 490 pg/mL

Method 1

Method 2: 600 pg/mL

Method 3: 400 pg/mL

Large Variabilities
Between-Methods
Between-Laboratories
Assay-Dependent
Reduction between vendor bias & Re-standardized CRM measurements close to target values (CV≥7%)
SOP for CSF-handling (Fresh samples)

ALZHEIMER’S ASSOCIATION CSF PRE-ANALYTICAL CONSENSUS PROTOCOL

Proposed Routine-use Pre-Analytical Protocol

**Collection Site**

- Sample Collection
- Collection
- Storage*
- Sample Handling

**Testing Site**

- Measurement

**Fresh samples (Routine)**

- Discard first 2 mL, then directly collect 1.5-2 mL by drip method directly into a Low Bind (i.e. Sarstedt) tube.
- No centrifugation, freezing, mixing/inverting or tube transfers
- Storage up to 15 days at 2-8 °C
- No mixing; immediate measurement
- Measure: Aβ_{1-42}, Aβ_{1-40}, tTau, pTau

*The Storage step assumes the transport of samples however the group does not yet have data on sample stability during transport.

SOP for frozen samples- Ongoing

https://www.alz.org/research/for_researchers/partnerships/biomarker_consortium/gbsc_working_groups
Blood-Based Biomarkers

Clinical usefulness in AT(N) pathophysiology

- Screening of patients for 2nd grade diagnostic evaluation (CSF, PET, MRI)
- To monitor drug effects on amyloid/tau pathology in clinical trials

Diagnostic performance requirements

- Distinguishable concentrations to identify cognitively unimpaired elderly
- Not required to be disease specific: Positive predictive value (PPV) >40-50%
  High negative predictive value >90-95%

Challenges to develop blood-based biomarkers

- Very low levels of brain-derived proteins in plasma
- High amount of plasma proteins (50 g/L) > risk for matrix effects

Adapted from Kaj Blennow presentation: PPSB LA 2018
Summary

- AT(N) classification system: definition of AD based on biomarkers
- Unified and coordinated approach for identification of candidate biomarkers
- Planning of study design, subjects, methodology, and producing outcomes representative of wider population
- **Concerted and Collaborative effort**: academia, diagnostic, and pharma industries
- Development as a companion biomarker useful to practitioners
- Paradigm shift from “one treatment fits all” > biomarker-guided “tailored therapies“

**IVD Industry Goals**

- Validation of sensitive, specific and reproducible biomarkers in neurodegeneration
- Focus on reliable, and inexpensive blood based biomarkers for screening large populations
Thank you for your attention!