Publishable Summary for 22HLT07 NEuroBioStand
Standardisation of measurements of neurodegenerative disease biomarkers

Overview

Neurodegenerative disorders (NDDs) affect over nine million Europeans and constitute a large economic burden to society. NDD biomarkers have transformed the research landscape by linking diseases to pathological processes and assisting drug development. NDD biomarkers improve the accuracy of diagnosis and prognosis with the use of non-invasive tools. However, their implementation in clinical practice is hampered by a lack of standardisation. This project will build on the knowledge and experience of EMPIR projects 15HLT04 NeuroMet, 18HLT09 NeuroMET2 and 18HLT10 CardioMet and the International Federation of Clinical Chemistry (IFCC) programme to standardise biomarker measurements, harmonise measurements across assay manufacturers as well as develop and implement new biomarkers.

Need

One out of eight people in Europe is affected by diseases of the nervous system (Alzheimer's and Parkinson's disease, multiple sclerosis, epilepsy, brain tumours, paraplegia etc) with costs associated to this group of diseases corresponding to one third of the healthcare expense in Europe before the pandemic. Early and accurate diagnosis of NDDs is crucial for effective management of diseases and early identification of patients who could most effectively benefit from new treatments. The number of fluid biomarkers available for early diagnosis of neurodegenerative diseases is growing together with the rapid development of assays that have the potential to be routinely used in clinic. Translational research and metrology have a key role to play in this landscape.

While research in NDDs has significantly progressed in the last few years through the discovery of new biomarkers and the development of non-invasive sensitive assays implemented on automated platforms, large-scale implementation of these biomarkers in routine clinical settings necessitates standardisation. To date, most initiatives striving for standardisation of NDD biomarkers are focused measurements performed in cerbrospinal fluid (CSF), including Amyloid Beta 1-40 (Aβ 1-40), Amyloid Beta 1-42 (Aβ 1-42), total tau (t-tau) and Neuro Filament Light chain (NfL). To meet the need for minimally invasive assays, there is an urgent need to standardise measurements of NDD biomarkers in blood. Building a European infrastructure for NDD biomarker standardisation is of utmost importance to rapidly implement new NDD biomarkers in clinical practice. Due to the structural heterogeneity of these biomarkers, detailed molecular characterisation of the analytes requires metrology to define the measurand and develop adequate reference method procedures (RMPs) and certified reference materials (CRMs).

Objectives

This project will develop a metrological research framework for standardising biomarkers of neurodegenerative diseases (NDDs), including Alzheimer's disease, dementia with Lewy bodies, frontotemporal dementia and Parkinson's disease, the fast implementation and commercialisation of promising assays for NDD biomarkers will be promoted through compliance to the EU IVDR 2017/746. The specific objectives are:

1. To standardise the measurement results of phospho-tau (P-tau) in plasma. This will include the development of new metrological strategies addressing multiple phosphorylation sites in biological matrices. This will be achieved by developing candidate reference measurement procedures with a target uncertainty of less than 15 %. Prototype reference materials will be developed, and their commutability will be assessed through inter-laboratories studies enabling plasma P-tau measurements performed in clinical practice to be standardised.

2. To standardise the measurement of neurofilament light chain (NfL) in plasma or serum by establishing reference measurement procedures and developing prototype reference materials of which commutability will be evaluated. This will require defining the appropriate target analyte/measurand and establishing and validating traceability chain strategies to reach a limit of quantification (LOQ) of 0.5 pg/mL in blood derivatives with target measurement uncertainty of less than 15 %. Additionally, an LC-MS (Liquid Chromatography coupled to Mass Spectrometry) based clinical assay will be developed and its correlation with immunoassays will be established.

3. To implement clinical measurement of the glial acidic fibrillary protein (GFAP) as an emerging biomarker candidate by developing an SI-traceable LC-MS/MS method in biological fluids. This will include the development of primary calibrators of well characterised purity with an uncertainty of less than 15 %. A feasibility study will be carried out to evaluate the applicability to patient samples.

4. To develop effective and fit-for-purpose metrological approaches to overcome the challenges in standardising NDD fluid biomarkers. This will include the development of new metrological strategies to address protein conformational states, post-translational modifications and uncertainty frameworks.

5. To facilitate the take up of the technology and measurement infrastructure developed in the project by the measurement supply chain (EMN-TLM, JCTLM, ICHCLR), standards developing organisations (IFCC WG-BND, Diagnostic Devices Regulation 2017/746 (IVDR), ISO TC12, ISO TC212), and end users (e.g., clinical stakeholders, manufacturers of medical and healthcare products).

Progress beyond the state of the art and results

Previous standardisation efforts have been pioneered by the IFCC and through the EMPIR projects NeuroMet and NeuroMET2 in CSF. Today, sensitive assays for P-tau forms and NfL are available from several manufacturers and a great foreseeable utility of these biomarkers will be achieved through the development, standardisation and harmonisation of results from different assay platforms. Metrology plays a key role in supporting the implementation of those biomarkers into clinics, by providing the required traceability for calibrators, frameworks for measurement uncertainty and reference measurement systems to enable measurement standardisation and establishment of relevant clinical thresholds.

Objective 1. Standardisation of P-tau measurement results

Together with the Aβ42/Aβ40 ratio, CSF t-tau and P-tau181 have been proposed as biomarkers to biologically define Alzheimer's Disease (AD). A RMP for t-tau in CSF has been developed through NeuroMet and NeuroMET2 and the development of commutable CRMs is ongoing. A candidate RMP for P-tau181 in CSF is under validation. Recently, the development of ultrasensitive assays to measure P-tau forms in plasma has led to a breakthrough, with the finding of increased levels of tau phosphorylated at Thr-181 in AD plasma compared with control samples, and a good correlation with CSF P-tau levels, tau PET, and amyloid PET, indicating that plasma P-tau is a good biomarker for brain AD pathology. Mass spectrometry-based methods for some P-tau forms have also been developed, providing a basis for developing RMPs. SI-traceable RMPs and prototype RMs for different phosphorylated isoforms will be developed in plasma by ultrasensitive LC-MS methods to underpin a worldwide standardisation of results.

Objective 2. To standardise the measurement of neurofilament light chain in plasma or serum

A large number of studies in recent years have established NfL as a robust biomarker of neuroaxonal damage, in both acute neurological conditions, such as traumatic and vascular brain injury infections as well as in neurodegenerative and neuroinflammatory conditions. A candidate primary calibrator has been developed during NeuroMET2 and the development of an RMP and secondary CRMs in CSF is ongoing. SI-traceable

RMPs and prototype RMs for NfL will be developed in blood-derivatives by using ultrasensitive mass spectrometry.

Objective 3. To develop clinical measurement procedures for emerging biomarker candidates

GFAP is an emerging biomarker involved in several pathological states from traumatic injuries to dementia. This project will contribute to develop a prototype primary calibrator and a SI-traceable LC-MS method, to pave the road towards the establishment of traceability chains for standardisation of this novel biomarker. This will allow routes for standardisation in an early phase of the implementation of GFAP in clinical practice to be explored.

Objective 4. To develop effective and fit-for-purpose metrological approaches to overcome the challenges in standardising NDD fluid biomarkers

The accumulation of toxic aggregates of proteins in the brain is one of the basis of some neurodegenerative diseases like AD and PD. This project will use chemical cross-linking coupled to mass spectrometry and native mass spectrometry to elucidate clinically relevant protein conformational states by contributing to the definition of the measurand, as well as post-translational modifications.

Outcomes and impact

Outcomes for industrial and other user communities

Standardisation of biomarker measurements will greatly benefit assay manufacturers by facilitating the transfer of their assays from research to clinical practice. The development of SI-traceable calibration materials will improve comparability of test results across platforms, which will enable assay manufacturers to define standardised biomarker cut-off for patients' stratification criteria. The pharmaceutical industry will also benefit from the availability of more accurate and reliable biomarker data for clinical trials. For patients and care providers, access to standardised fluid biomarkers will enable better diagnosis regardless of the region where they live, improving the quality of life of patients and their relatives and reducing health care costs.

The project will benefit from the tight cooperation with the IFCC Working Group for Biomarkers of Neurodegenerative Diseases in which assay manufacturers are actively involved, as well as clinicians and representatives from the metrological community.

This project, in line with the priorities of the EMN-Traceability in Laboratory Medicine, will engage with instrument manufacturers and underpin their regulatory approval by ensuring compliance with the in vitro diagnostic regulations (IVDR). It is also envisaged that the consortium's knowledge of the project renders Europe an ideal choice for carrying out clinical trials and new therapies. Finally, the involvement of specialised hospitals linked to universities, industries and metrology institutes will ensure the training of a new generation of skilled scientists for neurodegeneration and make Europe a suitable location for diagnostic, biopharma and gene therapy industries.

Outcomes for the metrology and scientific communities

The project will enhance the international metrological capabilities through the development of new approaches that address for the first time the development of reference measurement procedures targeting post-translationally modified markers and protein conformational states. The scientific and metrological community will also benefit from the outputs of this project by the examples provided in defining clinical measurands vs the target analytes. These concepts will apply to biomarkers of other diseases towards personalised medicine, improve the tests' specificity and provide new tools for a better understanding of pathologies. The outputs of the project will be disseminated to the broader metrological community through the CCQM (Consultative Committee for the Amount of Substance: Metrology in Chemistry and Biology) and JCTLM (Joint Committee for Traceability in Laboratory Medicine). The proteomics/mass spectrometry community will be addressed.

Outcomes for relevant standards

The project will impact several standards and guidelines, including from ISO TC212, the IFCC, the Global Biomarker standardisation consortium of the Alzheimer's Association, the Society of CSF analysis and clinical neurochemistry, JCTLM and BIPM's Consultative Committees for Amount of Substance: protein analysis working group. Activities between the committees and the project will be aligned to improve the standardisation of units, biotechnology processes and laboratory medicine. Standardisation of biomarkers such as NfL and P-tau will enable those biomarkers to be included in international guidelines for diagnosing and managing dementia and other NDD disorders, such as NICE and the European Medicine Agency guidelines.

Longer-term economic, social and environmental impacts

In 2021, the first disease-modifying drug against AD was granted a partial approval by the American Food and Drug Administration (FDA), and several other drug candidates are currently at different stages of clinical trials. In an era when NDDs become treatable, it will be of paramount importance to identify patients at risk at an early stage, so that the right treatment can be administered before neurodegeneration has caused severe damage to the brain. In this context, fluid biomarkers will most likely play an important role as cost-effective, non-invasive tools for early diagnosis of NDD. Thereby, this project may in long-term help to decrease the socioeconomic burden represented by NDD, reduce the resources spent by the pharma industry, and most importantly improve the quality of life of NDD patients and their caregivers.

The use of non-invasive, blood-based and widely accessible tools will reduce the recourse to imaging for large-scale screenings, thus limiting costs for the healthcare system. The standardisation effort carried out during this project could lead to the implementation of multiple assays coming from different companies for the same biomarker in a competitive perspective that will contribute to limiting the prices of assays on the market.

The outputs of the project align and move forward some of the European priorities published on the "Driving policy to optimise care for people with Alzheimer's Disease in Europe today and tomorrow" [22], where supporting research into the early detection and diagnosis of AD and its implementation is not only a priority to ensure that the European healthcare system is prepared to embrace new therapeutics, but also plays an important role in upholding the right to dignity and combating stigma of patients with dementia.

List of publications

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This list is also available here: https://www.euramet.org/repository/research-publications-repository-link/

Project start date and duration:	1 October 2023, 36 months	
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Internal Beneficiaries: 1. LNE, France 2. PTB, Germany 3. TUBITAK, Turkiye	External Beneficiaries: 4. Charité, Germany 5. CHUM, France 6. ESPCI, France 7. UGOT, Sweden 8. VUmc, Netherlands	Unfunded Beneficiaries: 9. ADx, Belgium 10. JRC, Belgium
Associated Partners: 11. LGC Limited		
Affiliated Entities: 12. CNRS, France (linked to ESPCI)		