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Elecsys® Total-Tau and Phospho-Tau (181P) CSF assays: Analytical performance of the novel, fully automated immunoassays for quantification of tau proteins in human cerebrospinal fluid

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ABSTRACT

Background: Total tau (tTau) and phosphorylated 181P tau (pTau) are supportive diagnostic cerebrospinal fluid (CSF) biomarkers for Alzheimer's disease. Manual CSF tau assays are limited by lot-to-lot and between-laboratory variability and long incubation/turnaround times. Elecsys® Total-Tau CSF and Phospho-Tau (181P) CSF immunoassays were developed for fully automated **cobas e** analyzers, allowing broader access in clinical practice and trials.

Methods: Analytical performance, reproducibility, method comparisons with commercially available assays, and lot-to-lot and platform comparability (**cobas e** 601/411) of the Elecsys® CSF assays were assessed. Tau distributions and concentration ranges were evaluated in CSF samples from two clinical cohorts.

Results: Both assays showed high sensitivity (limit of quantitation [LoQ]: 63 pg/mL [tTau]; 4 pg/mL [pTau]) and linearity over the measuring range (80–1300 pg/mL; 8–120 pg/mL), which covered the entire concentration

Abbreviations: Aβ42, amyloid-β (1–42); AD, Alzheimer's disease; ADNI, Alzheimer's Disease Neuroimaging Initiative; ANOVA, analysis of variance; CLSI, Clinical and Laboratory Standards Institute; CN, cognitively normal; CSF, cerebrospinal fluid; CV, coefficient of variation; ELISA, enzyme-linked immunosorbent assay; FDA, US Food and Drug Administration; HPLC, high-performance liquid chromatography; Ig, immunoglobulin; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LoB, limit of blank; LoD, limit of detection; LoQ, limit of quantitation; MCI, mild cognitive impairment; MCS, mild cognitive symptoms; NC, normal cognition; NIST, National Institute of Standards and Technology; PET, positron emission tomography; pTau, phosphorylated 181P tau; QC, quality control; SCD, subjective cognitive decline; SD, standard deviation; SMC, significant memory concern; tTau, total tau; UPenn, University of Pennsylvania

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range measured in clinical samples. Lot-to-lot and platform comparability demonstrated good consistency (Pearson's *r*: 0.998; 1.000). Multicenter evaluation coefficients of variation (CVs): repeatability, < 1.8%; intermediate precision, < 2.8%; between-laboratory variability, < 2.7% (both assays); and total reproducibility, < 6.7% (tTau) and < 4.7% (pTau). Elecsys® CSF assays demonstrated good correlation with commercially available tau assays.

Conclusions: Elecsys® Total-Tau CSF and Phospho-Tau (181P) CSF assays demonstrate good analytical performance with clinically relevant measuring ranges; data support their use in clinical trials and practice.

1. Introduction

Accumulation of amyloid plaques, and neurofibrillary tangles composed of hyperphosphorylated tau protein, are pathological hallmarks of Alzheimer's disease (AD) [1–7]. Tau phosphorylation and aggregation lead to neuronal dysfunction and neuronal death, with neurofibrillary tangle density correlating with severity of cognitive deterioration in patients with mild cognitive impairment or AD [6,7]. Due to its role in neurodegenerative processes, tau may have clinical utility as a diagnostic biomarker and therapeutic target. Disease-modifying therapies designed to target tau pathology are currently in development, including monoclonal antibodies in ongoing phase II and III studies [8], and small molecules that inhibit tau phosphorylation or aggregation [8]. Potential availability of anti-tau therapies has generated a need for quick, reliable, low-cost, and widely available diagnostic methods to support accurate identification of patients with tau pathology [2,9,10].

In clinical trials, cerebrospinal fluid (CSF) tau concentration is often used to characterize neurodegeneration, and tau proteins may be used as a basis for patient recruitment and prediction or monitoring of anti-tau treatment response. This aligns with National Institute on Aging and Alzheimer's Association guidelines, which recommend use of genetic risk and biomarkers to complement neuropathological data for AD diagnosis [11]. Additionally, the International Working Group for New Research Criteria for the Diagnosis of AD advise that amyloid-β (1–42; Aβ42) and tau (total tau [tTau] or phosphorylated [181P] tau [pTau]) should be used in combination, and the CSF AD signature, which combines low amyloid-β (1–42) and high tTau or pTau concentrations, significantly increases diagnostic accuracy [12]. CSF measurements of tTau and pTau are used in clinical practice as supportive biomarkers for AD diagnosis, and have demonstrated potential clinical utility in AD dementia, prodromal AD, and differential diagnosis of dementias [9]. Assessment of tau pathology alone and in combination with amyloid pathology has been demonstrated to aid AD diagnosis and predict the risk of cognitive decline [13]. CSF biomarkers offer potential advantages compared with imaging techniques for the assessment of neurodegenerative disease pathology, including the ability to measure multiple analytes reflecting distinct pathological hallmarks in the same sample [14]. CSF biomarkers and imaging techniques are likely synergistic and reflect different aspects of AD pathophysiology. CSF pTau is a disease-state marker for the intensity of neurodegeneration and tau pathology, whereas imaging techniques are disease-stage markers for neurodegeneration and tau pathology [15–17]. Tau positron-emission tomography (PET) tracers are currently in clinical development, but so far none have received US Food and Drug Administration (FDA) approval.

The utility of CSF biomarker assays based on the commonly used enzyme-linked immunosorbent assay (ELISA) approach is limited by high variability in measured analyte concentrations in a given sample [14,18], especially across laboratories and between batches of plates and reagents. This variability is partly due to assay-related factors, but manual assays are also associated with significant between-laboratory variability, due to differences in laboratory procedures and analytical techniques [14,18–22]. Thus, there is a need to improve quality control (QC) standards in assay production, to ensure low overall calibration variability and strict variability limits across lots. Improved standardization and value assignment processes have been established to minimize lot-to-lot variation for ELISAs [18,23,24].

Two novel, fully automated electrochemiluminescence immunoassays (for use on **cobas** analyzers) have been developed for the quantitation of tTau and pTau in CSF: the Elecsys® Total-Tau CSF and Elecsys® Phospho-Tau (181P) CSF assays (hereafter referred to as Elecsys® tTau CSF and Elecsys® pTau CSF assays). The aim of this study was to evaluate the analytical performance of the Elecsys® tTau and pTau CSF assays and to assess correlation with commercially available assays for the quantification of CSF tau proteins.

2. Materials and methods

Further details of materials and methods relevant to each subsection are provided in the online Supplementary Material.

2.1. Materials

2.1.1. Elecsys® tTau and pTau CSF immunoassays

The Elecsys® tTau and pTau CSF immunoassays are intended for the quantitative determination of tTau and pTau in human CSF, on the **cobas e** 601 and **cobas e** 411 analyzers. Each assay uses monoclonal antibodies (see Supplementary Material for epitopes) in a sandwich format, and has a total run duration of 18 min. Both immunoassays were standardized gravimetrically with weighted in-house tTau and pTau reference materials.

Recalibration frequency (2-point calibration curve) is every 4 weeks when using the same reagent lot and every 7 days when using the same reagent kept on the analyzer.

2.1.2. CSF samples

CSF samples for design verification and validation experiments were obtained from leftover samples purchased from commercial vendors; samples used for method comparisons are described in the relevant subsection. Study protocols for each external study were approved by the local ethics committee/Institutional Review Board according to institutional guidelines, and patients provided written informed consent to participate.

2.2. Methods

Four separate production lots (A, B, C, and D) of each of the final Elecsys® tTau and pTau CSF immunoassay compositions were produced and evaluated for analytical performance, according to design control and commercialization and Clinical and Laboratory Standards Institute (CLSI) guidelines. For experiments conducted at Roche Diagnostics GmbH, Penzberg, Germany, acceptance criteria were: 100 ± 10% for tTau ≥ 250 pg/mL and pTau ≥ 25 pg/mL; ± 25 pg/mL for tTau < 250 pg/mL; and ± 3 pg/mL for pTau < 25 pg/mL.

2.2.1. Limits, linearity, and high-dose hook effect

Limit of blank (LoB), limit of detection (LoD), and limit of quantitation (LoQ) were determined according to CLSI EP17-A2.

Linearity was determined according to CLSI EP06-A, and assessed using three different reagent lots and a dilution series of three individual CSF samples with analyte concentrations slightly above the

measuring range.

Additional analyses assessed whether any false-low results due to high-dose hook effect occurred up to an analyte concentration > 3-fold higher than the upper measuring range (tTau, > 4800 pg/mL; pTau, > 450 pg/mL).

2.2.2. Interference and cross-reactivity

The potential for interference by endogenous substances and drugs with Elecsys® tTau and pTau CSF assays was tested according to CLSI EP07-A2 and I/LA30-A with human CSF pools in the presence/absence of potentially interfering substances.

The potential for cross-reactivity of the Elecsys® pTau CSF assay with non-phosphorylated tau peptide was tested according to CLSI EP07-A2.

2.2.3. Stability of reagent, calibration, and sample

Reagent and calibration stability were assessed according to CLSI EP25-A by storing reagents at 2–8 °C, 20–25 °C, and 35 °C for varying durations up to 24 months.

Sample stability experiments were performed using at least once-frozen CSF sample pools (−80 ± 10 °C) under the following storage conditions: at room temperature, 20–25 °C (8, 24, 120 h), 2–8 °C (1, 7, 14 days), −20 ± 5 °C (3 months), −80 ± 10 °C (6, 12 months), and upon freezing/thawing (three cycles).

2.2.4. Lot-to-lot and platform comparability

For each assay at one site (Roche Diagnostics GmbH, Penzberg, Germany), the comparability of four reagent production lots, A, B, C, and D (A vs B, A vs C, and C vs D), and platform comparability (**cobas e** 601 vs **cobas e** 411, measured using one lot), were tested according to CLSI EP09. A series of individual frozen CSF samples, with tTau and pTau concentrations covering the entire measuring range, was assessed with each reagent lot (n > 130; A, B, C, and D) or platform (n > 95; **cobas e** 601 and **cobas e** 411). Bias between lots/platforms was investigated using Passing-Bablok regression. Correlation was assessed using Pearson's and Spearman's correlation coefficients.

2.2.5. Precision: repeatability (within-run) and intermediate (within-laboratory)

Precision was assessed according to CLSI EP05-A3 using two artificial samples (controls) and at least six human CSF sample pools. Intermediate precision for the **cobas e** 601 and **cobas e** 411 analyzers was assessed at one site (Roche Diagnostics GmbH, Penzberg, Germany) over 21 days (two runs per day, two replicates per run), using one reagent lot. For each sample, variance components (within-run, between-run, between-day, and intermediate within-laboratory) were calculated as coefficient of variation (CV) (%) according to CLSI-EP05, using the analysis of variance (ANOVA) Type 1 approach for unbalanced data.

Inter-module (instrument-to-instrument) precision was evaluated at one site (Roche Diagnostics GmbH, Penzberg, Germany) on four **cobas e** 601 analyzers (one run on both measuring cells of each instrument, using two artificial samples [controls] and five human CSF sample pools). For each sample, CV (%) was calculated.

2.2.6. Reproducibility (multicenter evaluation study)

The multicenter performance evaluation study investigated reproducibility of the Elecsys® tTau and pTau CSF assays for different sites and lots, at three sites: Amsterdam University Medical Center, Vrije Universiteit, Amsterdam, The Netherlands; Washington University, St Louis, MO, USA; and University of Maryland School of Medicine, Baltimore, MD, USA. The study was designed and performed in accordance with CLSI-EP05. Three different assay lots (A, C, and D) were used; two reagent lots were run at each site. At least five different human CSF sample pools covering the measuring range of the assays and two artificial samples (controls) were analyzed. Each sample was measured at each site over 5 days with one run per day, five repetitions per run using two lots; variance components (within-run, between-day, within-laboratory, between-laboratory, lot-to-lot) and total reproducibility were calculated as CV (%).

2.2.7. Method comparisons with commercially available assays for quantification of tau proteins in CSF

Several method comparison experiments were performed at Roche Diagnostics GmbH, Penzberg, Germany (details in online Supplementary Material). Method comparisons performed at external sites were retrospective analyses of routine leftover patient samples. Details are described below.

The Elecsys® tTau CSF assay was compared against INNOTEST® hTAU Ag (116 samples, collected and measured at University Hospital Ulm [25–27]), INNO-BIA AlzBio3 tau (233 samples, collected and measured at University of Pennsylvania [UPenn]), and EUROIMMUN ADx Total-Tau ELISA (49 samples, from the AIBL study [28]). EUROIMMUN ADx ELISA measurements in samples from AIBL were known from previous studies. Elecsys® measurements were performed at Sahlgrenska Academy, University of Gothenburg.

The Elecsys® pTau CSF assay was compared against INNOTEST® PHOSPHO-TAU(181P) (110 samples, collected and measured at University Hospital Ulm) and INNO-BIA AlzBio3 P-tau(181P) (228 samples, collected and measured at UPenn). All assays were used according to manufacturer's instructions.

All samples were measured over ≥4 days (except AIBL samples, which were measured on 2 days) under routine conditions. Bias between measurements obtained with Elecsys® tTau and pTau CSF assays and commercially available tau assays was investigated using weighted Deming regression. Correlation was assessed using Pearson's and Spearman's coefficients.

2.2.8. Distribution and concentration range of CSF tTau and pTau measured in clinical CSF samples from two different cohorts

tTau and pTau concentrations were measured in a subset of retrospective CSF samples from the Alzheimer's Disease Neuroimaging Initiative (ADNI) and BioFINDER cohorts using the Elecsys® tTau and pTau CSF assays to determine the clinically relevant range.

3. Results

3.1. Limits, linearity, and high-dose hook effect

For the Elecsys® tTau CSF assay LoB, LoD, and LoQ were determined with lot A as 6.73, 18.6, and 62.6 pg/mL, respectively; these outperformed the prespecified values: 30, 60, and 80 pg/mL, respectively. For the Elecsys® pTau CSF assay LoB, LoD, and LoQ were determined with lot A as 1.55, 1.96, and 3.90 pg/mL, respectively, thereby outperforming the prespecified values: 4, 8, and 8 pg/mL, respectively. Variability at the LoQ was lower (tTau, 1.8%; pTau, 10.9%) than the prespecified acceptable CV (20%). Similar results were observed using two additional lots for each assay.

Linearity was demonstrated throughout the entire measuring range of the Elecsys® tTau (80–1300 pg/mL) and pTau (8–120 pg/mL) CSF assays. Results were consistent across the three separate assay lots (see Supplementary Fig. S1, example lot).

No falsely low results due to high-dose hook effect were observed up to 4834 pg/mL for tTau and up to 459 pg/mL for pTau, exceeding the upper measuring range of the Elecsys® tTau and pTau CSF assays by approximately fourfold (Supplementary Fig. S2). Results were reproduced for two further lots of each assay.

3.2. Interference and cross-reactivity

No interference was observed by the endogenous compounds or drugs (including biotin), up to the concentrations tested (Supplementary Fig. S3).

No significant cross-reactivity was observed with the Elecsys® pTau CSF assay (< 0.3%) at 50-fold excess concentrations of non-phosphorylated tau (see Results section of Supplementary Material).

3.3. Stability of reagent, calibration, and sample

High reagent and calibration stability were observed (Supplementary Table S2).

tTau and pTau stability across a range of storage conditions and durations is shown in Fig. 1. Both analytes were stable (change in measured concentration within acceptable limits) in CSF for 5 days at 20–25 °C (a decline in stability was observed, but recovery remained within limits), 14 days at 2–8 °C, 3 months at −20 ± 5 °C, 12 months at −80 ± 10 °C, and after three freeze/thaw cycles at −20 ± 5 °C.

3.4. Lot-to-lot and platform comparability

High lot-to-lot comparability in terms of correlation (Pearson's *r*, > 0.998 for all comparisons) and bias was observed between all four lots for both the Elecsys® tTau and pTau CSF assays (Fig. 2). The estimates of slope and percentage bias at medical decision point (tTau: 300 pg/mL and pTau: 27 pg/mL) were 0.961 and − 0.1%, respectively (tTau) and 0.996 and − 1.7%, respectively (pTau). Similar results were observed for all other pairwise lot-to-lot comparisons.

Good platform comparability was observed between the **cobas e** 601 and **cobas e** 411 analyzers for both assays (Pearson's *r*, 1.000; Supplementary Fig. S4). Biases at the medical decision point were 3.2% (tTau) and − 0.7% (pTau).

3.5. Precision

High within-run, between-run, between-day, and intermediate precision were observed on the **cobas e** 601 analyzer; for human CSF pools, intermediate precision was ≤ 2.0% for tTau and ≤ 3.5% for pTau (Table 1). Estimated between-instrument variability was ≤ 2.4% for tTau and ≤ 2.6% for pTau (data not shown). On the **cobas e** 411 analyzer, intermediate precision was slightly lower (tTau, ≤ 3.4%; pTau, ≤ 6.7%) (Table 1). Precision was also assessed in three CSF samples (measured over 5 days with three repetitions per day) at UPenn; intermediate precision was < 2.7% for the Elecsys® tTau and < 2.0% for the Elecsys® pTau CSF assay in all samples. Using INNO-BIA AlzBio3, intermediate precision was < 6.1% for tTau and 11–56% for pTau.

3.6. Reproducibility

In the multicenter evaluation study, high within-run and between-day precision were observed. For pooled human CSF samples, CV values for within-run precision were < 1.8%, and for between-day precision < 2.3%, for both assays (Table 2). High between-lab consistency (tTau, < 1.6%; pTau, < 2.6%) and good lot-to-lot variability (tTau, < 5.9%; pTau, < 3.1%) were observed. Estimated CV for total reproducibility were < 6.7% (Elecsys® tTau CSF assay) and < 4.7% (Elecsys® pTau CSF assay).

3.7. Method comparisons with available assays for quantification of tau proteins in CSF

Elecsys® Tau CSF assay (**cobas e** 601) results were well correlated with INNOTEST/Fujirebio ELISA measurements for tTau (weighted Deming regression: y = 0.45× + 58.46; Pearson's *r*, 0.941) and pTau (y = 0.38× + 0.84; Pearson's *r*, 0.940; Fig. 3). Similar results were observed in in-house design verification experiments (Pearson's *r*, Elecsys® tTau CSF vs INNOTEST® hTAU Ag, 0.963; Elecsys® pTau CSF vs

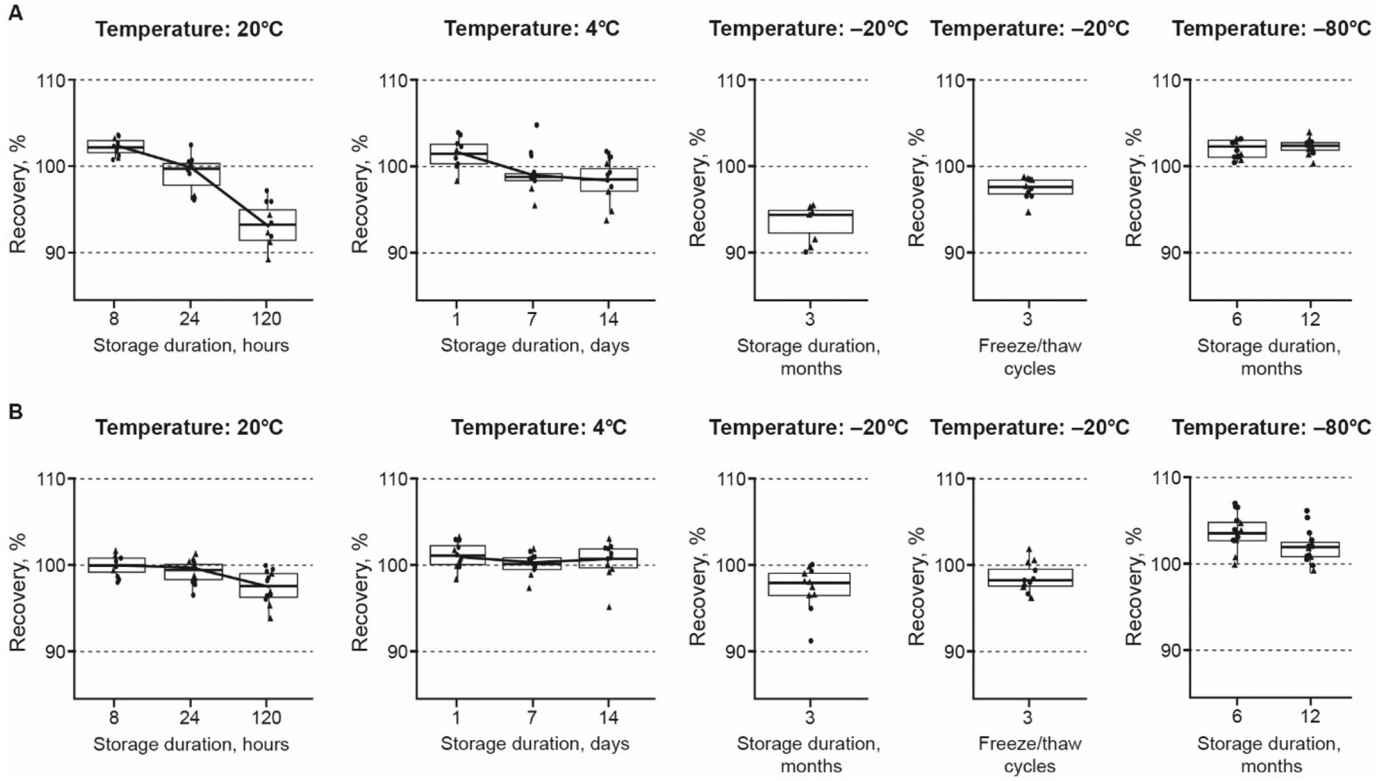

Fig. 1. Sample stability of tTau and pTau measured with the (A) Elecsys® tTau CSF and (B) Elecsys® pTau CSF assays, assessed by recovery after storage at 20–25 °C, 2–8 °C, −20 °C (± 5 °C), and − 80 °C (± 10 °C) and after repeated freeze/thaw cycles. Circles correspond to samples with baseline concentration ≥ 250 pg/mL (tTau) and ≥ 25 pg/mL (pTau). Triangles correspond to the samples with lower concentrations.
pTau, phosphorylated 181P tau; tTau, total tau.

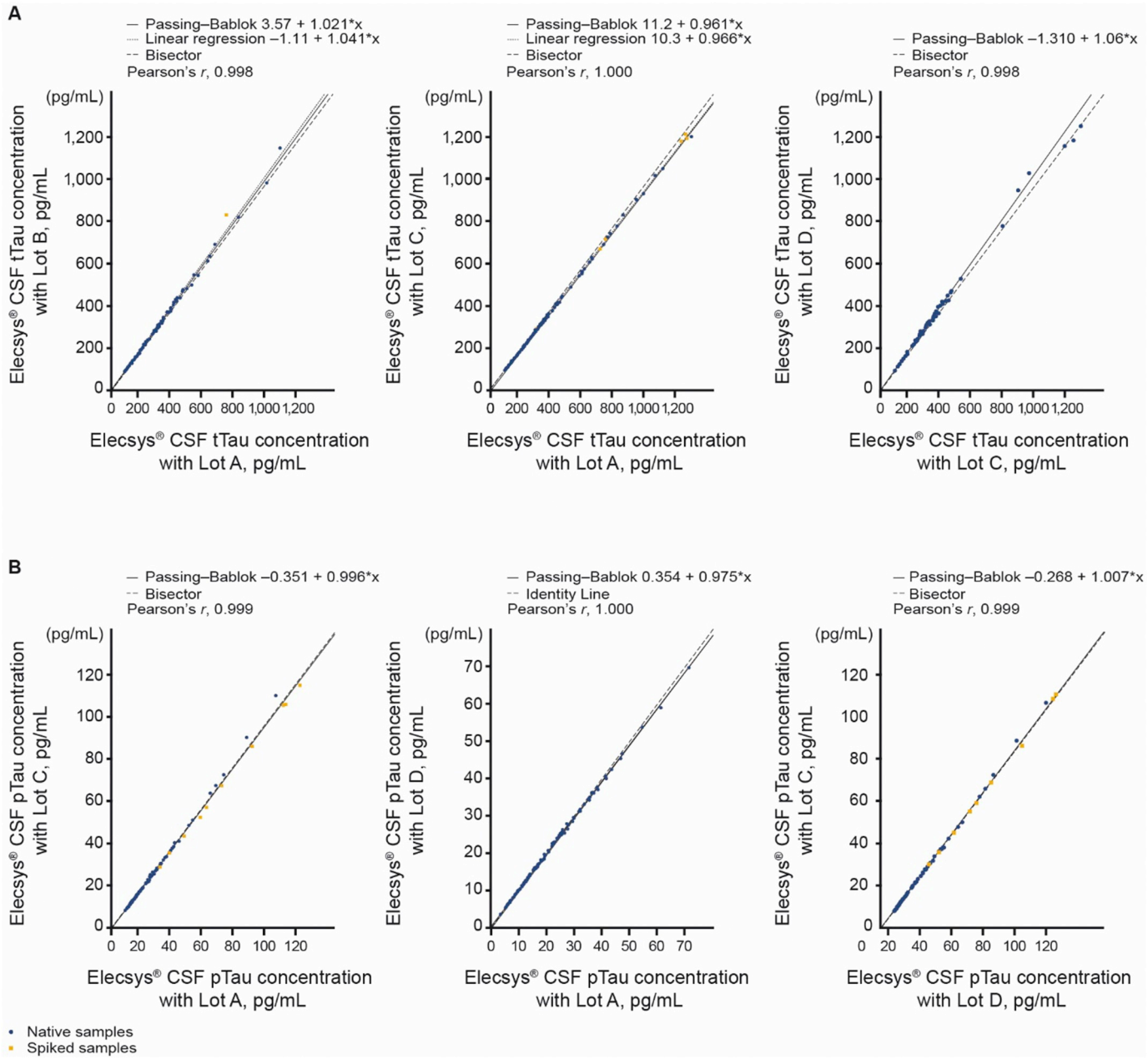

Fig. 2. Lot-to-lot comparability of the (A) Elecsys® tTau CSF and (B) Elecsys® pTau CSF assays assessed on the **cobas e** 601 analyzer, by Passing–Bablok regression.

INNOTEST® PHOSPHO-TAU(181P), 0.979); data not shown.

High correlation and linearity were observed between tTau measurements obtained using Elecsys® tTau CSF and EUROIMMUN Total-Tau ELISA (weighted Deming regression: 0.54× + 17.21; Pearson's *r*, 0.933; Spearman's ρ, 0.90; Fig. 3). Elecsys® tTau CSF measurements were also highly correlated with INNO-BIA AlzBio3/Fujirebio Luminex, but were slightly nonlinear (weighted Deming regression: 2.76× + 47.51; Pearson's *r*, 0.931; Spearman's ρ, 0.94). Poor correlation was observed between Elecsys® pTau CSF and INNO-BIA AlzBio3/Fujirebio Luminex pTau measurements (Pearson's *r*, 0.458; Spearman's ρ, 0.38).

3.8. Distribution of CSF tTau and pTau concentrations in the ADNI and BioFINDER cohorts

Measuring ranges of Elecsys® tTau and pTau CSF assays completely covered the clinically relevant ranges, as demonstrated by the distribution of measured CSF tTau and pTau concentrations in the BioFINDER and ADNI cohorts (see Supplementary Material, including Supplementary Table S3 and Supplementary Fig. S5).

4. Discussion

There is a need for accurate and robust assays to measure CSF tau protein concentrations. This is driven by the development of potential treatment options for AD targeting tau pathology, recommendations for using CSF biomarkers to support AD diagnosis [12], and a lack of precise assays with low lot-to-lot variability [11]. The Elecsys® tTau and pTau CSF assays are novel, fully automated electrochemiluminescence immunoassays for the quantitation of CSF tTau and pTau phosphorylated at threonine 181. We demonstrated that the Elecsys® tTau and pTau CSF assays are sensitive, precise, and have broad measuring ranges that cover clinically relevant concentration ranges measured in CSF samples from two clinical cohorts.

Table 1
Precision of the Elecsys® tTau and pTau CSF assays[a] on the **cobas e** 601 and **cobas e** 411 analyzers (CLSI EP05-A3; 21 days).

Elecsys® tTau CSF assay						Elecsys® pTau CSF assay					
Sample	Mean, pg/mL	Precision, CV (%)				Sample	Mean, pg/mL	Precision, CV (%)			
		Within-run	Between-run	Between-day	Intermediate			Within-run	Between-run	Between-day	Intermediate
cobas e 601 analyzer						**cobas e** 601 analyzer					
Control						Control					
1	167	1.1	1.2	1.4	2.1	1	13.5	1.8	0.6	1.3	2.3
2	456	0.7	0.8	0.8	1.4	2	49.2	2.1	0.7	2.0	3.0
Human CSF						Human CSF					
1	93.3	1.2	0.4	1.2	1.7	1	10.1	2.7	1.0	1.9	3.5
2	151	0.9	0.8	0.9	1.5	2	17.6	1.8	1.1	1.6	2.6
3	220	0.9	1.2	1.2	2.0	3	25.1	2.3	0.0	0.9	2.5
4	272	0.9	0.9	1.0	1.6	4	30.1	1.5	0.8	0.8	1.9
5	341	0.9	0.8	1.1	1.7	5	58.7	1.9	0.0	1.5	2.4
6	414	0.8	1.0	1.0	1.6	6	108	2.2	1.7	1.6	3.2
7	626	0.7	0.7	1.1	1.5	7	116	2.1	1.1	0.4	2.4
8	717	1.0	0.6	1.0	1.6						
9	1044	0.7	0.7	1.0	1.4						
10	1192	1.0	0.5	1.2	1.6						
cobas e 411 analyzer						**cobas e** 411 analyzer					
Control						Control					
1	211	1.2	2.1	2.6	3.5	1	12.2	1.1	1.5	1.4	2.3
2	481	1.3	2.1	1.7	2.9	2	46.7	1.6	1.5	2.4	3.3
Human CSF						Human CSF					
1	132	2.7	1.4	0.7	3.1	1	9.77	5.8	0.0	1.2	6.0
2	236	1.0	1.8	1.3	2.4	2	17.5	5.4	0.0	0.0	5.4
3	280	1.4	1.9	1.0	2.6	3	25.6	1.4	1.0	1.3	2.1
4	325	2.3	0.4	2.1	3.1	4	29.3	1.0	0.9	1.7	2.2
5	771	1.1	1.6	0.9	2.2	5	57.4	6.0	3.0	0.4	6.7
6	1197	1.4	1.8	2.4	3.4	6	110	2.8	1.9	1.0	3.5

CLSI, Clinical and Laboratory Standards Institute; CSF, cerebrospinal fluid; CV, coefficient of variation; pTau, phosphorylated 181P tau; tTau, total tau.
[a] For both assays, two samples in the cutoff area were measured (tTau, 300 pg/mL; pTau, 27 pg/mL).

Table 2
Multicenter evaluation of the precision and reproducibility of the Elecsys® tTau and pTau CSF assays on the **cobas e** 601 analyzer.

Sample	Mean, pg/mL	Repeatability (within-run) (CV, %)	Between-day	Intermediate precision	Between-laboratory	Lot-to-lot	Total reproducibility
Elecsys® tTau CSF assay							
Control							
1	172	1.2	2.0	2.3	2.7	5.0	6.1
2	450	1.2	1.9	2.2	2.2	4.3	5.3
Human CSF							
1	211	1.5	2.3	2.7	0.9	5.8	6.4
2	289	1.6	2.2	2.7	1.1	5.6	6.3
3	414	1.3	2.0	2.4	1.0	5.4	6.0
4	638	1.7	2.1	2.8	1.1	5.6	6.4
5	1084	1.8	2.0	2.7	1.6	5.9	6.7
Elecsys® pTau CSF assay							
Control							
1	14.2	1.6	1.3	2.0	2.2	3.3	4.5
2	33.2	1.3	1.5	2.0	2.4	2.1	3.7
Human CSF							
1	19.8	1.4	1.9	2.3	1.1	1.8	3.1
2	28.8	1.3	2.0	2.4	1.4	2.0	3.4
3	40.7	1.4	2.2	2.6	2.5	2.8	4.6
4	61.9	1.8	1.6	2.4	2.4	3.1	4.5
5	102	1.5	2.1	2.6	2.5	3.0	4.7
6	109	1.5	2.3	2.8	2.6	2.8	4.7

For both assays, one sample in the cutoff area was measured (tTau, 300 pg/mL; pTau, 27 pg/mL). CSF, cerebrospinal fluid; CV, coefficient of variation; pTau, phosphorylated 181P tau; tTau, total tau.

Elecsys® tTau and pTau CSF assays demonstrated good repeatability, intermediate precision, accuracy, and consistency between **cobas e** 601 and 411, as well as good total reproducibility across multiple sites on **cobas e** 601. High lot-to-lot consistency was demonstrated, due to the lot standardization process developed at Roche Diagnostics, which involves target values traceable to internal reference material. In contrast, CSF tau measurement using INNOTEST® hTAU Ag, INNOTEST® PHOSPHO-TAU(181P), INNO-BIA AlzBio3 P-tau(181P), and INNO-BIA AlzBio3 tau assays exhibits high lot-to-lot and between-laboratory variability [18, 29–31]. The Elecsys® tTau and pTau CSF assays have been evaluated in the Alzheimer's Association Quality Control Program [31] since 2017. During four rounds at seven laboratories (eight

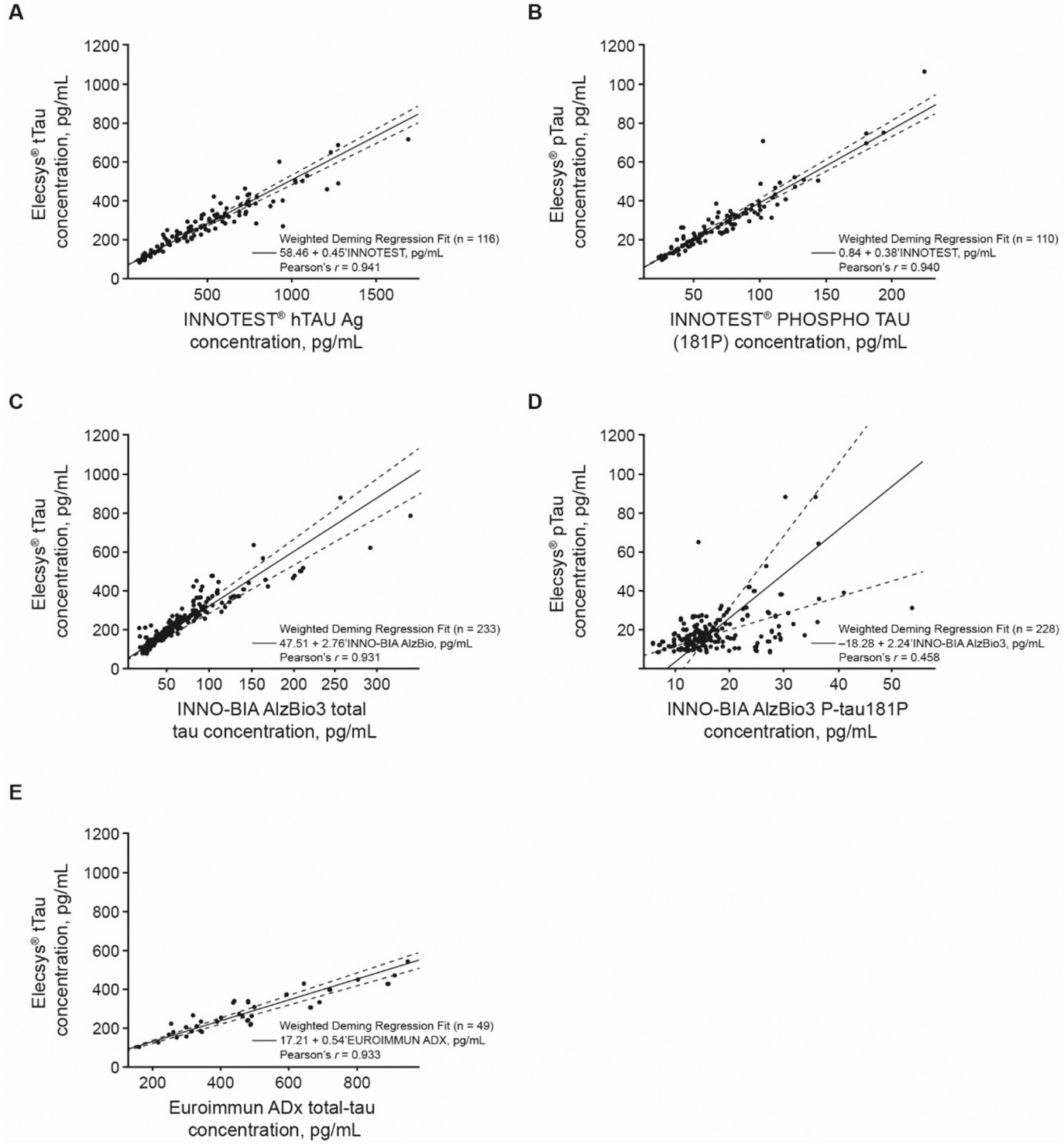

Fig. 3. Method comparison of (A) Elecsys® tTau CSF versus INNOTEST® hTAU Ag; (B) Elecsys® pTau CSF versus INNOTEST® PHOSPHO-TAU (181P); (C) Elecsys® tTau CSF versus INNO-BIA AlzBio3 tau; (D) Elecsys® pTau CSF versus INNO-BIA AlzBio3 P-tau(181P); (E) Elecsys® tTau CSF versus EUROIMMUN ADX Total-Tau ELISA, assessed by weighted Deming regression.

unique QC samples), between-laboratory CVs were 3.9% (tTau) and 2.1% (pTau), substantially lower than for the respective INNOTEST assays (tTau, 13.0%; pTau, 11.1%; data not shown).

High correlation was demonstrated between Elecsys® tTau and pTau CSF assays and commercially available tau assays, except between Elecsys® pTau CSF and INNO-BIA AlzBio3 P-tau(181P). This could be explained by the lower precision of INNO-BIA AlzBio3 P-tau(181P) and different binding properties of the antibodies used in each assay. Systematic deviations between assays are expected and can be explained by different reference standardization. For each method comparison, regression parameters were estimated using data from one site using one reagent lot per assay; results may therefore be biased and should be carefully interpreted.

No interference was observed at evaluated concentrations for the endogenous substances tested, including biotin. Very low (median, < 0.1 ng/mL; maximum, 1.43 ng/mL) biotin concentrations were

measured in > 280 CSF clinical leftover samples. A total of 99% measurements were < 0.28 ng/mL; thus, the current biotin threshold concentration for interference (50 ng/mL) exceeds the 99th percentile of the biotin distribution in these samples by > 170-fold.

Absence of a certified reference material or physicochemical reference method meant it was not possible to use such a material or method for standardization in this study. The lack of standardization of current assays to a certified reference material is a major unmet need in CSF tTau and pTau quantification [32]. Without standardization, the apparent concentration of biomarkers will likely vary between assays, leading to complications with interpretation and comparison of results between laboratories, and thereby preventing the introduction of universal cutoff concentrations to aid diagnosis of AD and other neurodegenerative diseases. A collaborative project is currently ongoing with the International Federation of Clinical Chemistry and Laboratory Medicine [33], to develop certified reference material for CSF tTau assays.

Limitations of this study include that data were generated using once-frozen CSF samples; results may therefore differ for fresh CSF samples, but no supporting evidence was found in fresh/frozen experiments. Furthermore, variation in preanalytical handling did not impact measured tTau and pTau concentrations [34]. This, along with high reagent and analyte stability, contributes to the robustness of the Elecsys® tTau and pTau CSF assays. Furthermore, lot-to-lot consistency data are currently available for four lots and demonstrated consistency within a 2-year period; longitudinal data are not yet available.

Strengths of the methodology include adherence to manufacturing standards and the Roche-specific standardization concept, with ~6000 determinations before the introduction of a new reagent lot, to ensure high lot-to-lot consistency and minimal batch variation. Additionally, robust reference standardization was applied to internally purified, highly characterized reference material, traceable to amino acid calibrators from the National Institute of Standards and Technology. Implementation of enhanced QC concept, where each laboratory establishes its own QC rules based on laboratory-specific bias and CV, ensured high precision and accuracy of measurements with Elecsys® tTau and pTau CSF assays.

The evaluation of CSF biomarkers has numerous advantages over imaging techniques in neurodegenerative disease diagnosis, including lower cost, wide availability, short testing time, multi-biomarker analysis from the same CSF sample, and longitudinal monitoring during disease development. Evaluation of CSF tTau or pTau in combination with CSF Aβ42 improves concordance of CSF Aβ42 with amyloid-PET, and predicts risk of clinical decline [10,35,36]. Development of the Elecsys® tTau and pTau CSF assays for use on fully automated **cobas** analyzers is a major advance from ELISAs in terms of laboratory workflow, due to substantially shorter incubation times per single sample, the possibility for random sample access, and higher throughput [29,37]. Elecsys® tTau and pTau CSF assays are widely available, and demonstrated a high consistency of results on both small- (**cobas e** 411) and mid-scale (**cobas e** 601) analyzers for laboratory-tailored throughput. The clinical applicability of Elecsys® tTau and pTau CSF assays has also been shown in BioFINDER and ADNI, two large independent cohorts of clinical samples [13].

5. Conclusion

The novel Elecsys® Total-Tau CSF and Elecsys® Phospho-Tau (181P) CSF assays demonstrated good analytical performance, with results showing high correlation with commercially available tau assays. Elecsys® tTau and pTau CSF assays will be beneficial to laboratories, clinicians, and patients due to their simple workflow and accurate, precise, and reproducible measurement of tau CSF biomarkers. Our findings support use of Elecsys® Tau CSF assays for determining tTau and pTau concentrations in clinical trials, and decision-making in clinical practice across a broad spectrum of neurodegenerative disorders.

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Disclosures

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clinbiochem.2019.05.005.

References

[1] I. Churcher, Tau therapeutic strategies for the treatment of Alzheimer's disease, Curr. Top. Med. Chem. 6 (2006) 579–595.
[2] D.M. Holtzman, M.C. Carrillo, J.A. Hendrix, L.J. Bain, A.M. Catafau, L.M. Gault, et al., Tau: from research to clinical development, Alzheimers Dement. 12 (2016) 1033–1039.
[3] Y. Huang, Z. Wu, B. Zhou, Behind the curtain of tauopathy: a show of multiple players orchestrating tau toxicity, Cell. Mol. Life Sci. 73 (2016) 1–21.
[4] M. Medina, F. Hernandez, J. Avila, New features about tau function and dysfunction, Biomolecules 6 6 (2) (2016) (pii:E21).
[5] E.J. Mufson, M.D. Ikonomovic, S.E. Counts, S.E. Perez, M. Malek-Ahmadi, S.W. Scheff, et al., Molecular and cellular pathophysiology of preclinical Alzheimer's disease, Behav. Brain Res. 311 (2016) 54–69.
[6] J.Z. Wang, Y.Y. Xia, I. Grundke-Iqbal, K. Iqbal, Abnormal hyperphosphorylation of tau: sites, regulation, and molecular mechanism of neurofibrillary degeneration, J. Alzheimers Dis. 33 (2013) S123–S139 Suppl. 1.
[7] P. Scheltens, K. Blennow, M.M. Breteler, B. de Strooper, G.B. Frisoni, S. Salloway, et al., Alzheimer's disease, Lancet 388 (2016) 505–517.
[8] J. Cummings, G. Lee, T. Mortsdorf, A. Ritter, K. Zhong, Alzheimer's disease drug development pipeline: 2017, Alzheimers Dement. 3 (2017) 367–384.
[9] K. Blennow, H. Hampel, M. Weiner, H. Zetterberg, Cerebrospinal fluid and plasma biomarkers in Alzheimer disease, Nat. Rev. Neurol. 6 (2010) 131–144.
[10] T.A. Pascoal, S. Mathotaarachchi, M. Shin, A.Y. Park, S. Mohades, A.L. Benedet, et al., Amyloid and tau signatures of brain metabolic decline in preclinical Alzheimer's disease, Eur. J. Nucl. Med. Mol. Imaging 45 (2018) 1021–1030.
[11] C.R. Jack, D.A. Bennett, K. Blennow, M.C. Carrillo, B. Dunn, S.B. Haeberlein, et al., NIA-AA research framework: toward a biological definition of Alzheimer's disease, Alzheimers Dement. 14 (2018) 535–562.
[12] B. Dubois, H.H. Feldman, C. Jacova, H. Hampel, J.L. Molinuevo, K. Blennow, et al., Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria, Lancet Neurol. 13 (2014) 614–629.
[13] O. Hansson, J. Seibyl, E. Stomrud, H. Zetterberg, J.Q. Trojanowski, T. Bittner, et al., CSF biomarkers of Alzheimer's disease concord with amyloid-beta PET and predict clinical progression: a study of fully automated immunoassays in BioFINDER and ADNI cohorts, Alzheimers Dement. 14 (2018) 1470–1481.
[14] S.J. Vos, P.J. Visser, F. Verhey, P. Aalten, D. Knol, I. Ramakers, et al., Variability of CSF Alzheimer's disease biomarkers: implications for clinical practice, PLoS One 9 (2014) e100784.
[15] K. Blennow, H. Hampel, CSF markers for incipient Alzheimer's disease, Lancet Neurol. 2 (2003) 605–613.
[16] N. Mattsson, M. Schöll, O. Strandberg, R. Smith, S. Palmqvist, P.S. Insel, et al., (18) FAV-1451 and CSF T-tau and P-tau as biomarkers in Alzheimer's disease, EMBO Mol. Med. 9 (2017) 1212–1223.
[17] M.W. Riepe, J. Karl, H. Tumani, C.A. von Arnim, Tau-proteins as gender-specific state markers in amnestic mild cognitive impairment, Dement. Geriatr. Cogn. Disord. 30 (2010) 93–100.
[18] N. Mattsson, U. Andreasson, S. Persson, M.C. Carrillo, S. Collins, S. Chalbot, et al., CSF biomarker variability in the Alzheimer's Association quality control program, Alzheimers Dement. 9 (2013) 251–261.
[19] L.M. Shaw, H. Vanderstichele, M. Knapik-Czajka, M. Figurski, E. Coart, K. Blennow, et al., Qualification of the analytical and clinical performance of CSF biomarker analyses in ADNI, Acta Neuropathol. 121 (2011) 597–609.
[20] T. Bittner, H. Zetterberg, C.E. Teunissen, R.E. Ostlund Jr., M. Militello, U. Andreasson, et al., Technical performance of a novel, fully automated electrochemiluminescence immunoassay for the quantitation of beta-amyloid (1-42) in human cerebrospinal fluid, Alzheimers Dement. 12 (2016) 517–526.
[21] M.C. Carrillo, K. Blennow, H. Soares, P. Lewczuk, N. Mattsson, P. Oberoi, et al., Global standardization measurement of cerebral spinal fluid for Alzheimer's disease: an update from the Alzheimer's Association global biomarkers consortium, Alzheimers Dement. 9 (2013) 137–140.
[22] J. Pannee, J. Gobom, L.M. Shaw, M. Korecka, E.E. Chambers, M. Lame, et al., Round robin test on quantification of amyloid-beta 1-42 in cerebrospinal fluid by mass spectrometry, Alzheimers Dement. 12 (2016) 55–59.
[23] A. Algeciras-Schimnich, D.E. Bruns, J.C. Boyd, S.C. Bryant, K.A. La Fortune, S.K. Grebe, Failure of current laboratory protocols to detect lot-to-lot reagent differences: findings and possible solutions, Clin. Chem. 59 (2013) 1187–1194.
[24] M. del Campo, W. Jongbloed, H.A.M. Twaalfhoven, R. Veerhuis, M.A. Blankenstein, C.E. Teunissen, Facilitating the validation of novel protein biomarkers for dementia: an optimal workflow for the development of sandwich immunoassays, Front. Neurol. 6 (2015) 202.
[25] K. Blennow, A. Wallin, H. Agren, C. Spenger, J. Siegfried, E. Vanmechelen, Tau protein in cerebrospinal fluid: a biochemical marker for axonal degeneration in Alzheimer disease? Mol. Chem. Neuropathol. 26 (1995) 231–245.
[26] E. Vanmechelen, H. Vanderstichele, P. Davidsson, E. Van Kerschaver, B. Van Der Perre, M. Sjogren, et al., Quantification of tau phosphorylated at threonine 181 in human cerebrospinal fluid: a sandwich ELISA with a synthetic phosphopeptide for standardization, Neurosci. Lett. 285 (2000) 49–52.
[27] S. Palmqvist, H. Zetterberg, K. Blennow, S. Vestberg, U. Andreasson, D.J. Brooks, et al., Accuracy of brain amyloid detection in clinical practice using cerebrospinal fluid beta-amyloid 42: a cross-validation study against amyloid positron emission tomography, JAMA Neurol. 71 (2014) 1282–1289.
[28] K.A. Ellis, A.I. Bush, D. Darby, D. De Fazio, J. Foster, P. Hudson, et al., The Australian imaging, biomarkers and lifestyle (AIBL) study of aging: methodology and baseline characteristics of 1112 individuals recruited for a longitudinal study of Alzheimer's disease, Int. Psychogeriatr. 21 (2009) 672–687.
[29] H. Vanderstichele, K. De Vreese, K. Blennow, N. Andreasen, C. Sindic, A. Ivanoiu, et al., Analytical performance and clinical utility of the INNOTEST PHOSPHO-TAU181P assay for discrimination between Alzheimer's disease and dementia with Lewy bodies, Clin. Chem. Lab. Med. 44 (2006) 1472–1480.
[30] N.A. Verwey, W.M. van der Flier, K. Blennow, C. Clark, S. Sokolow, P.P. De Deyn, et al., A worldwide multicentre comparison of assays for cerebrospinal fluid biomarkers in Alzheimer's disease, Ann. Clin. Biochem. 46 (2009) 235–240 Pt 3.
[31] N. Mattsson, U. Andreasson, S. Persson, H. Arai, S.D. Batish, S. Bernardini, et al., The Alzheimer's Association external quality control program for cerebrospinal fluid biomarkers, Alzheimers Dement. 7 (2011) 386–395 (e6).
[32] K. Blennow, H. Zetterberg, The past and the future of Alzheimer's disease fluid biomarkers, J. Alzheimers Dis. 62 (2018) 1125–1140.
[33] J. Kuhlmann, U. Andreasson, J. Pannee, M. Bjerke, E. Portelius, A. Leinenbach, et al., CSF Aβ1-42 – an excellent but complicated Alzheimer's biomarker – a route to standardisation, Clin. Chim. Acta 467 (2017) 27–33.
[34] O. Hansson, E. Stomrud, S. Rutz, V. Lifke, E. Bauer, U. Eichenlaub, et al., Novel pre-analytical protocol for handling of cerebrospinal fluid samples for the analysis of Alzheimer's disease biomarkers in clinical practice, J. Prev. Alz. Dis. 5 (2018) S1–S196.
[35] R.S. Desikan, L.K. McEvoy, W.K. Thompson, D. Holland, J.B. Brewer, P.S. Aisen, et al., Amyloid-β associated clinical decline occurs only in the presence of elevated p-tau, Arch. Neurol. 69 (2012) 709–713.
[36] P. Buchhave, L. Minthon, H. Zetterberg, A.K. Wallin, K. Blennow, O. Hansson, Cerebrospinal fluid levels of beta-amyloid 1-42, but not of tau, are fully changed already 5 to 10 years before the onset of Alzheimer dementia, Arch. Gen. Psychiatry 69 (2012) 98–106.
[37] N.A. Verwey, F.H. Bouwman, W.M. van der Flier, R. Veerhuis, P. Scheltens, M.A. Blankenstein, Variability in longitudinal cerebrospinal fluid tau and phosphorylated tau measurements, Clin. Chem. Lab. Med. 46 (2008) 1300–1304.