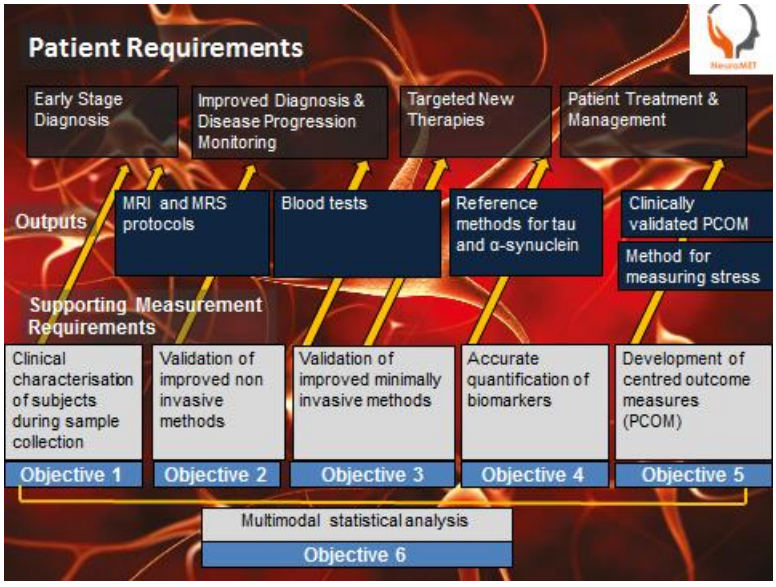
Publishable Summary for 15HLT04 NeuroMET
Innovative measurements for improved diagnosis and management of neurodegenerative diseases

Overview

Neurodegeneration is an incurable, debilitating process which presents a growing global challenge due to the increasingly ageing population. Alzheimer's disease (AD) and Parkinson's disease (PD) are the two most common neurodegenerative diseases (NDD). Both involve the build-up of specific proteins in the brain and subsequent neurodegeneration leading to physical and mental impairment including dementia. Currently, there are no clinically validated, minimally invasive diagnostic tools which allow the early diagnosis and/or monitoring of disease progression in AD and PD patients and available therapeutics only offer transient symptomatic relief. This project aims to develop tools to address the major NDD patient needs including less invasive, more accurate diagnostic measurement, and improved treatment and anxiety monitoring. Measurement comparability through SI (System of International Units) traceability and uncertainty analysis is an, as yet, unmet requirement for regulatory approval of NDD biomarkers, patient centred outcome measures (PCOM), clinical thresholds and new therapeutic drugs. Therefore, the development of reference methods to underpin the production of calibrators and improve measurement comparability of established biomarkers in the NDD area will significantly move the field forward.

Need

The "Implementation report on the Commission Communication on a European initiative on Alzheimer's disease and other dementias" (2014) highlighted the importance of early diagnosis in NDD. Established biomarkers of AD from cerebrospinal fluid (CSF) have been used to differentiate between subjects with mild cognitive impairment (MCI) who have progressed to AD and those MCI patients who have not. However, the lumbar puncture procedure for CSF sample collection is time consuming, invasive and therefore limited in terms of the possibility of widespread application for early AD diagnosis. Furthermore, although AD biomarker detection from less invasive diagnostic procedures such as blood samples and neuroimaging (i.e. Magnetic Resonance Imaging (MRI) has significant advantages, the limits in measurement sensitivity and high measurement variability of recognised and novel biomarkers for AD and PD have constrained the development of clinical thresholds for NDD early diagnosis. Major impacts of NDD, particularly decline in cognitive function, as well as increase in psychological symptoms (agitation, anxiety, etc.), can be captured in patient centred outcome measures (PCOMs). These PCOMs need to be correlated with the various biomarkers and objectively monitored and managed. The NeuroMET project aims to address these needs for measurement improvement and standardisation of NDD non- and minimally invasive biomarker measurements to develop PCOMs, which will be validated by laboratory data, as well as establishling reference measurement procedures to underpin



measurement comparability. These are fundamental requirements for the reliable development of minimally invasive early diagnostic and patient management procedures but also to support therapeutic discovery.

Objectives

This project combines the diverse expertise of a number of metrology laboratories together with clinicians and academics, to overcome specific measurement issues currently constraining clinical innovation and uptake in NDD diagnosis and treatment. This will provide routes to directly translate this research into the clinic. Unusually for a metrology project, patients play a central role in the NeuroMET project, thus ensuring that metrology is correlated to patients' clinical status and is relevant to patient and clinical measurement requirements. The specific objectives of the project are:

1. **To establish patient cohorts** to be employed in the validation of innovative tools for early diagnosis.
2. **To develop and validate non-invasive magnetic resonance imaging and spectroscopy (MRI and MRS) approaches** for *in vivo* characterisation of AD and MCI patients and healthy matched controls.
3. **To develop minimally invasive methods for AD and PD early diagnosis and drug therapeutic monitoring** based on immunoassay, digital PCR and liquid chromatography mass spectrometry technology.
4. **To improve NDD biomarker measurement comparability through the establishment of SI traceability chains** by developing a reference method for tau (AD biomarker) and α-synuclein (PD biomarker).
5. **To determine and characterise PCOMs for NDD** by developing improved clinical assessment questionnaires focusing on decline in cognitive function using invariant measurement theory. Tools for measuring stress will be also evaluated.
6. **To develop, validate and verify multimodal statistical analyses** for (i) the correlation of cognitive task difficulty with instrument parameters (e.g. sequence entropy) and (ii) the correlation of cognitive health status of the patients with the data obtained from biomarker analyses and MRI obtained from this project and other relevant studies. In both cases, causal Rasch models are developed which yield successful prediction of the observed outcome (count correct) as prototype metrological references for cognition.
7. **To form a NeuroMET Stakeholder Network** in Europe for neurodegenerative disease diagnosis and disease progression monitoring which includes members of the consortium and stakeholders.

Progress beyond the state of the art

The performance of a number of minimally/non-invasive approaches is challenged and applied to the NeuroMET project patient cohorts to enable disease state related differentiation for comparative experiments through validated statistical analysis:

- Proton MR spectroscopy (7T) and imaging is being used to provide both metabolic and anatomical information with the final aim to improve sensitivity, resolution and delineation for volume quantification and to define transferrable protocols to 3T scanners.

- Open platform immunodetection array technologies are being optimised for the quantification of recognised AD and PD protein biomarkers in blood. The methods developed are then applied to clinical samples to improve sensitivity and reduce measurement uncertainty with the final goal to identify stress and diagnostic biomarker trends in the patient cohort samples.
- Digital polymerase chain reaction (PCR), is being developed to enable accurate quantification of candidate AD microRNA (miRNA) biomarkers in blood samples and will be applied to clinical samples.
- Liquid chromatography–mass spectrometry (LC-MS) methods are being developed for monitoring the PD pathological protein α-synuclein in CSF to overcome the issues associated with immunoassay measurements of this recognised target for pharmaceuticals. Furthermore a method for quantification of α-synuclein in saliva will be developed to exploit the potential use of this non- invasive marker for early diagnosis of PD and AD.

Progress is being made towards establishing SI traceability of NDD biomarker measurements. Reference methods for tau (AD biomarker) and α-synuclein are being developed and calibration approaches are being implemented for tau to facilitate immunoassay measurement comparability.

The application of novel statistical and metrological approaches to the integration of data from the NeuroMET biomarker approaches into a PCOM of NDD is a key innovation for the project and will test the concept of 3rd generation standards (beyond traditional products or management standards) by associating the results from the analysis of clinical biomarkers with the results of clinical assessments.

Overall this innovative multidisciplinary approach, within the NeuroMET project, will enable the development of a clinical and metrological framework to improve the quality of life of NDD patients and their carers, by improving accuracy in diagnosis, facilitating the development of new therapeutics and enabling more effective treatment.

Results

1. *To establish patient cohorts to be employed in the validation of innovative tools for early diagnosis*.

Ethical approval for the recruitment of patients for two cohorts was obtained and recruitment is close to completion. Cohort 1 is to be used to develop validated PCOM for AD and MCI patients through MRI and magnetic resonance spectroscopy (MRS), protein, microRNA and statistical analysis, while cohort 2 was used to assess cortisol as a potential marker for stress in PD and AD patients. Samples and cognitive assessment data from cohort 1 have been distributed to the consortium and analysis is on-going as described in objective 2,3 and 4. The recruitment, stratification and sampling of cohort 2 has been completed and samples were analysed by immunoassay for free and total cortisol as markers of stress as part of objective 3.

2. *To develop and validate non-invasive MRI and MRS approaches for in vivo characterisation of AD and MCI patients and healthy matched controls*.

MRI and MRS protocols were defined and the scan parameters were optimised on a 7T scanner. These protocols were applied to scan 77 subjects from cohort 1. The acquired data was analysed for structural, functional and metabolic information. Correlations between different volumes of subcortical structures (e.g. the hippocampus) and the concentrations of the neurotransmitters glutamate and GABA in the posterior cingulate cortex have been observed. The resulting data was sent for statistical analysis together with the cognitive assessment data as part of objective 6.

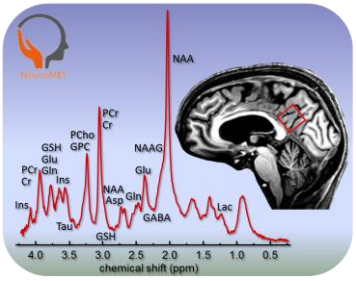


Figure 1: Magnetic resonance spectrum acquired at 7 T from the posterior cingulate gyrus (red square) of a 77-year-old male with annotated metabolites to be quantified. Insert shows the T1-weighted image used for voxel identification and later volumetric analysis.

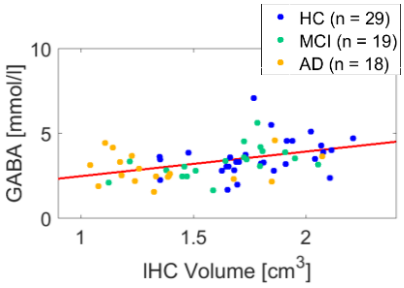


Figure 2: Correlation between volume of left hippocampus and the concentration of the neurotransmitter GABA measured in the posterior cingulate cortex. (from: A. Fillmer et al. "Correlations Between Brain Structural Volumes and Brain Metabolite Concentrations in Alzheimer's Disease: Preliminary Results from the NeuroMET Project", Proc. Int. Soc. Magn. Reson. Med. 27: 3903 (2018))

3. *To develop minimally invasive methods for AD and PD early diagnosis and drug therapeutic monitoring based on immunoassay, digital PCR and LC-MS technology*.

A number of proteins and miRNA biomarkers were selected by the consortium and in consultation with stakeholders as the most promising biomarkers in plasma. Based on this selection, a new generic approach to overcome matrix effects in immunoassay measurements was developed and implemented for cortisol, Aβ40 and Aβ42 peptides[1]. The method developed was applied to the samples from patient cohort 1 and the data generated are being used in objective 6. The same method was also applied to the samples from cohort 2 and data obtained showed the potential of using cortisol as a marker of stress. However, due to the small number of patients in the cohort, limited conclusions could be drawn. Alongside the standard addition approach, commercially available methods have been applied to generate biomarker data on the samples from cohort 1 (i.e. Aβ40, Aβ42, t-tau, neurofilament and α-synuclein). A digital droplet PCR method for miRNA purification and quantification and its application to samples from cohort 1 is also on-going. All the results on cohort 1 from objective 3 are being used with the results from objectives 1 and 2 to develop mathematical models to predict cognitive patient abilities.

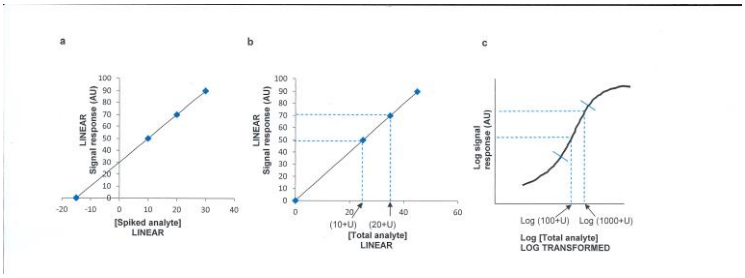


Figure 3: Illustrations depicting conventional standard additions for chemical analytes and biological targets by immunoassay [2]. Fig 3a and 3b represent plots for traditional standard additions applicable to chemical analyses, whereas 3c represent the plot for a standard additions approach implemented for immunoassays. .

4. *To improve NDD biomarker measurement comparability through the establishment of SI traceability chains by developing a reference method for tau (AD biomarker) and α-synuclein (PD biomarker)*.

The specifications for tau and α-synuclein primary calibrators have been defined and experiments are currently on-going to finalise their quantification traceable to the SI. Two primary calibrators have been selected for quantification of t-tau in CSF, and the method for quantification of tau in CSF was applied on three CSF pooled samples for method validation. Furthermore to evaluate the potential commutability of certified reference materials valued assigned by using the NeuroMET reference method for t-tau, three pools with low, medium and high values of tau were prepared to be SI traceably quantified by using the reference measurement procedure developed. In addition, 40 individual CSF samples with tau values covering the detectable range of concentration have been prepared for distribution to immunoassay laboratories. Progress was also made towards the validation of the method for quantification of α- synuclein in CSF and saliva. Experiments are planned for correlating the results from the method with immunoassays results on the CSF and saliva samples from cohort 1, as well as 10 CSF and plasma Parkinson's disease samples and 3 pooled samples

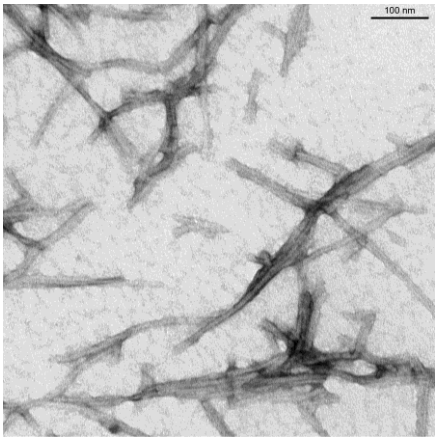


Figure 4: Electron-microscopy image of wild type α-synuclein fibrils generated by using the primary standard (scale bar, 100 nm).

5. *To determine and characterise PCOMs for NDD by developing improved clinical assessment questionnaires focusing on decline in cognitive function using invariant measurement theory.*

Improved cognitive assessment protocols are currently being developed and their use assessed on relevant published data. Development of new cognitive assessment instruments (specially for memory) are underway for (i) improved, metrological evaluation of cognitive scores; and (ii) the development of so-called 'specification equations' for a variety of cognitive protocols (such as the relationship between cognitive task difficulty and protocol attributes, i.e. the number of blocks tapped). Studies have been performed on the correlation of the NeuroMET data with cognitive task difficulty and instrument parameters (e.g. sequence entropy) and it was

shown how the mathematical models developed can be used to predict for example task difficulty. Figure 5 gives an example where task difficult (zR) for a sequence of increasing difficulty in the Knox cube test is explained as a sum of three terms: entropy; number of reversals and the average distance covered in each sequence, where the specification equation $zR_i = Intercept + 1(1) \cdot Entropy_i + 0{,}6(1{,}3) \cdot Reversals_i + 0{,}4(9) \cdot Distance_i$ (coverage factor $k = 2$) can be used to predict the difficulty of sequence *i*.

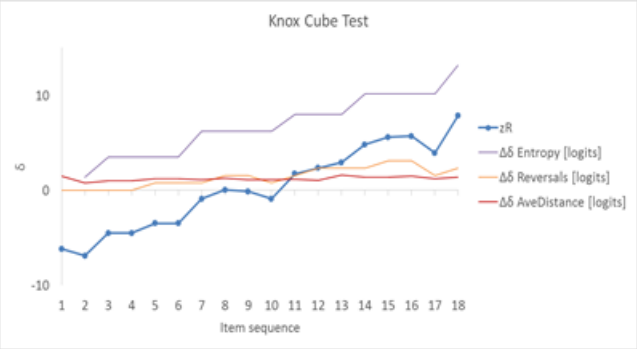


Figure 5: Cognitive difficulty of tasks in Knox Cube Test as a function of a number of explanatory variables

6. *To develop, validate and verify multimodal statistical analyses for the correlation of the cognitive health status of the patients with the data obtained from biomarker analyses and MRI obtained from this project and other relevant studies*

Following initial work[2] on correlating Mini Mental State Examination results with MRI estimates of grey matter volume by using relevant published data, the first set of construct specification equations where person cognitive (memory) ability is described as a function of several explanatory variables such as biomarkers has been formulated. Multivariate principal component regression has been applied to the NeuroMET data for biomarkers in plasma, CSF and saliva together with MRI/MRS data. Formulation of causal Rasch models as prototype metrological references for cognition is being guided by data from other studies such as the ADNI (Alzheimer Disease Neuroimaging Initiative) relating to disease state indices, while using the new cognitive assessment instruments (specially for memory) being developed in objective 5.

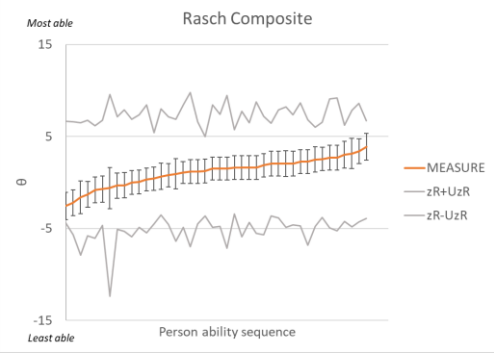


Figure 6: Cognitive (memory) ability of persons from NeuroMET Composite Score as a function of a number of explanatory variables (biomarkers in plasma + MRI/MRS)

Impact

The ultimate goal of the NeuroMET project is to improve the diagnosis and drug therapeutic monitoring of NDD by developing assays, protocols and PCOM, which can be directly implemented in the clinical settings such as those of the project partner's hospitals and laboratories.

The NeuroMET consortium has so far organised three stakeholder workshops and the project has been strongly represented at a number of metrological and NDD conferences. Details can be found on the project webpage (http://www.lgcgroup.com/our-science/national-measurement-laboratory/european-metrology-programme-for-innovation-and-re/neuromet/).

Impact on the metrology and scientific communities
The NeuroMET project is expected to have an impact on establishing links between the metrological community and the biological/clinical community in the NDD area by translating physical (MRI) and bio-organic (t-tau, p-tau and α-synuclein) reference methods and procedures into clinical practice. Progress were made through the direct involvement of NeuroMET consortium partners in NDD standardisation initiatives (such as the IFCC working group for standardisation of CSF protein biomarkers and EUFIND: European Ultrahigh-Field Imaging Network for Neurodegenerative Diseases that aims at drawing roadmaps for implementing and reporting harmonised ultrahigh-field MRI in dementia) and dissemination of the project to standardisation/metrological workshops and NDD meetings. Those include the IFCC EuroMEDLAB, the Joint Committee for Traceability in Laboratory Medicine (JCTLM), the International Measurement Conference (IMEKO), the Protein and Peptides Therapeutic Drug workshop organised under the auspice of JCTLM, the Alzheimer's & Parkinson's Diseases Congress (AD/PD) and the Alzeihmer's Association International Conference. In addition, the progress and approaches developed for purity assessment of primary calibrators have been regularly discussed within the CCQM metrological community and the metrological reference measurement procedures from NeuroMET (biomarkers and mathematical) have been linked to two recently formed EURAMET European Metrology Networks (EMN): the EMN on "Traceability in Laboratory Medicine" and "MATHMET".

Impact on relevant standards

The NeuroMET consortium are involved in a number of ISO technical committees including TC12 on Quantities and Units, TC 212 on Clinical laboratory testing and in-vitro diagnostic test systems and TC 215 on Health Informatics. Their regular attendance to meetings and events enable the application of the multidisciplinary NeuroMET metrological and clinical expertise to improve standardisation documents and current guidelines.

Impact on industrial and other user communities
The improved cognitive and anxiety clinical tests and rating scales developed within the NeuroMET project will provide guidance and tools to clinicians for improved diagnosis, better prediction of future patient decline and will be made available to industry to be used for improved patient recruitment in clinical trials and data analysis. Three training courses were so far organised addressing predominantly industry stakeholders. These 3 training courses were on (1) Assuring quality in person-centred healthcare; (2) How to calibrate a questionnaire: quality assuring categorical data with psychometric measurement theory and (3) Quality-assured categorical data. The in-vitro diagnostic (IVD) industry will also benefit from the project by gaining additional information on instrument performance and by the availability of the developed reference measurement procedures for regulatory approaval of new instruments and compliance with the IVD Directive 98/79/EC.

Longer-term economic, social and environmental impacts

Overall the NeuroMET project will combine the strengths of NMIs and NDD clinicians to establish an infrastructure, that will provide guidance and measurement tools to NDD clinicians, academics and pharmaceutical companies for:

- Appropriate study design and definition of the uncertainty of NDD clinical assessment protocols to improve diagnosis and NDD progression monitoring;
- More accurate stratification of patient cohorts, with respect to NDD status, for enrolment into clinical trials and for more informed patient management;
- Improved measurement comparability for NDD biomarkers through optimised measurement procedures and development of SI traceable reference methods for key biomarkers.

List of publications

[1]. S. Pang and S. Cowen, Scientific Reports, https://doi.org/10.1038/s41598-017-17823-y

[2]. L. Pendrill 2017, Measurement Science and Technology, special issue Metrologie, https://doi.org/10.1088/1361-6501/aa9cd2

[3]. G. Franceschi 2017, JBC http://www.jbc.org/content/292/17/6927.full.pdf

Project start date and duration:	July 2016, 36 months

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Project website address: http://www.lgcgroup.com/our-science/national-measurement-laboratory/european-metrology-programme-for-innovation-and-re/neuromet/#.WRHEO2dYrcs

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