The EMPIR NeuroMET project

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

Innovative measurements for improved diagnosis and management of neurodegenerative diseases

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

NeuroMET

July 2016-June 2019

NeuroMET 2

July 2019-June 2022

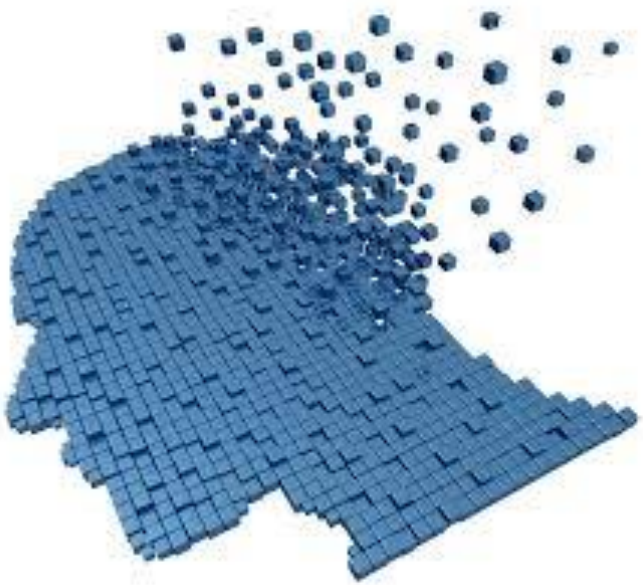
Why better measurements?

NeuroMET

"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 :

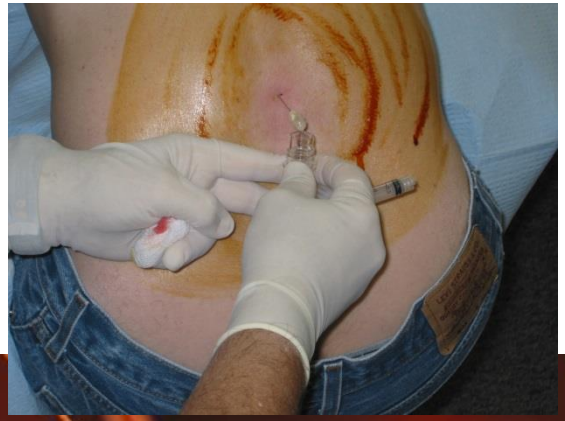
- clinical diagnostic criteria have a low diagnostic performance
- neuropathogenic changes occur at least 20 years before symptoms onset
- clinical symptoms and neuropathologies frequently overlap among the NDDs and with non-NDDs, thus leading to misdiagnosis



AD and NDD diagnosis

Cognitive assessments

MRI and MRS



Clinical biomarkers (Aβ
α-synuclein)_immunoassay_bloo

T-tau, alpha syn, NFL in CSF

Patient diagnosis

NeuroMET consortium (2016-2019)

National Measurement Institutes

UK coordinator	France	Sweden	Germany	Italy
Reference methods Immunoassay	Reference methods	Cognitive analysis	MRI/MRS	ddPCR

Clinical partners

CHARITÉ UNIVERSITÄTSMEDIZIN BERLIN

CHRU MONTPELLIER CENTRE HOSPITALIER REGIONAL UNIVERSITAIRE

Royal Free London NHS Foundation Trust

UEA University of East Anglia

MODUS OUTCOMES

NeuroMET consortium (2019-2022)

NeuroMET

PTB Physikalisch-Technische Bundesanstalt Braunschweig und Berlin

LGC

LABORATOIRE NATIONAL DE MÉTROLOGIE ET D'ESSAIS LNE

RISE

LGC

Patient cohort/ cognitive assessment

MRI/MRS

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

Construct specification equations

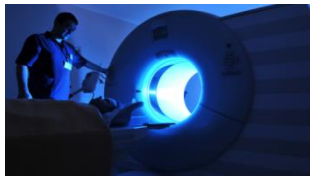
CHARITÉ UNIVERSITÄTSMEDIZIN BERLIN

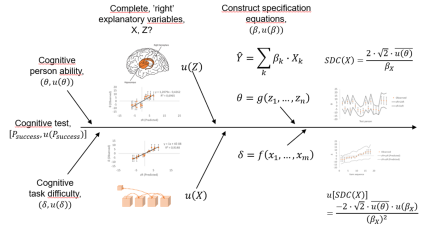
UNIVERSITÄT GREIFSWALD Wissen lockt. Seit 1456

MONTPELLIER CHU CENTRE HOSPITALIER UNIVERSITAIRE

Amsterdam UMC University Medical Centers

MODUS OUTCOMES





NeuroMET cohort

In-/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

HC	MCI	AD
N = 39	N = 22	N = 26

NeuroMET2

HC	SCD	MCI	AD
N = 21	N = 13	N = 14	N = 17

CHARITÉ
UNIVERSITÄTSMEDIZIN BERLIN

Medical Data

	Baseline	Follow Up
Medical history (incl. comorbidities, risk factors, medication list)	x	x
Physical examination (incl. height, weight, blood pressure, heart rate)	x	x
Blood sampling	x	-
Saliva extraction	x	x
Liquor extraction	(x)	(x)
7T MRT/MRS	x	-

Questionnaires

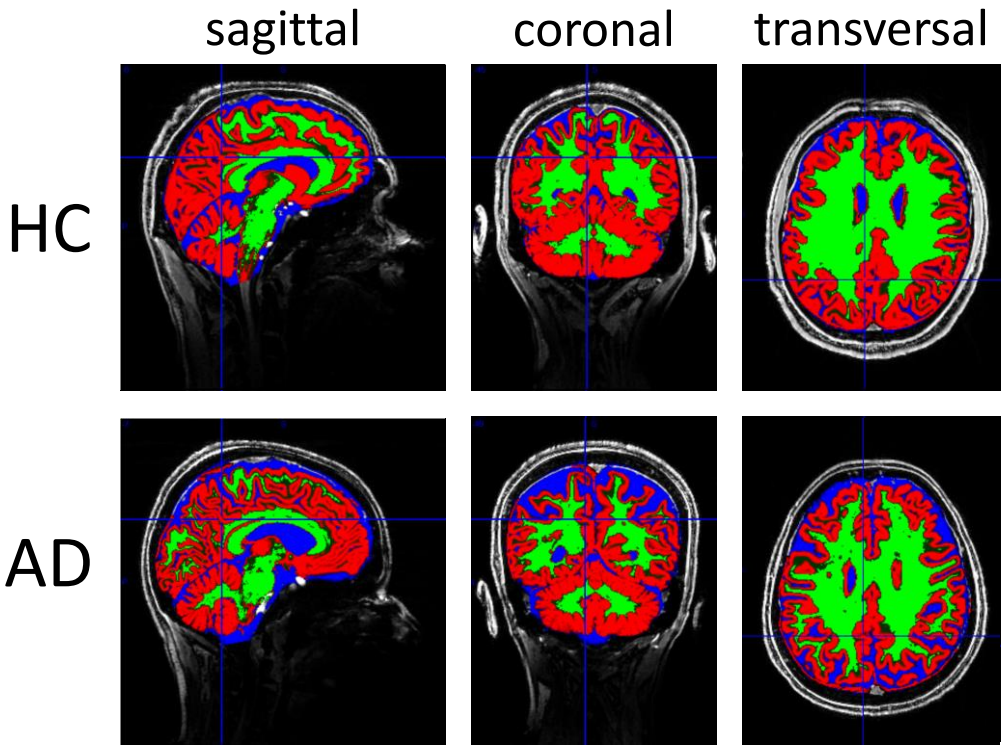
	Baseline	Follow Up
Clinical Dementia Rating (CDR)	x	x
Geriatric Depression Scale (GDS)	x	x
Instrumental Activities of Daily Living Scale (IADL)	x	x
Positive and Negative Affect Schedule (PANAS)	x	x
Questionnaire on physical activity, nutrition, alcohol and nicotine consumption (FKA)	x	x
Oldfield hand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

Neuropsychological Tests

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

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

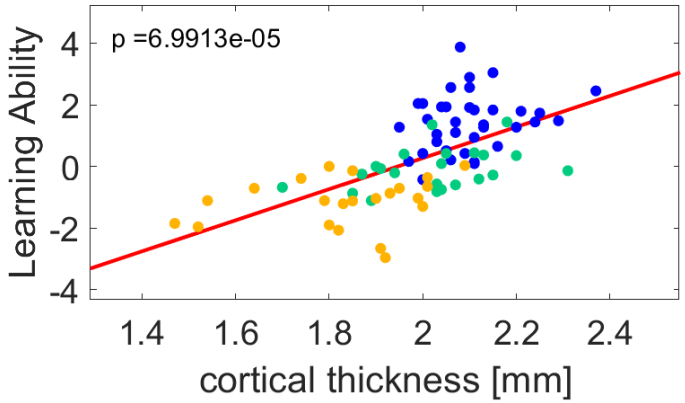
→ Segmentation using CAT12

J.P.Marquess et al., NeuroImage 49: 1271-1281 (2010)
C.Gaser et al., HBM 2016: 33-348 (2016)

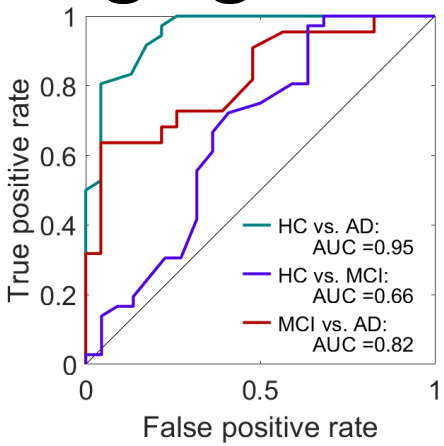
Physikalisch-Technische Bundesanstalt
Braunschweig und Berlin

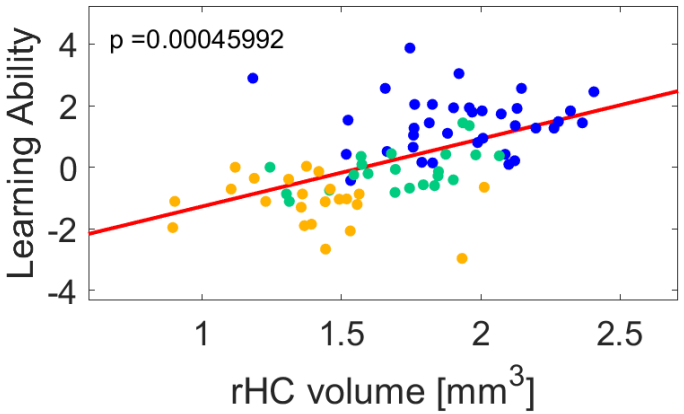
MRI and MRS

High Resolution Anatomical Imaging

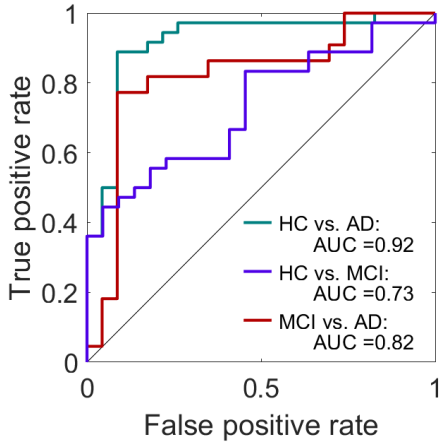


AUROC cortical thickness





AUROC rHC volume



- HC (n = 36)
- MCI (n = 22)
- AD (n = 23)

rHC: right hippocampus

Learning Ability: Rasch transformed results from AVLT

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

Physikalisch-Technische Bundesanstalt
Braunschweig und Berlin

Immunoassays biomarkers

Plasma samples	SIMOA	SIMOA	SIMOA	SIMOA	MSD	EUROIMMUN
Subject ID	NFL plasma pg/mL	Tau plasma pg/mL	Ab42 plasma pg/mL	Ab40 plasma pg/mL	Alpha-synuclein plasma MSD pg/mL	Alpha-synuclein plasma Euroimmmun pg/mL
NeuroMet 01	86.4	4.29	10.0	319	5015	
NeuroMet 02	20.6	1.84	10.0	217	2640	

CSF samples (1 mL aiquot)	SIMOA	SIMOA	SIMOA	SIMOA	MSD	EUROIMMUN
Subject ID	NFL CSF pg/mL	Tau CSF pg/mL	Ab42 CSF pg/mL	Ab40 CSF pg/mL	Alpha-synuclein CSF MSD pg/mL	Alpha-synuclein CSF Euroimmmun pg/mL
NeuroMet 01	2711	272	521	17006	839	
NeuroMet 02	1042	121	460	12552	761	
NeuroMet 03	1925	126	690	14637	769	
NeuroMet 23	1978	215	351	10019	1287	

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 bio-marker in plasma

Cortisol in plasma showed promising results as biomarker by using a standard addition approach

Candidate reference methods development

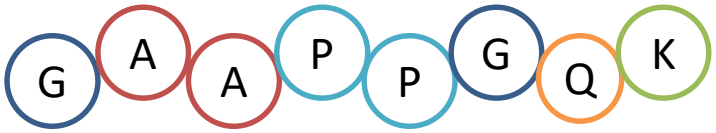
t-tau primary calibrator:

evaluation of protein vs peptide as calibrators

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

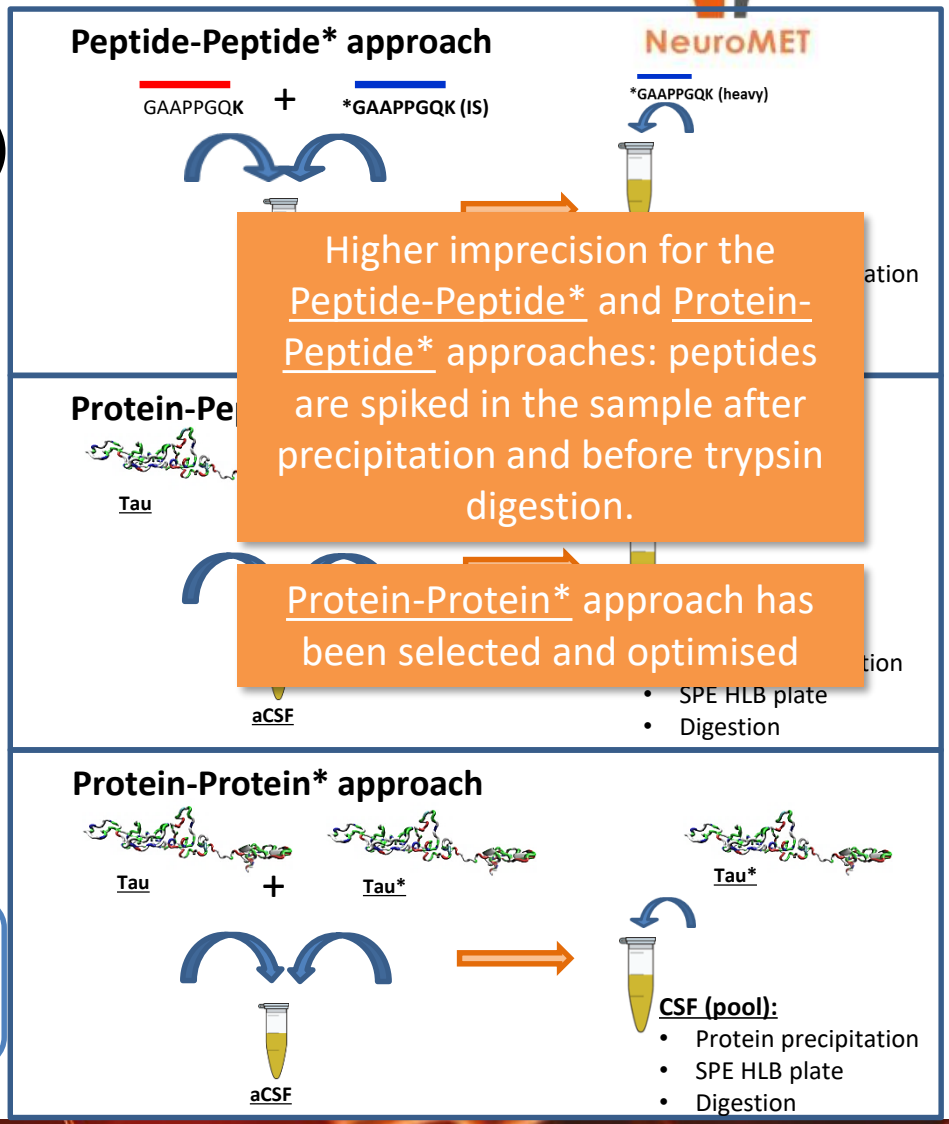
[rTau] (µg/g)=263±16 (6,09%), k=2

Peptide:
Signature peptide (Ala, Pro)



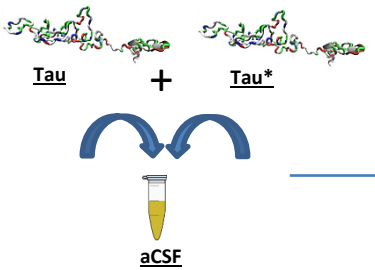
AAA on Alanine and Proline

[rGAAPPGQK] (µg/g)=755±48.3 (6,4%), k=2

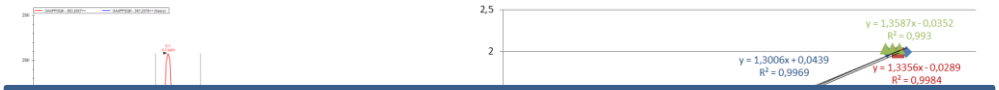


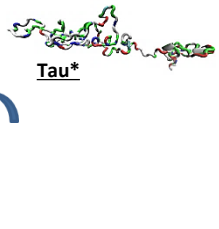
LABORATOIRE NATIONAL DE MÉTROLOGIE ET D'ESSAIS

t-tau: LC-MS method



Preparation of calibration blends





Quantification of tau in CSF

POSTER P-09
Helene Vaneeckhoutte

CSF Low :

Aliquot 5.1.2 : 1 041 pg

Aliquot 5.2.1 : 868 pg/

Aliquot 5.2.2 : 867 pg/

n :

5.1.2 : 3 780 pg/mL

5.2.1 : 3 687 pg/mL

5.2.2 : 3 736 pg/mL

CV 10,8%

CV 3,3%

CV 1,2 %

Comparison with immunoassay data
IFCC-CSF WG round robin

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

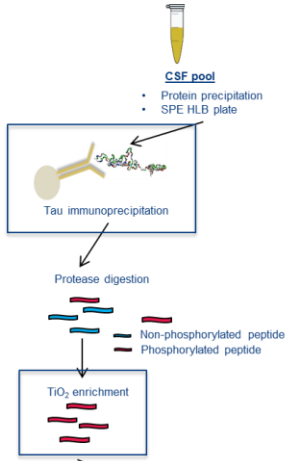
↓

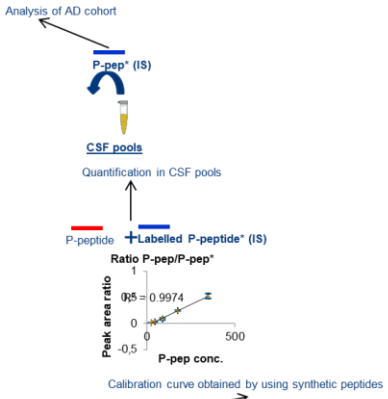
Virtual recalibration of immunoassays for quantification of tau

↓

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

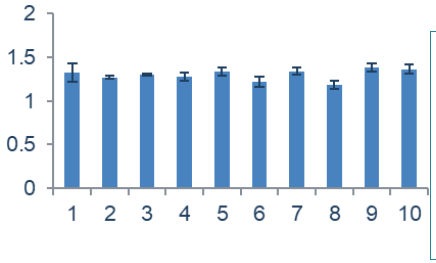
No clinical thresholds based on immunoassays have been established due to poor measurement performance

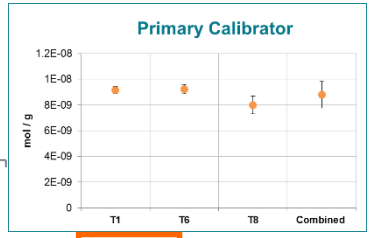
MS methods also suffered of poor measurement comparability (M.J.Fox study data not published)

RT-QUIC measurements show promising results, but implementation difficult due to calibrators

α-synuclein primary calibrator

Peptide standards	Certificate	Crude AAA	AAA corrected	qNMR	qNMR corrected
MDVFMK	≥95%	71.1	**68.5**	68.0	**68.0**
QGVAEAAGK	≥95%	63.4	**49.9**	60.9	**50.2**
EGVLYVGSK	≥95%	68.7	**65.5**	66.8	**66.6**
TVEGAGSIAAATGFVK	≥95%	26.1	**21.4**	25.2	**21.8**





Recombinant primary calibrator from UCL:

√ Purified

√ Protocol for dilution and storage developed

√ Quantified traceable to the System of International Units

MS clinical routine method (Shimazu)

RT-QuiC

Structural analysis characterisation

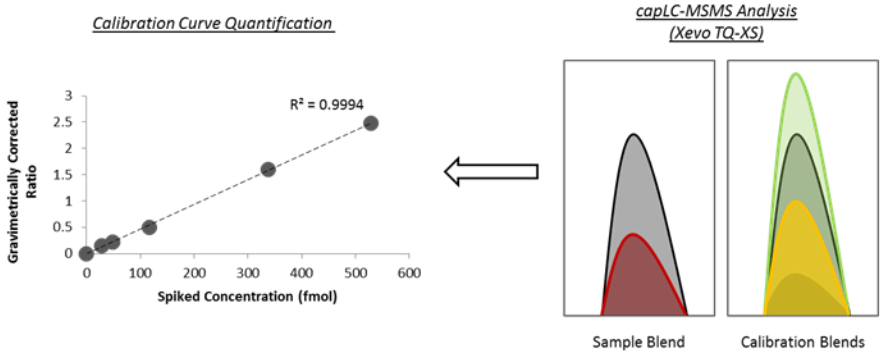
UCL

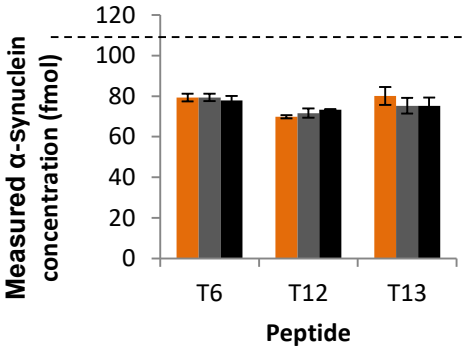
CHU Montpellier – Centre Hospitalier Universitaire

Amsterdam UMC – University Medical Centers

LGC

α-synuclein
LC-MS method traceable to SI





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

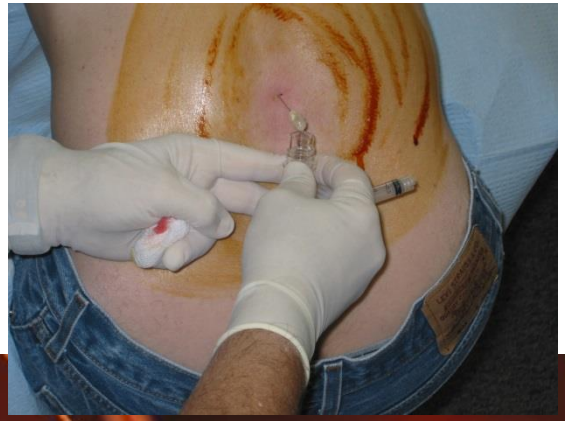
CRM?

AD and NDD diagnosis

Cognitive assessments

Magnetic resonance imag

Patient diagnosis

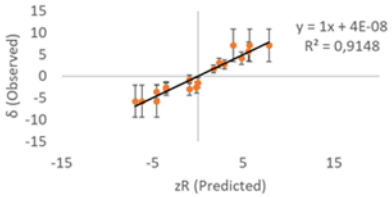


Clinical biomarkers (Aβ 1-42, 1-40, tau, NFL, α-synuclein)

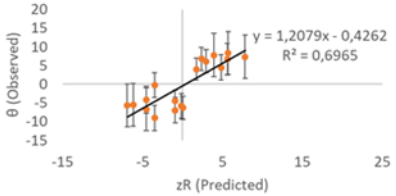
Construct Specification Equation

NeuroMET

Difficulty, δ

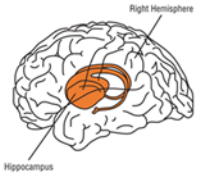


Cognition, θ



Memory test

Biomarkers



Principal component analysis

$$\mathbf{X}' = \mathbf{T} = \mathbf{X} \cdot \mathbf{P}$$

$$Cov(\mathbf{X}) \cdot \mathbf{p}_n = \lambda_n \cdot \mathbf{p}_n$$

Regression

$$\delta = f(x_1, \ldots, x_m)$$

$$\widehat{\mathbf{C}} = \left(\mathbf{T}^{\mathbf{T}} \cdot \mathbf{T}\right)^{-1} \cdot \mathbf{T}^{\mathbf{T}} \cdot \mathbf{Y}$$

$$\mathbf{Y} = \mathbf{T} \cdot \mathbf{C} + \varepsilon_y$$

$$\theta = g(z_1, \ldots, z_n)$$

RI. SE

NeuroMET

Complete, 'right' explanatory variables, X, Z?

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

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

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

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

$u(Z)$

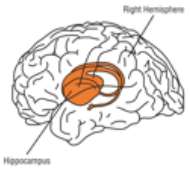
$$\hat{Y} = \sum_k \beta_k \cdot X_k$$

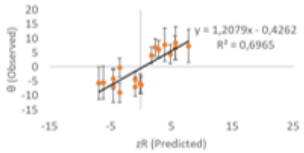
$$SDC(X) = \frac{2 \cdot \sqrt{2} \cdot \overline{u(\theta)}}{\beta_X}$$

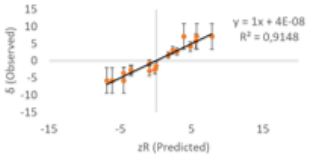
$$\theta = g(z_1, \ldots, z_n)$$

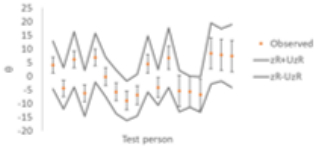
$$\delta = f(x_1, \ldots, x_m)$$

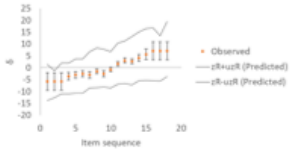
$$u[SDC(X)] = \frac{-2 \cdot \sqrt{2} \cdot \overline{u(\theta)} \cdot u(\beta_X)}{(\beta_X)^2}$$











MODUS OUTCOMES

The first Magento 2 POS app for iPad/tablet with PWA technology

(also works perfectly on PC and laptop)

RI. SE

LGC

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

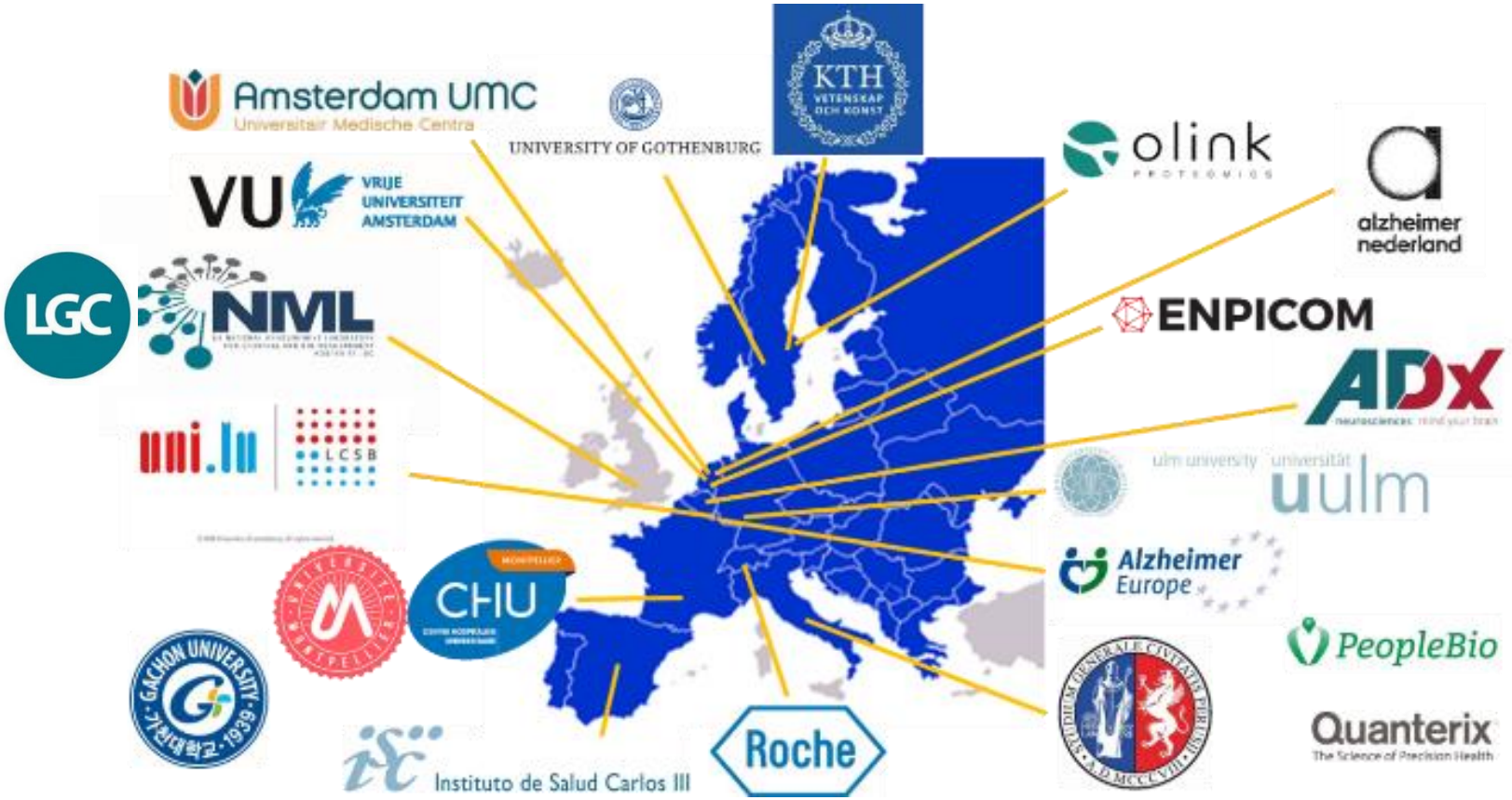
Promising biomarkers and methods for early AD diagnosis were identified and need to be validated through longitudinal studies

MIRIADE

Marie Curie program (2019-2022)

NeuroMET

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 Tecnologies de Saclay (IBITECS - CEA)

This project has received funding from the EMPIR programme co-financed by the Participating States and from the European Union's Horizon 2020 research and innovation programme