Innovative measurements for improved diagnosis and management of neurodegenerative diseases

The EMPIR NeuroMET project

M. Quaglia, LGC, Queens Road, Teddington, UK
Aim

To develop **metrological tools** to underpin the development and validation of **minimally/not invasive tools** for **early diagnosis** of Alzheimer's and Parkinson's diseases.

July 2016-June 2019

July 2019-June 2022
Why better measurements?

“The current medications for Alzheimer’s disease are approved, essentially, because it's better than nothing. There's nothing else at the moment. These drugs were pioneered in the '70s and '80s and they treat the symptoms, as opposed to the underlying biology.” (Joseph Jebelli, Jan 2018)

The current approaches for diagnosis and recruitment for clinical trials are not satisfactory:

- Clinical diagnostic criteria have a low diagnostic performance

- Neuropathogenic changes occur at least 20 years before symptoms onset

- Clinical symptoms and neuropathologies frequently overlap among the NDDs and with non-NDDs, thus leading to misdiagnosis
AD and NDD diagnosis

Cognitive assessments

MRI and MRS

Clinical biomarkers (Aβ42, Aβ40, tau, NFL, α-synuclein)_immunoassay_blood

T-tau, alpha syn, NFL in CSF
NeuroMET consortium (2016-2019)

**National Measurement Institutes**
- UK coordinator: LGC
- France: LNE
- Sweden: RISE
- Germany: PTB
- Italy: INRiM

**Reference methods**
- Immunoassay

**Cognitive analysis**
- MRI/MRS
- ddPCR

**Clinical partners**
- Charité
- CHRU Montpellier
- Royal Free London NHS
- UEA
- Modus Outcomes
NeuroMET consortium (2019-2022)

Patient cohort/cognitive assessment

MRI/MRS

α-synuclein; neurofilament light chain RMP; t-tau

Construct specification equations
Inclusion/Exclusion

- Aged 55-90
- Ability to consent
- Suitable for MRI
- AChE inhibitors / Memantine / Antidepressive therapy only if stable > 3 months
- No Stroke/ Parkinson’s/ Severe depression/ other neurologic disorders

NeuroMET cohort

HC

N = 39

MCI

N = 22

AD

N = 26

HC

N = 21

SCD

N = 13

MCI

N = 14

AD

N = 17

NeuroMET

N = 39

NeuroMET2

N = 21

NeuroMET N = 39

MCI N = 22

AD N = 26

HC N = 21

SCD N = 13

MCI N = 14

AD N = 17

HC N = 21

SCD N = 13

MCI N = 14

AD N = 17

NeuroMET

CHARITÉ

UNIVERSITÄTSMEDEZIN BERLIN
Medical Data

<table>
<thead>
<tr>
<th>Medical Data</th>
<th>Baseline</th>
<th>Follow Up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medical history (incl. comorbidities, risk factors, medication list)</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Physical examination (incl. height, weight, blood pressure, heart rate)</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Blood sampling</td>
<td>x</td>
<td>-</td>
</tr>
<tr>
<td>Saliva extraction</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Liquor extraction</td>
<td>(x)</td>
<td>(x)</td>
</tr>
<tr>
<td>7T MRT/MRS</td>
<td>x</td>
<td>-</td>
</tr>
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</table>
## Questionnaires

<table>
<thead>
<tr>
<th>Questionnaire</th>
<th>Baseline</th>
<th>Follow Up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical Dementia Rating (CDR)</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Geriatric Depression Scale (GDS)</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Instrumental Activities of Daily Living Scale (IADL)</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Positive and Negative Affect Schedule (PANAS)</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Questionnaire on physical activity, nutrition, alcohol and nicotine consumption (FKA)</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Oldfield hand preference questionnaire (Edinburgh Inventory)</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Questionnaire for self-description (Stai-G Form X 1)</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>General health questionnaire SF12</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Day Sleepiness Questionnaire (Epworth Sleepiness Scale)</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Sleep Quality Questionnaire (PSQI)</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>World Health Organization Quality of Life (WHOQoL-BREF)</td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>
## Neuropsychological Tests

<table>
<thead>
<tr>
<th>Test</th>
<th>Baseline</th>
<th>Follow Up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consortium to Establish a Registry for Alzheimer’s Disease (CERAD)</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Wechsler Memory Scale (WMS) Logical Memory</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Multiple Vocabulary Test (MWT)</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Digit Span Test</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Block Tapping Test</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Stroop-Test (Farbe-Wort-Interferenztest)</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Word Fluency</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>TAP</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Auditory Verbal Learning Test (AVLT, German VLMT)</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Digit-Symbol</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Age and concentration test (AKT)</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Oral Trail Making Test (oTMT-B)</td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>
MRI and MRS

High Resolution Anatomical Imaging

- 7T (Siemens)
- MP2RAGE
- 0.75 mm iso
- TE = 2.51 ms
- TR = 5000 ms
- TI1 = 900 ms
- TI2 = 2700 ms
- FA1 = 7°
- FA2 = 5°
- Accel.: 2x

Segmentation using CAT12

C.Gaser et al., HBM 2016: 33-348 (2016)
MRI and MRS

High Resolution Anatomical Imaging

**AUROC cortical thickness**

**AUROC rHC volume**

Learning Ability: Rasch transformed results from AVLT

Correlations adjusted for: age, sex, education, gray matter fraction in MRS voxel

rHC: right hippocampus

<table>
<thead>
<tr>
<th>Group</th>
<th>Sample Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC</td>
<td>n = 36</td>
</tr>
<tr>
<td>MCI</td>
<td>n = 22</td>
</tr>
<tr>
<td>AD</td>
<td>n = 23</td>
</tr>
</tbody>
</table>
### Plasma samples

<table>
<thead>
<tr>
<th>Subject ID</th>
<th>NFL plasma pg/mL</th>
<th>Tau plasma pg/mL</th>
<th>Ab42 plasma pg/mL</th>
<th>Ab40 plasma pg/mL</th>
<th>Alpha-synuclein plasma MSD pg/mL</th>
<th>Alpha-synuclein plasma Euroimmun pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>NeuroMet 01</td>
<td>86.4</td>
<td>4.29</td>
<td>10.0</td>
<td>319</td>
<td>5015</td>
<td></td>
</tr>
<tr>
<td>NeuroMet 02</td>
<td>20.6</td>
<td>1.84</td>
<td>10.0</td>
<td>217</td>
<td>2640</td>
<td></td>
</tr>
</tbody>
</table>

### CSF samples (1 mL aliquot)

<table>
<thead>
<tr>
<th>Subject ID</th>
<th>NFL CSF pg/mL</th>
<th>Tau CSF pg/mL</th>
<th>Ab42 CSF pg/mL</th>
<th>Ab40 CSF pg/mL</th>
<th>Alpha-synuclein CSF MSD pg/mL</th>
<th>Alpha-synuclein CSF Euroimmun pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>NeuroMet 01</td>
<td>2711</td>
<td>272</td>
<td>521</td>
<td>17006</td>
<td>839</td>
<td></td>
</tr>
<tr>
<td>NeuroMet 02</td>
<td>1042</td>
<td>121</td>
<td>460</td>
<td>12552</td>
<td>761</td>
<td></td>
</tr>
<tr>
<td>NeuroMet 03</td>
<td>1925</td>
<td>126</td>
<td>690</td>
<td>14637</td>
<td>769</td>
<td></td>
</tr>
<tr>
<td>NeuroMet 23</td>
<td>1978</td>
<td>215</td>
<td>351</td>
<td>10019</td>
<td>1287</td>
<td></td>
</tr>
</tbody>
</table>

All CSF, plasma and saliva samples were analysed by using commercially available immunoassays for the recognised biomarkers and data were process vs person ability.

NFL most promising biomarker in plasma.

Cortisol in plasma showed promising results as biomarker by using a standard addition approach.
Candidate reference methods development
t-tau primary calibrator: evaluation of protein vs peptide as calibrators

Recombinant protein:
Amino Acid analysis (Ala, Ile, Leu, Phe, Val)

[rTau] (µg/g) = 263±16 (6.09%), k=2

Peptide:
Signature peptide (Ala, Pro)

[rGAAPPGQK] (µg/g) = 755±48.3 (6.4%), k=2

Higher imprecision for the Peptide-Peptide* and Protein-Peptide* approaches: peptides are spiked in the sample after precipitation and before trypsin digestion.

Protein-Protein* approach has been selected and optimised

Protein-Protein* approach

CSF (pool):
- Protein precipitation
- SPE HLB plate
- Digestion
t-tau: LC-MS method

CSF Medium:
- Aliquot 5.1.2: 2289 pg/mL
- Aliquot 5.2.1: 2153 pg/mL
- Aliquot 5.2.2: 2175 pg/mL

CSF Low:
- Aliquot 5.1.2: 1041 pg/mL
- Aliquot 5.2.1: 868 pg/mL
- Aliquot 5.2.2: 867 pg/mL

CSF High:
- Aliquot 5.1.2: 3780 pg/mL
- Aliquot 5.2.1: 3687 pg/mL
- Aliquot 5.2.2: 3736 pg/mL

CV 10.8%

CV 3.3%

CV 1.2%

Comparison with immunoassay data
IFCC-CSF WG round robin

POSTER P-09
Helene Vaneeckhoutte

Preparation of calibration blends

Quantification of tau in CSF

Determination of ratio to Std Preparation of calibration blens

POSTER P-09
Helene Vaneeckhoutte
Tau...on-going

EVALUATING COMPARABILITY OF DIFFERENT CANDIDATE REFERENCE METHODS FOR T-TAU

Round-Robin study in conjunction with the IFCC CSF-WG
- LNE
- CEA
- University of Goteborg
- University of Pennsylvania

Correlation between the MS method and the major immunoassays

Virtual recalibration of immunoassays for quantification of tau

Initiation of an external quality assurance (EQA) scheme with commutable CSF samples to assess the accuracy and reproducibility of common methods.

DEVELOPMENT OF A LC-MS METHOD TO DETECT AND QUANTIFY PHOSPHORYLATION

- Selection of the phosphorylated residues (p-tau 181)
- Source and characterization of primary calibrators (p-peptides) (purity of the material)
- Development of an experimental workflow to detect and localize phosphorylated sites (protein IP, TiO2 enrichment?)
- Development of a LC-MS method to detect and quantify phosphorylation (target uncertainty <15%) in CSF
**α-synuclein**

α-synuclein is the major constituent in Lewis body in Parkinsonism and Parkinson’s disease.

Target of bio-products, but no reliable methods are available to measure drug-efficacy.

No clinical thresholds based on immunoassays have been established due to poor measurement performance.

MS methods also suffered poor measurement comparability (M.J. Fox study data not published)

RT-QUIC measurements show promising results, but implementation difficult due to calibrators.
α-synuclein primary calibrator

<table>
<thead>
<tr>
<th>Peptide standards</th>
<th>Certificate</th>
<th>Crude AAA</th>
<th>AAA corrected</th>
<th>qNMR</th>
<th>qNMR corrected</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDVFMK</td>
<td>≥95%</td>
<td>71.1</td>
<td><strong>68.5</strong></td>
<td>68.0</td>
<td>68.0</td>
</tr>
<tr>
<td>QGVAEAAGK</td>
<td>≥95%</td>
<td>63.4</td>
<td><strong>49.9</strong></td>
<td>60.9</td>
<td>50.2</td>
</tr>
<tr>
<td>EGVLYVGSK</td>
<td>≥95%</td>
<td>68.7</td>
<td><strong>65.5</strong></td>
<td>66.8</td>
<td>66.6</td>
</tr>
<tr>
<td>TVEGAGSIAATGFVK</td>
<td>≥95%</td>
<td>26.1</td>
<td><strong>21.4</strong></td>
<td>25.2</td>
<td>21.8</td>
</tr>
</tbody>
</table>

Recombinant primary calibrator from UCL:
- Purified
- Protocol for dilution and storage developed
- Quantified traceable to the System of International Units

Structural analysis characterisation

MS clinical routine method (Shimazu)

RT-QuiC
**α-synuclein**

**LC-MS method traceable to SI**

- **Calibration Curve Quantification**
  - \( R^2 = 0.9994 \)

- **captLC-MSMS Analysis**
  - (Xevo TQ-XS)

- **Measured α-synuclein concentration (fmol)**
  - U=12% (k=2)
  - LOD 0.5ng/g

- **Analysing patient samples** (AD_Charite’ cohort and PD_Montpellier data base)

- **Transfer of the method to CHUMpt to facilitate MS clinical assay CRM?**
AD and NDD diagnosis

Cognitive assessments

Magnetic resonance imaging

Clinical biomarkers (Aβ 1-42, 1-40, tau, NFL, α-synuclein)
Construct Specification Equation

Memorytest

Difficulty, $\delta$

Cognition, $\theta$

Biomarkers

Principal component analysis

$X' = T = X \cdot P$

$\text{Cov}(X) \cdot p_n = \lambda_n \cdot p_n$

Regression

$\hat{C} = (T^T \cdot T)^{-1} \cdot T^T \cdot Y$

$Y = T \cdot C + \epsilon$,
Complete, 'right' explanatory variables, \(X, Z\)?

Construct specification equations, \((\beta, u(\beta))\)

\[
\bar{Y} = \sum_{k} \beta_k \cdot X_k
\]

\[
SDC(X) = \frac{2 \cdot \sqrt{2} \cdot u(\theta)}{\beta_X}
\]

Cognitive person ability, \((\theta, u(\theta))\)

\[
\theta = g(z_1, \ldots, z_n)
\]

Cognitive test, \([P_{success}, u(P_{success})]\)

\[
\delta = f(x_1, \ldots, x_m)
\]

Cognitive task difficulty, \((\delta, u(\delta))\)

\[
u[SDC(X)] = \frac{-2 \cdot \sqrt{2} \cdot u(\theta) \cdot u(\beta_X)}{(\beta_X)^2}
\]
Summary

We developed a metrological/clinical infrastructure with routes to industry for translational research.

We have applied metrological concepts throughout the workflow for AD diagnosis.

New cognitive assessments and a prototype Memory score were developed through NeuroMET data and Rasch analysis.

Promising biomarkers and methods for early AD diagnosis were identified and need to be validated through longitudinal studies.
MIRIADE Marie Curie program (2019-2022)

Accelerating NDD biomarker development
Acknowledgments

Stakeholders

- International Federation of Clinical Chemistry (IFCC)
- Joint Committee for Traceability in Laboratory Medicine (JCTLM)
- European Commission Joint Research Centre, Geel
- Alzheimer's Research UK, Manchester
- Parkinson's UK
- UCB Celltech
- ISMRM-MR Spectroscopy Study Group
- Centre for Lifespan Psychology, Max Planck Institute for Human Development, Berlin
- Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE), Bonn
- National Institute for Standards and Technology (USA)

- Center for Healthy Brain Aging and Dementia Prevention, Munich
- Imperial College University, London
- Charité Universitätsmedizin Berlin, Department of Neurology with Experimental Neurology
- Royal Hospital, London
- Leiden University
- University Medical Centre Utrecht
- VUMC, Amsterdam
- Kristianstad University
- Institut de Biologie et de Technologies de Saclay (IBITECS - CEA)

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