European Platform for Neurodegenerative Diseases

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WP5 – Operationalization of the platform and ATN scheme

D5.1 – Report on ATN markers

Lead contributor (partner organisation)	9 – UGOT
Other contributors	22 – Roche
	1 – UM
	5 – Vumc

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Abstract

The main goal of work package (WP) 5 is to test the functionality of the EPND platform as a validation tool of biomarkers for drug development. This will be performed through case studies (CS) 1 to 5, where CS1 will focus on evaluating the basic functionalities of the EPND and progress to testing the full functionalities through the rest of case studies. For this, the same set of samples will be used across different case studies (1,2, and part of 4). In this report, we focus on CS1, which addresses the operationalization of the platform and ATN biomarker scheme. In this deliverable, we report on (1) the logistical steps related to transfer of CSF and plasma samples to the laboratories for analysis; (2) methods used for lab analyses of the ATN staging in the samples; (3) collection and harmonisation of associated clinical data collection; and (4) key results of CS1 data analyses; (5) making CS1 data available through the EPND platform for future reuse.

Introduction

Case study (CS) 1 is the first case study to test the functionality of the EPND platform and focusses on Amyloid-Tau-Neurodegeneration (ATN) biomarker classifications.

ATN biomarker classifications can be used to identify Alzheimer's disease (AD) at early stages and allows the investigation of non-AD pathophysiology. ATN CSF biomarker classification tests have previously shown good analytical performance and utility in the identification of amyloid pathology in clinical practice. Emerging plasma markers also allow ATN classification. To date, it remains unclear how ATN classifications in CSF and plasma are related to each other in neurodegenerative diseases, such as Parkinson's disease (PD) and Lewy body dementia (DLB).

The aim of CS1 was to assess ATN (amyloid, tau, neurodegeneration) markers in CSF and plasma in 350 cases with AD, DLB, PD and controls. Moreover, the relation of ATN biomarkers and α-synuclein with relevant clinical and genetic measures was studied. To this end, CSF and plasma samples from several cohorts were collected for central ATN lab analyses using the Roche NeuroToolKit. Additionally, the Janssen plasma pTau217 assay was used in an independent single cohort and compared to other relevant ATN related measures.

Methods

1. CSF and plasma Roche NeuroToolKit (NTK) panel

The methodological steps are visualized below.

1.1. Sample collection (UM, VUMC)

350 AD, DLB, PD and control cases were selected from 7 centers (10 cohorts) by WP4, as reported in D4.1. A detailed description of logistics to coordinate transfer of samples and data for case studies is reported in D4.3. Shortly, steps included coordination of (A) case selection, (B) finalization of case study methods and logistics, (C) biobank approval, (D) ethical approval, (E) MDTA drafting and signature, (F) sample and data preparation, (G) sample shipment, (H) sample aliquoting redistribution, and (I) data sharing. As part of steps F), G) and H), the 350 CS1 CSF and plasma samples and 163 DNA samples (subset) were prepared according to instructions by each center and shipped to VUmc for randomization and aliquoting. Subsequently, CSF and plasma samples were shipped in one batch by VUmc to UGOT in June 2023 for ATN measurement with NTK, and, if applicable, DNA samples were shipped to Rotterdam for GWAS analyses.

1.2. Laboratory analyses (UGOT, Roche, VUmc/Rotterdam)

The Roche NeuroToolKit (NTK) panel has been tested and set up by UGOT. Accordingly, the sample measurements were performed by UGOT under the NTK study protocol. At UGOT, 350 paired CSF and plasma samples from participants with AD (45 preclinical, 52 prodromal, 54 dementia) PD (n=75), DLB (n=67) and 57 controls (selected by WP4) were measured with NTK in July and August 2023. Measurements were performed after valid calibration and QC. Aß42 CSF and p-tau181 CSF are both CE-marked IVD assays. Plasma p-tau181 and NfL-hs are clinical trial assays and all other assays Aß40 CSF, α-Syn, NfL CSF, plasma Aß40, plasma Aß42 are Robust Prototype assays. The CSF samples were measured with the Aß40, Aß42 and p-tau181 immunoassays on the cobas e 601 instrument; α-Syn and NfL immunoassays were measured on the cobas e 411 instrument. The plasma samples were measured with the Aß40, Aß42 and p-tau181 immunoassays on the cobas e 601 instrument and the NfL-hs immunoassay was measured on the cobas e 801 instrument.

To test genetic associations with ATN measures, GWAS analyses will be performed. 163 DNA samples have been shipped to Rotterdam for GWAS analyses and will be merged with 144 cases for which GWAS data is already available.

1.3. Data collection and harmonization (UM)

Clinical data associated to the samples were collected in parallel. Approximately 120 clinical data items were requested by WP4 from each cohort. These included variables previously identified by WP3 (T3.3 – SOP Minimal dataset) as key disease variables. A subset of variables was selected by WP4 disease experts and WP5 case study leads for WP5 case study analyses purposes. Shortly, these encompassed relevant data on demographics, clinical factors (diagnosis, medical history), lifestyle, rating scales, cognition (global and neuropsychological tests), samples (CSF, plasma, serum, DNA), genetics (APOE, GBA), and imaging (MRI, amyloid PET, DaT SPECT) – altogether here referred to as “clinical data”.

Cohorts sent the clinical data to UM for manual harmonization by WP4 (UM) using R programming to merge data from all cohorts together. An EPND data form was set up to facilitate data transfer and communication with the centers around relevant information on the provided variables. CS1 generated sample data (UGOT/Roche) and unblinded sample lists (VUmc) were also shared with WP4 (UM) for merging.

1.4. CS1 statistical analyses (UM)

Initial data analyses included:

(1) Comparison of levels of CSF Aß42/40, p-tau181, NfL (ATN) and α-Syn between AD, PD, DLB and controls. This was conducted using multiple linear regressions, adjusted for age, sex, and global cognition level.

(2) Associations of CSF amyloid positivity (within DLB and PD) and tau positivity (within all groups) with CSF NfL and α-Syn, as well as age, sex, APOE-e4, motor function, and global cognition. Multiple linear or logistic regressions were used, adjusting for age and sex where appropriate.

(3) Spearman's correlations between CSF and plasma Aß42/40, p-tau181, and NfL (ATN) markers.

1.5. Making CS1 data available for reuse

All participating cohorts were asked for approval of making generated CS1 data available for future reuse by external users through the EPND platform via the AD Workbench (ADWB). More specifically, the CS1 data were made discoverable through metadata in the EPND Catalogue and requestable via data access request (DAR) processes. If a request is approved, data will be shared via the ADWB (in partnership with the Alzheimer's Disease Data Initiative, ADDI). The proposed approach is that UM will act as the controller under the GDPR for requests on the CS1 dataset.

2. Plasma Janssen pTau217 assay (VUmc)

997 plasma samples were selected from patients with AD or controls from the Amsterdam Dementia cohort. pTau217 (Janssen antibodies on Simoa HD-X instrument) was first analytically validated, according to Andreasson et al (Frontiers in Neurology 2015), meeting all acceptance criteria for precision, linearity, parallelism. Next pTau217 was measured in the 997 plasma samples of the Amsterdam Dementia cohort. Initial ROC-curve analysis was performed to assess accuracy for detection of amyloid positivity.

Results

1. CSF and plasma Roche NeuroToolKit (NTK) panel

1.1. Sample and data collection

An overview of final cohorts and cases is displayed in Table 1. Case selection, MDTA drafting and signature, and data sharing took longer than anticipated especially due to the inclusion of 3 third-party centers to EPND to fulfil the number requirements for the rarer DLB cases. This led to long MDTA contracting processes and revision rounds, which, together with the long data preparation timelines by these centers, caused significant delays to CS1 (see D4.3). The aliquoting process for DNA samples was also challenging, given the high variability in sample concentration that was received.

Table 1. Diagnosis cases per center.

	AHUS	**DZNE**	**FNM**	**SUS**	**UNIGE**	**UNILU**	**VUMC**	**Total**
AD	5	56	0	0	36	3	51	151
DLB	0	3	14	12	0	0	38	67
PD	0	46	0	0	0	29	0	75
Control	7	13	10	0	7	7	13	57
Total	12	118	24	12	43	39	102	350

Center abbreviations: AHUS = Akershus University Hospital (Norway); DZNE = Deutsches Zentrum für Neurodegenerative Erkrankungen (Germany); FNM = Motol University Hospital (Czech Republic); SUS = Stavanger University Hospital (Norway); UNIGE = University of Geneva (Switzerland); UNILU = University of Luxembourg (Luxembourg); VUMC = VU University Medical Center Amsterdam (The Netherlands).

1.2. Laboratory analyses

Concentrations for the ATN markers were obtained. Five of the CSF samples were above the measuring range of Aß42. Five different CSF samples were below the measuring range of p-tau. One CSF sample was above the measuring range of NfL. There were two CSF samples that had inadequate sample volume for all assays.

1.3. Data harmonization

Clinical data were harmonized and merged with the CS1 sample results via the unblinded sample lists by WP4 (UM), and a merged dataset was obtained ("CS1 dataset"). Clinical data structure, types, and availability varied widely across cohorts, posing a significant challenge for harmonization. Interaction with centers was often needed to clarify aspects around the data they provided (e.g., missing or incomplete data or metadata, incongruencies, foreign languages, data spread throughout multiple sources), leading to prolonged waiting times and significant efforts by WP4 (UM). Due to this complexity and related technical challenges, automation of the whole data harmonization process proved to be unfeasible (as explored by WP1). On the other hand, the format of CS1 sample generated data and unblinded sample lists was clear, thus the merging with the harmonized data was relatively straightforward. The harmonization exercise provided important insights and learnings that will inform future case studies (e.g., harmonization of CS3 data) and the design of future EPND platform services.

1.4. CS1 data analyses

Controls were defined as individuals with normal cognition and negative CSF Aß42/40 status. AD was defined as negative CSF Aß42/40 status without meeting clinical criteria of DLB or PD. The previously validated cutoffs of < 0.06 pg/mL for CSF Aß42/40 and of > 24 pg/mL for CSF p-tau181 (Blennow et al 2019) were used to determine AT marker positivity. Due to the novelty of plasma markers, Gaussian mixture models are currently being explored to delineate potential cutoffs for plasma AT markers.

Baseline sample characteristics are shown in Table 2.

Table 2. Baseline sample characteristics

	Control	AD	DLB	PD	Total sample
N	57	151	67	75	350
Age	62.8 (9.17)	69.4 (8.23)	70.2 (6.78)	62.9 (10.19)	67.1 (9.2)
Female sex (%)	23 (40%)	68 (45%)	16 (24%)	24 (32%)	131 (37%)
Education years	14.6 (3.3)	12.8 (3.4)	12.4 (3.2)	13.2 (3.5)	13.0 (3.4)
APOE-ε4 carriership (%)	5 (9%)	98 (65%)	28 (47%)	16 (25%)	147 (45%)
MMSE score	29.0 (1.10)	25.3 (3.9)	23.8 (3.9)	28.5 (1.7)	26.3 (3.8)
MDS-UPDRS-III score	2.9 (2.6)	6.0 (7.8)	20.7 (14.1)	31.7 (15.3)	25.5 (16.8)

Education years were available for N=346, APOE-ε4 carriership for N=333, MMSE score for N=342, MDS-UPDRS-III score for N=116. Values represent mean (SD) for continuous variables or N (%) for dichotomous variables.

Levels of CSF ATN and α-Syn in the different groups are shown in Figure 3. AD individuals showed lower CSF Aß42/40 levels compared to all other groups. DLB individuals displayed lower Aß42/40 levels than those with PD and controls, whereas levels of PD individuals did not differ from controls. CSF p-tau181 levels were higher in AD relative to all other groups, while no differences were observed between DLB, PD and controls. CSF NfL levels were higher in AD and PD compared to controls, whereas no differences were found between AD, PD, and DLB. These latter results were significantly influenced by global cognitive performance. Finally, no differences between groups were observed in CSF α-Syn levels.

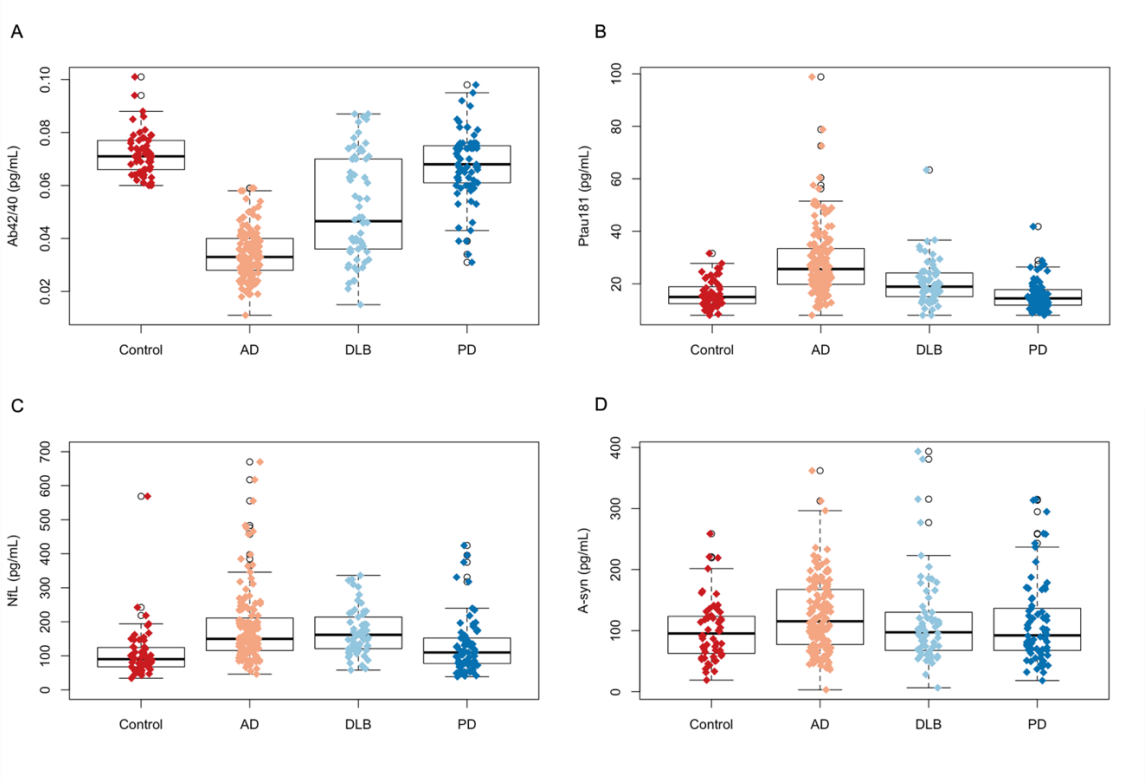

Figure 3. CSF ATN and α-Syn levels in AD, DLB and PD and controls.

61% of DLB and 20% of PD were amyloid-positive in CSF. Amyloid-positive and -negative DLB and PD individuals showed similar levels of CSF α-Syn and NfL. Compared to amyloid-negative ones, amyloid-positive DLB and PD individuals were older, and more often APOE-e4 carriers (a trend in PD). No differences between amyloid-positive and -negative DLB and PD individuals were observed in sex, MMSE/MoCA and motor function.

57% of AD, 26% of DLB, 9% of PD, and 9% of controls were tau-positive in CSF. Within all groups, tau-positive individuals had lower levels of CSF α-Syn than tau-negative ones (Figure 4). Compared to tau-negative ones, tau-positive AD and DLB individuals had higher levels of CSF NfL. Tau-positive DLB individuals were older than tau-negative ones, and tau-positive AD individuals had a higher MMSE/MoCA than tau-negative ones. No differences between tau-positive and -negative individuals were observed in sex, APOE-e4 carriership, and motor function (latter only tested for DLB and PD groups).

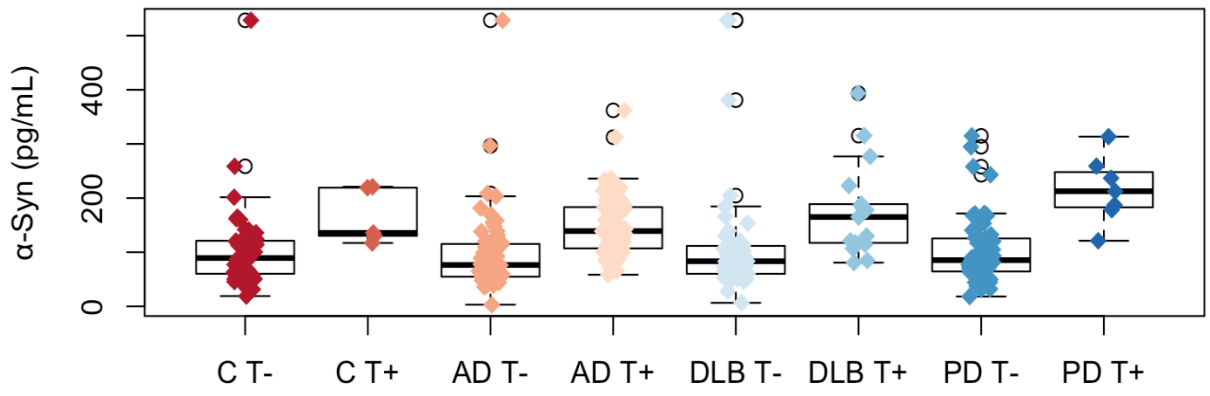

Figure 4. CSF α-Syn levels in CSF p-tau181 negative (T-) and positive (T+) controls (C), AD, DLB, and PD individuals.

Initial results of correlation analysis between CSF and plasma ATN markers are shown in Figure 5. Aβ42/40, p-tau181 and NfL in CSF and plasma showed a correlation of 0.60 for Aβ42/40, 0.55 for p-tau181, and 0.69 for NfL.

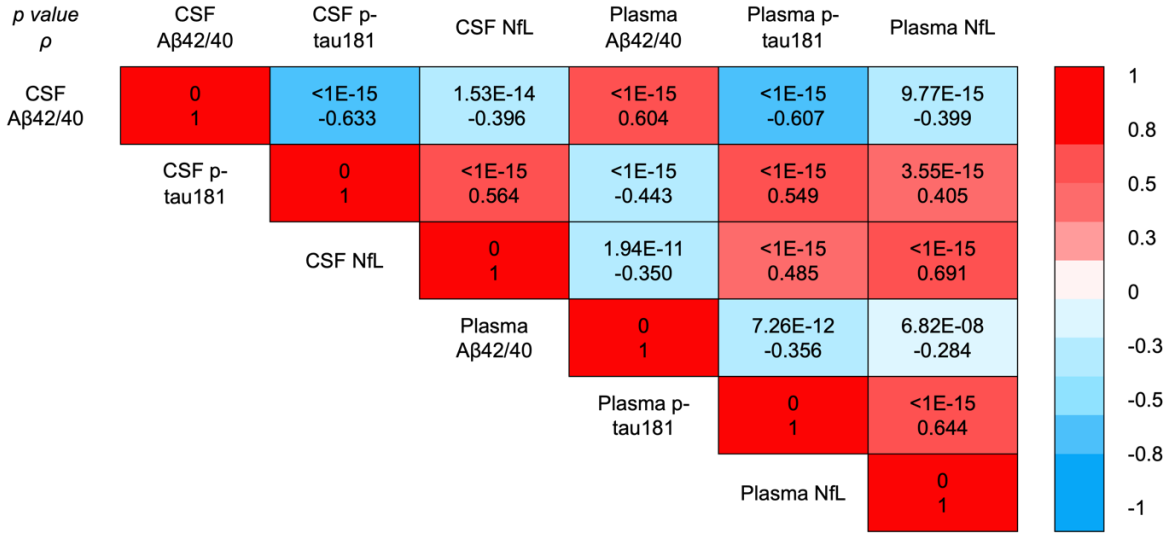

Figure 5. Spearman's correlation of CSF and plasma ATN markers. P values and ρ are reported for each comparison.

1.5. Making CS1 results available for reuse

CS1 metadata were pre-loaded in the EPND catalogue. Legal agreements between UM and ADDI were arranged and CS1 data are now available on the EPND platform AD Workbench. Procedures for permissioning of CS1 data are currently being put in place. While awaiting cohort approval, CS1 data were concurrently uploaded into the Maastricht Data Repository for storage, in collaboration with WP1 (UM).

2. Plasma Janssen pTau217 assay

pTau217 was successfully measured in 997 plasma samples of patients with AD or control from the Amsterdam Dementia cohort, as indicated in Table 3. Initial ROC-curve analysis of accuracy for detection of amyloid positivity, as defined by CSF pTau181/amyloidbeta42 or amyloid PET analysis was performed. The data in Figure 6 show a higher AUC (0.92) for plasma pTau217 compared to other plasma biomarkers measured in the same samples, such as pTau181 (AUC 0.84), GFAP (AUC 0.78), abeta42/40 (AUC 0.75) and NfL (AUC 0.59).

Table 3. Demographics of patients included in the plasma pTau217 biomarker analyses

	Amyloid negative n=469 (47)	**Amyloid positive n=528 (53)**
Age (mean ± SD in years)	61 ± 8	66 ± 7
Female sex (n, %)	172 (37%)	264 (50%)
MMSE (mean ± SD)	26 ± 4	23 ± 5
Plasma pTau217 (mean ± SD in pg/mL)	0.04 ± 0.02	0.11 ± 0.07

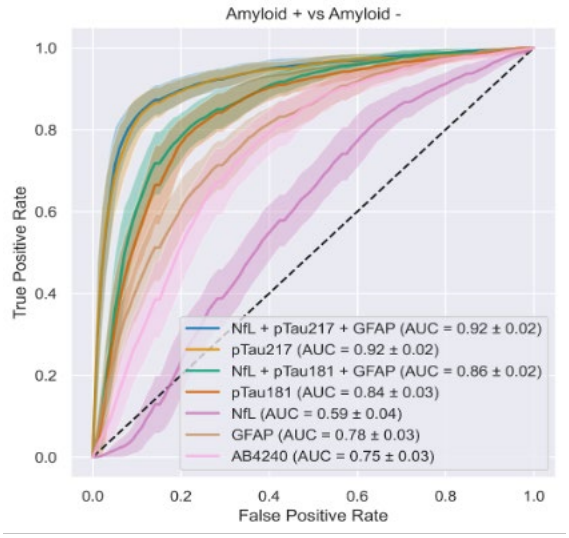

Figure 6. AUC curve analysis of plasma pTau217 compared and combined with other AD relevant plasma biomarkers.

Discussion

We successfully operationalized key aspects of the EPND platform and could generate new insights into neurodegenerative disorders through large-scale analyses.

We obtained the CS1 dataset and established the framework for its reuse by external researchers. By virtue of pooling multicenter data, we were able to observe overlapping pathologies in AD, DLB and PD in key CSF markers, and a good correlation between CSF and plasma modalities. This is relevant for disease pathophysiology and the design of clinical trials.

While centres were interested in participating in CS1, data and sample collection was a time-consuming process, mainly due to the lengthy timelines associated with the inclusion of third-party centres for the rarer DLB cases. As the EPND platform was still in the initial stages of development, its full desired functionalities could not be tested, including for example automated tools for data annotation or tools for central coordination of logistics. Together, this has led to a significant delay of this deliverable.

The learnings from CS1 are being used to shorten timelines of newer case studies (e.g., CS3, CS5), although some processes are expected to remain time consuming, due to different characteristics and organizations of European centres that are independent of EPND, as well as the often lengthy timelines of legal teams reviews. Insights from CS1 are also being used to define services that EPND can offer which ensure both meaningfulness and feasibility. These will significantly contribute to streamline and thus promote processes for multicentre research, which will allow crucial insights into neurodegenerative disorders.

Conclusion

We successfully operationalized key aspects of the EPND platform and could generate new insights on neurodegenerative diseases though large-scale analyses. Learnings from this case study will serve future studies using the EPND platform. As the platform continues to develop, future case studies will be able to test its novel functionalities.

Repository for primary data (if applicable)

UM servers, Maastricht Data Repository, EPND platform, ADWB