Henrik Schinke*, Magnus Förnvik Jonsson, Mayme Gummesson, Rikard Nilsson, Stefanie Gaupp, Ekaterina Manuilova, Silja McIlwrick, Jan-Philipp Weinberger, Sandra Rutz, Margherita Carboni and Erik Stomrud

Concordance between the updated Elecsys cerebrospinal fluid immunoassays and amyloid positron emission tomography for Alzheimer's disease assessment: findings from the Apollo study

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Abstract

Objectives: The Apollo study was designed to support the clinical performance verification of the adjusted cutoffs of the Elecsys® β-Amyloid(1–42) ($A\beta_{42}$) cerebrospinal fluid (CSF) II, β-Amyloid(1–40) ($A\beta_{40}$) CSF, Phospho-Tau (181P) (pTau) CSF and Total-Tau (tTau) CSF immunoassays (Roche Diagnostics International Ltd) for measuring fresh CSF samples, and assess the concordance of the Elecsys CSF pTau/$A\beta_{42}$, tTau/$A\beta_{42}$ and $A\beta_{42}/A\beta_{40}$ ratios, as well as $A\beta_{42}$ alone, with amyloid positron emission tomography (PET) visual read status.

Methods: The primary study endpoint was to assess the concordance of the Elecsys CSF ratios and $A\beta_{42}$ alone with amyloid PET visual read status using fresh CSF samples collected from individuals with subjective cognitive decline or mild cognitive impairment, handled with a new routine-use pre-analytical procedure and measured with the Elecsys CSF immunoassays. The sample stability after 1- to 13-week storage at −20 °C was also investigated in an exploratory analysis.

Results: Of 108 screened individuals, 91 met the eligibility criteria, of whom 44.0 % were amyloid PET-positive and 56.0 % amyloid PET-negative. Positive percent agreement (PPA) and negative percent agreement, respectively, were 0.800 and 0.882 for pTau/$A\beta_{42}$, 0.775 and 0.902 for tTau/$A\beta_{42}$, and 0.950 and 0.824 for $A\beta_{42}/A\beta_{40}$. For $A\beta_{42}$, PPA was 0.975 and negative likelihood ratio was 0.039. Overall, 33 samples (36.3 %) were frozen at −20 °C for 1–13 weeks. All concentration recoveries were within 100 ± 10 % when stored at −20 °C for ≤8 weeks.

Conclusions: Elecsys CSF ratios and $A\beta_{42}$ alone may be reliable alternatives to amyloid PET for identifying amyloid positivity in clinical practice.

Keywords: amyloid positivity; amyloid PET; clinical performance; cerebrospinal fluid biomarkers; routine-use pre-analytical protocol; sample stability

Introduction

Alzheimer's disease (AD) is a progressive brain disease accounting for 60–80 % of dementia cases in the United States [1, 2]. Globally, the prevalence of AD and other dementias is estimated to increase from 57.4 million cases in 2019, to 152.8 million by 2050 [3].

AD pathology involves accumulating amyloid-β (Aβ) plaques and the hyperphosphorylation of tau proteins (pTau) [4]. The recent development of disease-modifying treatments targeting the pathophysiology of AD, such as donanemab and lecanemab, has highlighted the need for accurate diagnostic tests [5–8]. Recommendations provided by the International Working Group on the clinical diagnosis of AD suggest that the assessment of biological parameters, such as cerebrospinal fluid (CSF) biomarkers and amyloid positivity by positron emission tomography (PET) imaging, may help detect biological

***Corresponding author: Henrik Schinke**, Roche Diagnostics GmbH, Nonnenwald 2, 82377 Penzberg, Germany,
E-mail: henrik.schinke@roche.com
Magnus Förnvik Jonsson, Department of Clinical Chemistry and Pharmacology, Skåne University Hospital, Lund, Sweden; and Department of Translational Medicine, Lund University, Malmö, Sweden
Mayme Gummesson and Rikard Nilsson, Department of Clinical Chemistry and Pharmacology, Skåne University Hospital, Lund, Sweden
Stefanie Gaupp, **Ekaterina Manuilova**, **Silja McIlwrick**, **Jan-Philipp Weinberger and Sandra Rutz,** Roche Diagnostics GmbH, Penzberg, Germany
Margherita Carboni, Roche Diagnostics International Ltd, Rotkreuz, Switzerland
Erik Stomrud, Clinical Memory Research Unit, Department of Clinical Sciences Malmö, Lund University, Malmö, Sweden; and Memory Clinic, Skåne University Hospital, Malmö, Sweden

changes before symptom onset and aid in early AD diagnosis [9].

According to recent AD diagnostic criteria, the levels of β-amyloid(1–42) ($A\beta_{42}$), tau phosphorylated at a threonine residue at position 181 ($pTau_{181}$) and total tau (tTau) in CSF play a crucial role in the timely and accurate diagnosis of AD [10, 11]. $A\beta_{42}$ levels are inversely correlated with amyloid plaque burden, while $pTau_{181}$ and tTau levels are markers for tangle formation and neuronal degeneration, respectively [4]. Early-stage studies have shown that the ratios of $A\beta_{42}$ with $pTau_{181}$ and tTau may have increased performance in predicting clinical decline and cognitive impairment in AD, compared with each biomarker alone [12–15]. Although the levels of β-amyloid(1–40) ($A\beta_{40}$) have been found to remain unaltered in AD [16], the CSF $A\beta_{42}/A\beta_{40}$ ratio has also demonstrated better diagnostic performance than $A\beta_{42}$ alone [16–20].

The fully automated Elecsys® β-Amyloid (1–42) CSF, Elecsys Phospho-Tau (181P) CSF and Elecsys Total-Tau CSF immunoassays (Roche Diagnostics International Ltd, Rotkreuz, Switzerland) are *in vitro* diagnostic (IVD)-certified electrochemiluminescence immunoassays that employ a quantitative sandwich principle and were developed to aid amyloid pathology detection [21]. Since the initial clinical validation, all three immunoassays have been updated resulting in second-generation immunoassays (Elecsys β-Amyloid (1–42) CSF II [$A\beta_{42}$ Gen2], Elecsys Phospho-Tau (181P) CSF [pTau] and Elecsys Total-Tau CSF [tTau]), which are IVD-certified for their intended use, have higher thresholds for biotin interference and run on a broader range of analyzers than previously [21]. The updated immunoassays have also been recently approved by the US Food and Drug Administration (FDA) due to their concordance with amyloid PET visual read status and ability to identify the presence of amyloid pathology [22, 23].

The initial Elecsys CSF immunoassay clinical cutoff values were established using CSF samples stored at −80 °C in a research setting [21]. To suit clinical routine testing requirements, a new, simplified pre-analytical procedure has been developed to ensure standardization and reduce pre-analytical variability when handling fresh CSF samples [24]. The new handling procedure and updated Elecsys CSF immunoassays, when used in combination, offer improved robustness in measuring CSF biomarkers [21]. However, due to the susceptibility of $A\beta_{42}$ to differences in pre-analytical handling, a shift in $A\beta_{42}$ levels is expected when different protocols are applied [25]. Therefore, the clinical cutoff values for the updated Elecsys $A\beta_{42}$ Gen2 immunoassay and its ratios with pTau, tTau and $A\beta_{40}$ were adjusted accordingly, as previously published [21].

The present study aimed to support clinical performance verification of the adjusted cutoffs of the Elecsys immunoassays in terms of their ability to correctly identify patients with subjective cognitive decline (SCD) and mild cognitive impairment (MCI) based on amyloid PET results.

Materials and methods

Study design

The Apollo study was a prospective, supportive verification study for the updated $A\beta_{42}$ Gen2 immunoassay and its ratios with the updated Elecsys pTau and tTau CSF immunoassays as well as the $A\beta_{40}$ immunoassay, used to measure fresh CSF samples handled according to the new routine-use pre-analytical procedure.

Individuals diagnosed as SCD/MCI were recruited for the Swedish BioFINDER-2 study (NCT03174938) based on previously described eligibility criteria at baseline or at the 2-year follow-up visit [26]. From this population, SCD/MCI individuals who had available amyloid PET scans and valid biomarker measurements in fresh CSF were eligible for Apollo. More details on the eligibility criteria for the Apollo study are described in the Supplementary Material.

The primary objective of the study was to investigate the concordance of the Elecsys CSF $pTau/A\beta_{42}$ and $tTau/A\beta_{42}$ ratios, as well as $A\beta_{42}$ alone, with amyloid PET visual read status (positive vs. negative). An exploratory analysis was conducted to demonstrate the concordance of amyloid status based on the Elecsys CSF $A\beta_{42}/A\beta_{40}$ ratio with amyloid PET visual read status. The $A\beta_{42}/A\beta_{40}$ ratio was determined using the updated $A\beta_{42}$ Gen2 immunoassay and an Elecsys $A\beta_{40}$ CSF assay, which was in early development during this study. Additionally, the stability of frozen CSF samples after storage at −20 °C for 1–13 weeks was explored.

Elecsys CSF immunoassays

The original clinical cutoff values for Elecsys $pTau/A\beta_{42}$, $tTau/A\beta_{42}$ and $A\beta_{42}$ alone were determined in frozen samples from the Swedish BioFINDER-1 study and their concordance with amyloid PET visual read status was validated in samples from the Alzheimer's Disease Neuroimaging Initiative (ADNI) study [15]. The immunoassays were then updated to eliminate potential interference and improve analytical performance [21]. Additionally, the Elecsys $A\beta_{42}$ immunoassay was

re-standardized using updated certified reference material recently introduced by the International Federation of Clinical Chemistry and Laboratory Medicine, the $A\beta_{42}$ measuring range was extended from 200–1,700 ng/L to 150–2,500 ng/L and the calibrator levels and control samples (PreciControl level 2) were updated.

The Elecsys β-Amyloid (1–40) CSF assay used in this study was at an early development stage, to be used for exploratory study measurements only. More details on the cutoff determination for $A\beta_{42}/A\beta_{40}$ are provided in the Supplementary material.

CSF measurements

All CSF samples in Apollo were collected for the Swedish BioFINDER-2 study at the Memory Clinic, Skåne University Hospital (Malmö, Sweden) [27] and handled according to the new routine-use pre-analytical procedure for fresh CSF samples [24]. The measurements of fresh and frozen CSF samples were performed using the updated Elecsys $A\beta_{42}$ Gen2, pTau and tTau CSF immunoassays as well as the Elecsys $A\beta_{40}$ CSF immunoassay on the Cobas® e 601 module (Roche Diagnostics International Ltd) at the Department of Clinical Chemistry and Pharmacology, Skåne University Hospital. No additional sample collections or measurements were performed under the Apollo study protocol.

Amyloid PET imaging and analysis

Amyloid PET scans for visual evaluation were collected under the Swedish BioFINDER-2 study, as previously described [27]. No additional PET scans were performed for the Apollo study. Further details on the expert amyloid PET visual read process can be found in the Supplementary material. The primary endpoint for all CSF biomarkers was the amyloid PET visual read outcome, determined as the majority vote from three independent readers, blinded to subject diagnosis and all other clinical and biomarker data.

Exploratory analysis of frozen samples (sample stability)

For the exploratory sample stability analysis, a subset of samples from individuals with available CSF samples were frozen at −20 °C and re-measured after storage for 1–13 weeks. For $A\beta_{42}$, pTau and tTau, six and 27 samples were stored for 1–8 and >8–13 weeks, respectively; for $A\beta_{40}$, six and 22 samples were stored for 1–8 and >8–13 weeks, respectively. After freezing, samples were thawed at a temperature between 20–25 °C for 30 min on a roller mixer. During rolling, the tube caps were placed slightly higher than the bottoms to prevent $A\beta_{42}$ from sticking to the tube lids and ensure measurement accuracy.

Statistical analysis

Primary analysis – concordance of pTau/$A\beta_{42}$, tTau/$A\beta_{42}$ and $A\beta_{42}$ in fresh CSF samples with amyloid PET visual read status

The primary analysis aimed to verify the performance at the pre-specified (adjusted) cutoffs ($pTau/A\beta_{42}$>0.023; $tTau/A\beta_{42}$>0.28; $A\beta_{42}$≤1,030 ng/L) for the new routine-use pre-analytical protocol using the updated Elecsys CSF immunoassays, by demonstrating the concordance of the CSF biomarker status (positive or negative), determined by the $pTau/A\beta_{42}$ and $tTau/A\beta_{42}$ ratios and $A\beta_{42}$ alone in fresh CSF, with amyloid PET visual read status (positive or negative). The minimum sample size for the analysis was determined to be at least 40 PET-positive and 40 PET-negative individuals with confirmed SCD/MCI to ensure a joint power of 90 % to meet the positive percent agreement (PPA), negative percent agreement (NPA) and negative likelihood ratio (LR–) acceptance criteria for an expected underlying performance of 0.85.

CSF biomarker concordance was tested using a fixed sequence approach based on the FDA Draft Guidance 'Multiple Endpoints in Clinical Trials' for the hypothesis testing of [28]: sensitivity (PPA) and specificity (NPA) for $pTau/A\beta_{42}$ and $tTau/A\beta_{42}$; PPA and the negative likelihood ratio (LR–=[1 – PPA]/NPA) for $A\beta_{42}$ alone.

For each biomarker, two joint hypotheses for PPA and NPA (or LR– for $A\beta_{42}$) had to be rejected (each with alpha level 0.05), so that the hypothesis testing was fulfilled. If a hypothesis for a biomarker was not rejected (e.g., the null hypothesis of non-concordance was accepted), hypothesis testing was terminated, and the subsequent biomarkers were considered non-concordant. For the test on PPA and NPA, the two-sided 95 % confidence interval (CI) was computed, and the acceptance criterion was met if the point estimate was >0.75 and the lower confidence limit was >0.60. For the test on LR–, the two-sided 95 % CI was computed, and the acceptance criterion was met if the upper confidence limit was <1.00.

Exploratory analysis – concordance of $A\beta_{42}/A\beta_{40}$ in fresh CSF samples with amyloid PET visual read status

The concordance of the dichotomized $A\beta_{42}/A\beta_{40}$ ratio values, measured with the $A\beta_{42}$ Gen2 and $A\beta_{40}$ immunoassays, with

visual amyloid PET readout status was investigated in an exploratory analysis.

Exploratory analysis – sample stability

The influence of storage at −20 °C was investigated in an exploratory analysis using a regression approach and description of concentration recoveries after freezing and storage. Concentration recoveries were described using boxplots and descriptive tables. Concentration measurements in fresh samples and frozen samples at baseline were compared after storage using scatter plots and Passing–Bablok regression analysis.

Ethics

This study was conducted according to the principles of the Declaration of Helsinki. All samples used were collected under the Swedish BioFINDER-2 study. Written informed consent was obtained from each participant prior to enrollment into the Swedish BioFINDER-2 study. All samples and required clinical information were pseudonymized. Ethics approval was received for the Swedish BioFINDER-2 study, including data shared in the Apollo study, from the Swedish Ethical Review Authority, Sweden.

Results

Concordance of pTau/Aβ$_{42}$, tTau/Aβ$_{42}$, Aβ$_{42}$/Aβ$_{40}$ and Aβ$_{42}$ in fresh CSF samples with amyloid PET visual read status

Baseline demographics and clinical characteristics

The Apollo study initially included 108 individuals selected from the BioFINDER-2 cohort based on the criteria described in the Supplementary material. Of the 108 individuals, 16 were excluded due to missing CSF biomarker measurement data and one was excluded during the monitoring process due to not fulfilling the inclusion criterion for Mini-Mental State Examination score (≥24) (Figure 1). Thus, data from 91 individuals were included in the primary analysis.

Individuals were enrolled from the Swedish BioFINDER-2 study at baseline (61/91; 67.0 %) or at the 2-year follow-up visit (30/91; 33.0 %). The demographic and clinical characteristics of the primary analysis population are summarized in Table 1. The primary analysis population comprised 40 (44.0 %) amyloid PET-positive and 51 (56.0 %) amyloid PET-negative individuals according to the majority vote of three independent readers.

Amyloid PET concordance analysis

Amyloid PET concordance analysis showed that the performance of the pTau/Aβ$_{42}$ and tTau/Aβ$_{42}$ ratios at the adjusted cutoffs was as expected and the pre-defined acceptance criteria were met (pTau/Aβ$_{42}$: PPA 0.800, NPA 0.882; tTau/Aβ$_{42}$: PPA 0.775, NPA 0.902; Figure 2; Table 2). The observed concordance between Aβ$_{42}$/Aβ$_{40}$, dichotomized at the previously published adjusted cutoff, and amyloid PET status was comparable with a PPA of 0.950 and an NPA of 0.824 (Figure 2).

Using the CSF pTau/Aβ$_{42}$-based classification, 38 individuals were scored as CSF-positive, of whom 32 were concordant with a positive PET result; 53 individuals were scored as CSF-negative, of whom 45 were concordant with a negative PET result. In total, 77/91 (84.6 %) individuals showed concordant CSF and amyloid PET visual read results (Table 3). Of the 14 individuals with discordant results, eight were CSF-negative with a positive PET result and six were CSF-positive with a negative PET result (Supplementary Table 1); for 6/8 and 2/6 individuals, biomarker values were within ±10 % of the cutoff value (0.023), respectively (Supplementary Table 2). Similar results were observed using the CSF tTau/Aβ$_{42}$-based classification, where in total 77/91 (84.6 %) individuals showed concordant CSF and amyloid PET visual read results, while 9/14 were CSF-negative with a positive PET result and 5/14 were CSF-positive with a negative PET result (Table 3; Supplementary Table 1). For 5/9 and 1/5 individuals with discordant results, biomarker values were within ±10 % of the cutoff value (0.28), respectively (Supplementary Table 2). Using the exploratory Aβ$_{42}$/Aβ$_{40}$-based classification, in total 80/91 (87.9 %) individuals showed concordant CSF and amyloid PET visual read results, while 2/11 were CSF-negative with a positive PET result and 9/11 were CSF-positive with a negative PET result (Table 3; Supplementary Table 1). Two of the nine individuals with CSF-positive and PET-negative results had biomarker values within ±10 % of the cutoff value (0.052) (Supplementary Table 2).

The concordance analysis also showed that the performance of Aβ$_{42}$ as a single biomarker at the adjusted cutoff was as expected and met the pre-defined acceptance criteria (PPA 0.975, LR– 0.039; Figure 3; Table 2). Of the 91 individuals tested, 57 individuals were scored as CSF-positive, of whom 39 were concordant with a positive PET result; 34 individuals were scored as CSF-negative, of whom 33 were concordant

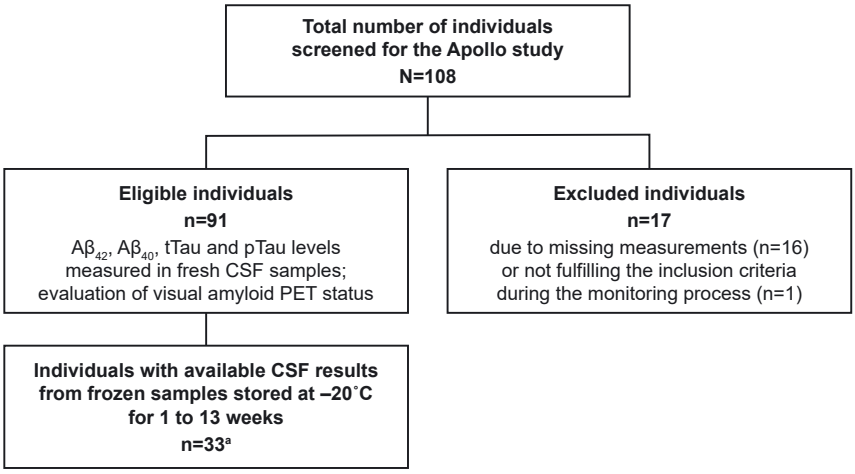

Figure 1: Enrollment summary. [a]The low number of frozen samples fulfilling the required conditions was due to many samples being excluded for exceeding 13 weeks of storage during the COVID-19 pandemic. $A\beta_{40}$, β-amyloid(1–40); $A\beta_{42}$, β-amyloid(1–42); CSF, cerebrospinal fluid; PET, positron emission tomography; pTau, phosphorylated tau; tTau, total tau.

Table 1: Demographic and clinical characteristics of the primary analysis population, total and split by amyloid PET visual read status.

	PET (visual)-positive (n=40)	PET (visual)-negative (n=51)	Total (n=91)
Age, years, mean (min–max)	72.9 (55.0–90.0)	68.2 (43.0–85.0)	70.3 (43.0–90.0)
Education, years, mean (min–max)	13.2 (7.0–31.0)	12.8 (7.0–22.0)	13.0 (7.0–31.0)
MMSE score, mean (min–max)	28.2 (25.0–30.0)	28.7 (24.0–30.0)	28.5 (24.0–30.0)
Sex, n (%)			
Female	15 (37.5)	23 (45.1)	38 (41.8)
Male	25 (62.5)	28 (54.9)	53 (58.2)
SCD/MCI, n (%)			
SCD	20 (50.0)	11 (21.6)	31 (34.1)
MCI	15 (37.5)	26 (51.0)	41 (45.1)
Missing	5 (12.5)	14 (27.5)	19 (20.9)
APOE genotype, n (%)			
E2/E2	1 (2.5)	1 (2.0)	2 (2.2)
E2/E3	2 (5.0)	3 (5.9)	5 (5.5)
E2/E4	0 (0.0)	1 (2.0)	1 (1.1)
E3/E3	9 (22.5)	29 (56.9)	38 (41.8)
E3/E4	21 (52.5)	16 (31.4)	37 (40.7)
E4/E4	7 (17.5)	1 (2.0)	8 (8.8)
Family history, n (%)			
Yes	17 (42.5)	25 (49.0)	42 (46.2)
No	20 (50.0)	23 (45.1)	43 (47.3)
Missing	3 (7.5)	3 (5.9)	6 (6.6)
Visit during the BioFINDER-2 study, n (%)			
Baseline visit	30 (75.0)	31 (60.8)	61 (67.0)
2-year follow-up	10 (25.0)	20 (39.2)	30 (33.0)

APOE, apolipoprotein E; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; PET, positron emission tomography; SCD, subjective cognitive decline.

with a negative PET result. In total, 72/91 (79.1 %) individuals had concordant CSF and amyloid PET visual read results using the updated $A\beta_{42}$ Gen2 immunoassay (Table 3). The NPA observed for the $A\beta_{42}$ Gen2 immunoassay was lower than the NPA of the pTau/$A\beta_{42}$ and tTau/$A\beta_{42}$ ratios, as expected, and 18/91 (35.3 %) individuals with negative PET scans were misclassified as positive by the $A\beta_{42}$ immunoassay (Table 3; Supplementary Tables 1 and 2). Nevertheless, the performance of pTau/$A\beta_{42}$, tTau/$A\beta_{42}$ and $A\beta_{42}$ met the pre-specified acceptance criteria for all three biomarkers (Table 2).

Stability analysis in frozen CSF samples

Of the 91 CSF samples, 33 samples (36.3 %) were frozen at −20 °C for 1–13 weeks and used to explore the effect of storage and one freeze-thaw cycle on the stability of frozen CSF samples; for $A\beta_{40}$, measurements were available for 28/33 samples. For all four biomarkers, the measurements in samples before and after freezing were highly correlated, with Pearson's R >0.99, and slope estimates were close to 1.000 (pTau: 0.973; tTau: 0.965; $A\beta_{42}$: 1.000; $A\beta_{40}$: 1.050; Figure 4, Supplementary Table 3). The largest bias estimate at the pre-specified concentration was observed for tTau (bias: −2.74 % [95 % CI −3.42; −1.17]) at a concentration of 300 ng/L, followed by $A\beta_{42}$ (bias: −2.64 % [95 % CI −6.08; −1.11]), $A\beta_{40}$ (bias: −1.6 % [95 % CI −5.6; 0.4]) and pTau (bias: −1.15 % [95 % CI −3.94; 0.62]) (Figure 4, Supplementary Table 3). The concentration recoveries for pTau and tTau were within 100 ± 10 % in all samples stored for 1–13 weeks (Figure 5). $A\beta_{42}$ and $A\beta_{40}$ recoveries were within 100 ± 10 % in all samples stored

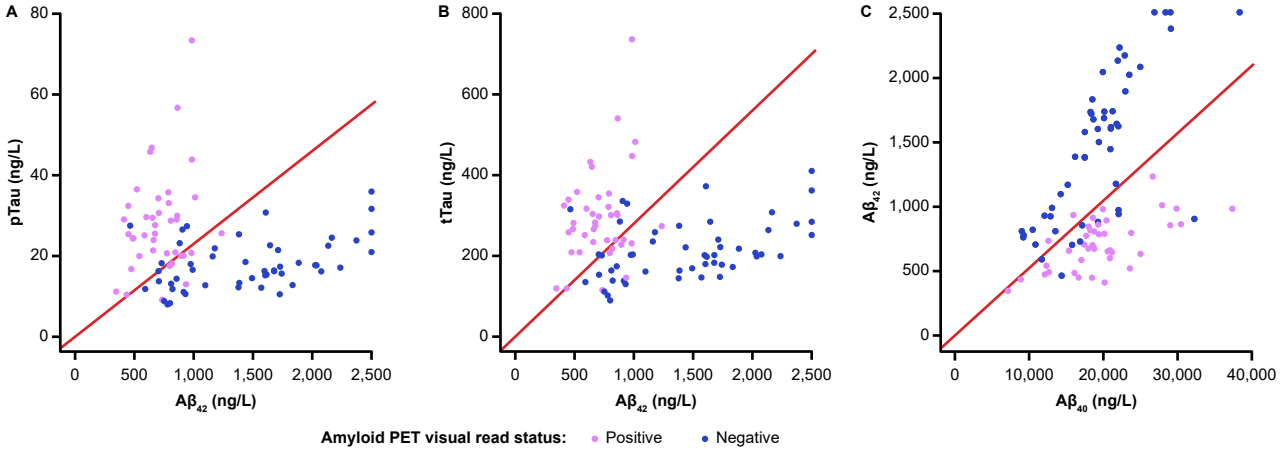

Figure 2: Joint distributions of the single biomarkers (A) pTau and $A\beta_{42}$, (B) tTau and $A\beta_{42}$ and (C) $A\beta_{42}$ and $A\beta_{40}$. Red lines indicate the respective cutoffs ($pTau/A\beta_{42}$>0.023; $tTau/A\beta_{42}$>0.28; $A\beta_{42}/A\beta_{40}$<0.052). Points are coloured by amyloid PET visual read status. $A\beta_{40}$, β-amyloid(1–40); $A\beta_{42}$, β-amyloid(1–42); PET, positron emission tomography; pTau, phosphorylated tau; tTau, total tau.

Table 2: Hypothesis testing of the pre-specified acceptance criteria for $pTau/A\beta_{42}$, $tTau/A\beta_{42}$ and $A\beta_{42}$.

Performance measure	Point estimate (95 % CI)	Acceptance criteria	Testing result
$pTau/A\beta_{42}$			
PPA	0.800 (0.652–0.895)	PPA >0.75 & LCL >0.60	Successful
NPA	0.882 (0.766–0.945)	PPA >0.75 & LCL >0.60	Successful
$tTau/A\beta_{42}$			
PPA	0.775 (0.625–0.877)	PPA >0.75 & LCL >0.60	Successful
NPA	0.902 (0.790–0.957)	PPA >0.75 & LCL >0.60	Successful
$A\beta_{42}$			
PPA	0.975 (0.871–0.996)	PPA >0.75 & LCL >0.60	Successful
LR–	0.039 (0.006–0.270)	UCL <1	Successful

$A\beta_{42}$, β-amyloid(1–42); CI, confidence interval; LCL, lower confidence limit; LR–, negative likelihood ratio; NPA, negative percent agreement; PPA, positive percent agreement; pTau, phosphorylated tau; tTau, total tau; UCL, upper confidence limit.

Table 3: Concordance tables of classification based on $pTau/A\beta_{42}$, $tTau/A\beta_{42}$, $A\beta_{42}/A\beta_{40}$ and $A\beta_{42}$ vs. amyloid PET visual read status.

	PET (visual)-positive (n=40)	PET (visual)-negative (n=51)	Total (n=91)
$pTau/A\beta_{42}$			
CSF-positive, n (%)	32 (35.2)	6 (6.6)	38 (41.8)
CSF-negative, n (%)	8 (8.8)	45 (49.5)	53 (58.2)
$tTau/A\beta_{42}$			
CSF-positive, n (%)	31 (34.1)	5 (5.5)	36 (39.6)
CSF-negative, n (%)	9 (9.9)	46 (50.5)	55 (60.4)
$A\beta_{42}/A\beta_{40}$			
CSF-positive, n (%)	38 (41.8)	9 (9.9)	47 (51.6)
CSF-negative, n (%)	2 (2.2)	42 (46.2)	44 (48.4)
$A\beta_{42}$			
CSF-positive, n (%)	39 (42.9)	18 (19.8)	57 (62.6)
CSF-negative, n (%)	1 (1.1)	33 (36.3)	34 (37.4)

$A\beta_{40}$, β-amyloid(1–40); $A\beta_{42}$, β-amyloid(1–42); CSF, cerebrospinal fluid; PET, positron emission tomography; pTau, phosphorylated tau; tTau, total tau. The cutoffs for CSF-positivity were as follows: $pTau/A\beta_{42}$ >0.023; $tTau/A\beta_{42}$ >0.28; $A\beta_{42}/A\beta_{40}$ <0.052; $A\beta_{42}$ ≤1,030 ng/L.

at –20 °C for 1–8 weeks (n=6). For $A\beta_{42}$ and $A\beta_{40}$, concentration recoveries for 6/27 and 2/22 samples, respectively, stored at the same temperature for >8–13 weeks, were below 90 %.

Discussion

This study supports the concordance of $pTau/A\beta_{42}$, $tTau/A\beta_{42}$ and $A\beta_{42}$ with amyloid PET visual reads and verifies that the performance of the adjusted cutoffs for the Elecsys ratios is as expected in CSF samples handled with the new routine-use pre-analytical procedure and measured with the updated CSF immunoassays. These results suggest that both biomarker ratios plus $A\beta_{42}$ alone could be used in clinical practice as reliable alternatives to amyloid PET imaging to aid in the diagnosis of amyloid pathology.

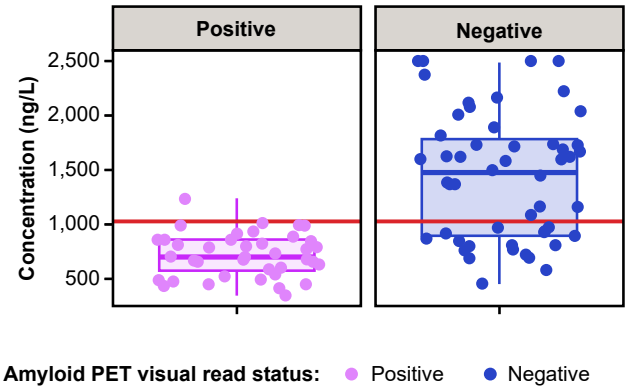

Figure 3: Box plot of $A\beta_{42}$ concentration (ng/L) by visual PET status. Red lines indicate the respective cutoff (≤1,030 ng/L). $A\beta_{42}$, β-amyloid(1–42); PET, positron emission tomography.

In this study, the pTau/$A\beta_{42}$ and tTau/$A\beta_{42}$ ratios met the pre-defined acceptance criteria for PPA and NPA and showed more than 80 % concordant positive and negative CSF and PET scan results, while only a low percentage (<16 %) were discordant. $A\beta_{42}$ alone also met the pre-defined acceptance criteria, and the LR– value was low (0.039). This indicated that the likelihood of an amyloid PET-positive individual having an $A\beta_{42}$ concentration greater than 1,030 ng/L is significantly smaller (by a factor of 0.039) compared with an amyloid PET-negative individual. Nevertheless, the NPA value of $A\beta_{42}$ as a single biomarker was substantially lower than the ratios, as biomarkers alone typically perform worse than combination ratios, and the original $A\beta_{42}$ cutoff value was set to fulfill a high PPA to ensure high sensitivity [29–31]. The exploratory analysis also indicated that normalization with $A\beta_{40}$ improved the performance of $A\beta_{42}$ alone, and the performance of the $A\beta_{42}/A\beta_{40}$ ratio was comparable to that of the pTau/$A\beta_{42}$ and tTau/$A\beta_{42}$ ratios, consistent with previously reported results [32–35], since the CIs of PPA and NPA overlapped.

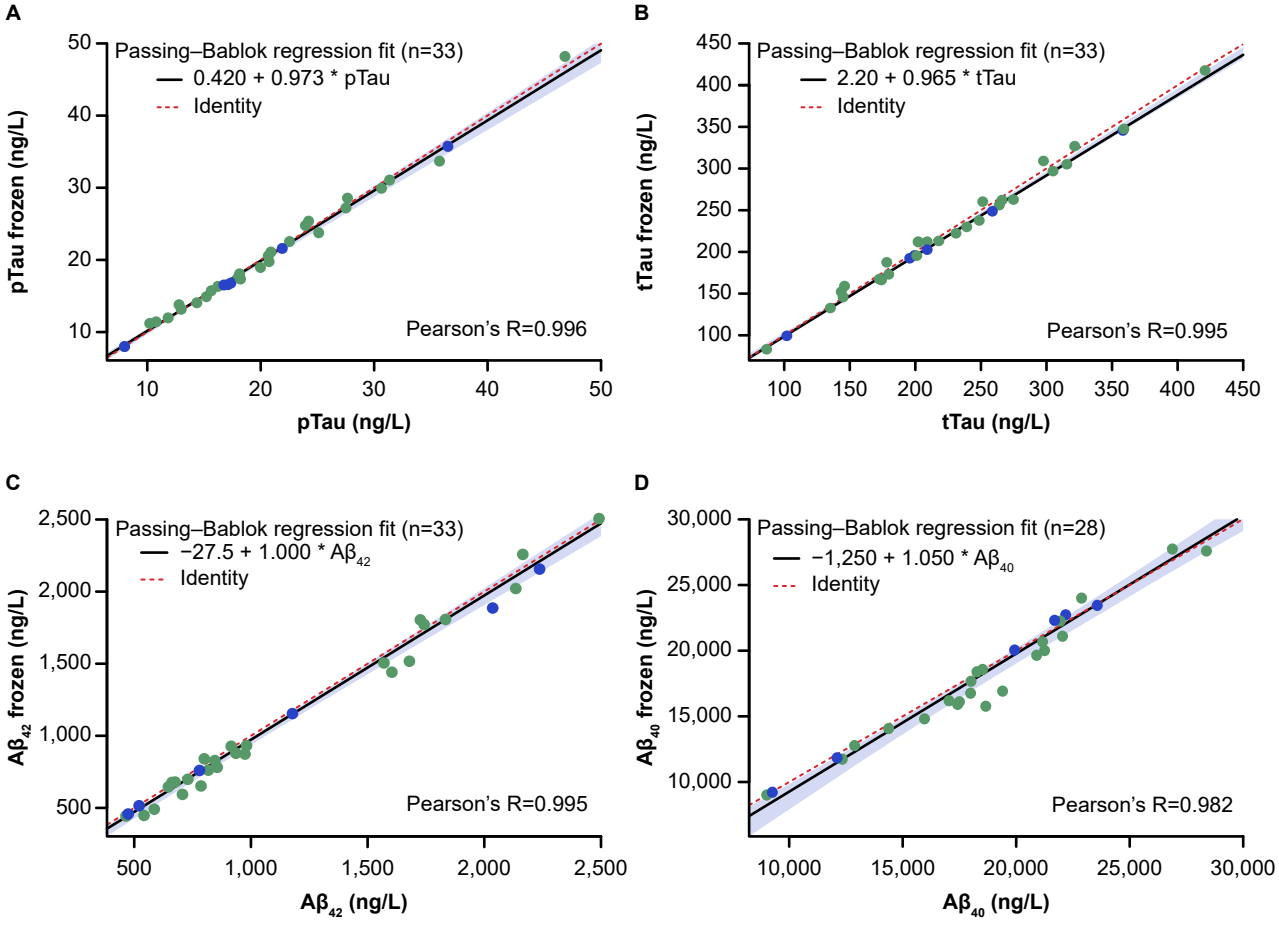

Figure 4: Stability of (A) pTau, (B) tTau, (C) $A\beta_{42}$ and (D) $A\beta_{40}$ at −20 °C for 1–8 and >8–13 weeks. Passing–Bablok regression fit is shown as a black line with 95 % confidence bounds (light blue shaded area). X-axes show concentrations in fresh samples and y-axes concentrations in frozen samples. Red dashed lines represent identity lines. Blue points indicate storage for 1–8 weeks and green points storage for >8–13 weeks. $A\beta_{40}$, β-amyloid(1–40); $A\beta_{42}$, β-amyloid(1–42); pTau, phosphorylated tau; tTau, total tau.

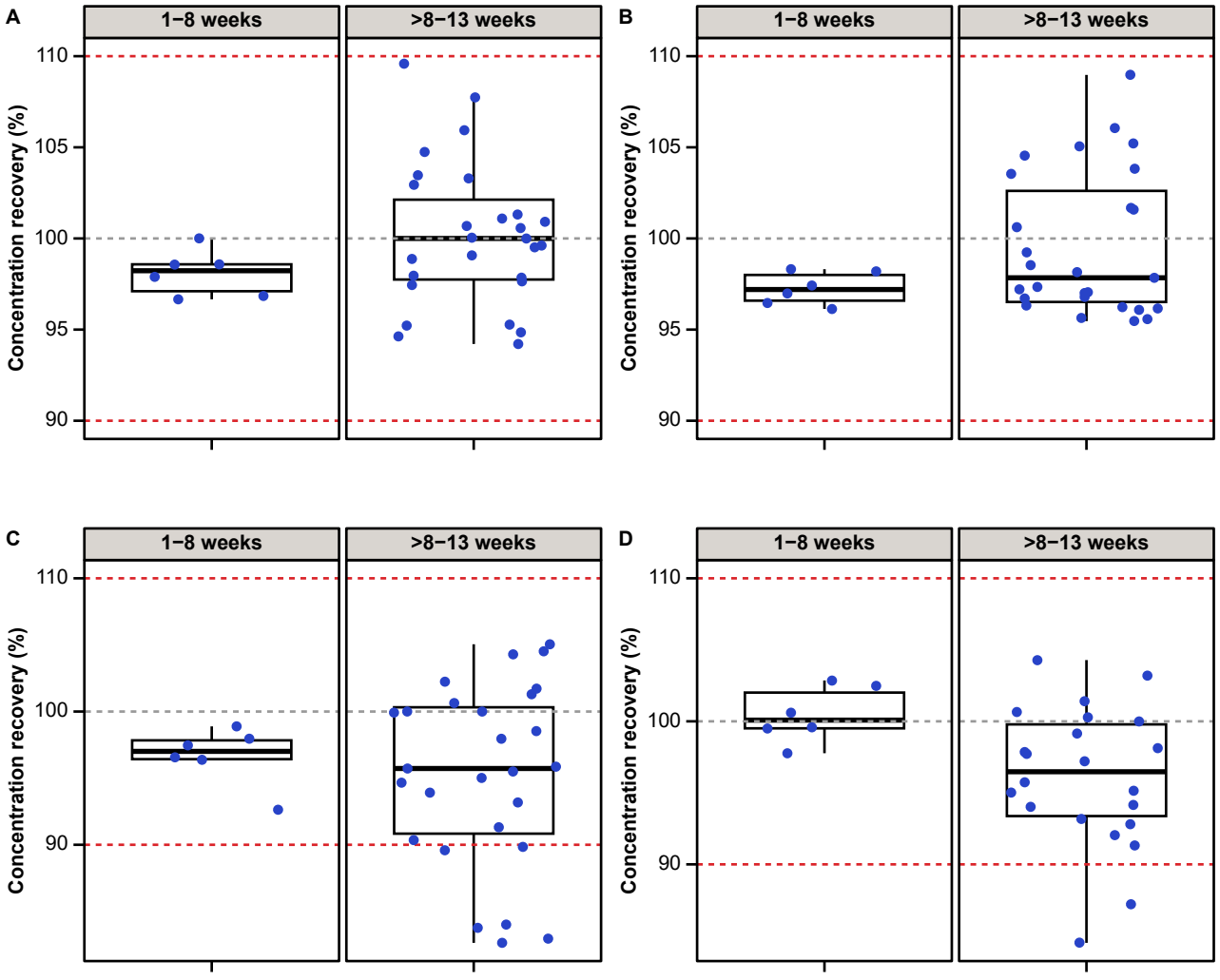

Figure 5: Concentration recoveries (%) for (A) pTau, (B) tTau, (C) $A\beta_{42}$ and (D) $A\beta_{40}$ observed after storage at −20 °C for 1–8 weeks and >8–13 weeks. Red dashed lines indicate 90 and 110 % recovery bounds. $A\beta_{40}$, β-amyloid(1–40); $A\beta_{42}$, β-amyloid(1–42); pTau, phosphorylated tau; tTau, total tau.

Storage at −20 °C for 1–8 weeks and one freeze-thaw cycle had no effect on any of the four biomarker concentration recoveries. Storage for >8–13 weeks also had no significant effect on pTau and tTau concentration recoveries, whereas a small effect was observed on $A\beta_{42}$ and $A\beta_{40}$, respectively, under the same conditions, with 6/27 and 2/22 samples showing concentration recoveries <90 %. It is therefore recommended to store CSF samples at −20 °C for ≤8 weeks to maintain stability.

This study supports the verification of the clinical performance of the updated Elecsys CSF immunoassays with the new routine-use pre-analytical procedure and their concordance with amyloid PET visual read status in distinguishing amyloid-positive individuals with early-stage AD, who are considered perhaps the most relevant but also the most diagnostically challenging group of the intended use population. Although early-stage disease PET scans are challenging to correctly classify as positive or negative, and biomarker levels are closer to the cutoff values, this study indicated a good performance of concordance with amyloid PET imaging. A better performance of the tests in terms of PPA and NPA is expected in individuals with Alzheimer's dementia (not included here) due to the more advanced amyloid pathology, which is more clearly reflected in the CSF biomarker levels and PET scans.

This study's findings are consistent with previous research using Elecsys and other platforms. Specifically, previous studies have shown that the Elecsys CSF pTau/$A\beta_{42}$, tTau/$A\beta_{42}$ and $A\beta_{42}/A\beta_{40}$ ratios, as well as $A\beta_{42}$ alone, are strongly concordant with PET imaging assessing Aβ burden in AD, supporting the use of CSF biomarkers in early amyloid identification [15, 34]. For instance, Hansson et al. indicated

that pTau/Aβ_{42} and tTau/Aβ_{42}, measured with the first-generation Elecsys CSF immunoassays, were highly concordant with amyloid PET visual reads across two different cohorts (BioFINDER and ADNI) comprising different populations and PET radiotracers [15]. Schindler et al. reported high concordance between Pittsburgh compound B PET imaging and pTau/Aβ_{42}, tTau/Aβ_{42} and Aβ_{42}/Aβ_{40} ratios, measured using the first-generation Elecsys CSF immunoassays, in discriminating PET-positive from PET-negative individuals [35]. Campbell et al. showed agreement between pTau/Aβ_{42} and Aβ_{42}/Aβ_{40} ratios, measured with the first-generation Elecsys and LUMIPULSE immunoassays, and amyloid PET classification, and the biomarker ratio results were superior to individual biomarkers [36]. In another study, Alcolea et al. reported that pTau/Aβ_{42}, tTau/Aβ_{42} and Aβ_{42}/Aβ_{40}, measured on the fully automated, Conformité Européenne-marked and FDA-approved LUMIPULSE G600II platform (Fujirebio), had good diagnostic agreement with ^{18}F-flutemetamol amyloid PET and the ratios were suggested to be more reliable in clinical practice than Aβ_{42} alone [31, 32, 37].

The future clinical application of these findings is expected to aid earlier diagnosis of patients with AD, giving them and their caregivers time to plan for the future and access potential treatments for early symptom management. Implementing the new pre-analytical procedure and recommended storage conditions for handling fresh CSF samples is expected to reduce the variability of assay measurements and enable comparison of CSF biomarker levels between different laboratories, thus increasing the utility of CSF biomarkers in research and routine clinical practice [24].

This study had some limitations, such as the relatively small number of individuals enrolled, which suggests that the results should be confirmed in a wider population. The enrolled population was not randomly selected from the intended use population, but was based on the Swedish BioFINDER-2 study cohort. Thus, the results of this study may be biased due to the inclusion and exclusion criteria of the BioFINDER-2 study. However, the BioFINDER-2 study includes participants from secondary care specialized memory clinics, and therefore does not differ substantially from an intended use population. Additionally, the concordance of Aβ_{42}/Aβ_{40} with amyloid PET visual reads was assessed using an early version of the Elecsys CSF Aβ_{40} immunoassay and the acceptance criteria as well as the adjusted cutoff for the Aβ_{42}/Aβ_{40} ratio were not pre-specified. Moreover, the objectives for the frozen sample analysis were limited to exploratory due to the low number of frozen samples available. The number of samples stored up to 8 weeks was small, suggesting that the results of the exploratory analysis under these storage conditions will need to be confirmed in a larger sample size. It is also worth noting that although the pTau/Aβ_{42}, tTau/Aβ_{42}, Aβ_{42}/Aβ_{40} ratios and Aβ_{42} alone can successfully identify individuals with positive amyloid PET results, their performance does not establish a diagnosis of AD or other cognitive disorder and cannot be used for predicting the development of dementia or other neurological conditions, or to monitor responses to therapies.

Conclusions

CSF biomarker status, determined by the pTau/Aβ_{42}, tTau/Aβ_{42} and Aβ_{42}/Aβ_{40} ratios and Aβ_{42} alone in fresh CSF, is concordant with amyloid PET visual read status. All three ratios can be used to identify amyloid PET positivity in individuals with SCD/MCI with high sensitivity and specificity, and Aβ_{42} alone can distinguish amyloid PET-positive individuals with high sensitivity. As a conservative approach, CSF samples should be stored at −20 °C for ≤8 weeks to maintain stability before testing. The new routine-use pre-analytical procedure and the updated Elecsys Aβ_{42} Gen2, pTau and tTau CSF immunoassays could be used in clinical practice as alternatives to amyloid PET imaging to identify amyloid positivity in SCD/MCI individuals, thus contributing to the accurate and timely diagnosis of AD.

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Research ethics: The study was conducted according to the principles of the Declaration of Helsinki. All samples used were prospectively collected for the Swedish BioFINDER-2 study. Ethics approval was received for the Swedish BioFINDER-2 study, including the data shared in the Apollo study, from the Swedish Ethical Review Authority, Sweden.

Informed consent: Written informed consent was obtained from each participant prior to enrollment into the Swedish

BioFINDER-2 study. All sample information and all required clinical information were pseudonymized.

Author contributions: Henrik Schinke: data curation, formal analysis, software, supervision, visualization, writing – original draft, writing – review & editing. Magnus Förnvik Jonsson: investigation, resources, supervision, validation, writing – review & editing. Mayme Gummesson: investigation, resources, software, validation, writing – review & editing. Rikard Nilsson: investigation, resources, software, validation, writing – review & editing. Stefanie Gaupp: supervision, writing – review & editing. Ekaterina Manuilova: conceptualization, methodology, validation, writing – review & editing. Silja McIlwrick: conceptualization, investigation, methodology, supervision, writing – review & editing. Jan-Philipp Weinberger: conceptualization, software, validation, writing – review & editing. Sandra Rutz: investigation, resources, writing – review & editing. Margherita Carboni: conceptualization, writing – original draft, writing – review & editing. Erik Stomrud: conceptualization, methodology, project administration, supervision, writing – review & editing. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

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Data availability: Requests concerning the data supporting the findings of this study can be directed to rotkreuz.datasharingrequests@roche.com for consideration.

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