Comparing CSF amyloid-beta biomarker ratios for two automated immunoassays, Elecsys and Lumipulse, with amyloid PET status

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Abstract

Introduction: We evaluated for two novel automated biomarker assays how cerebrospinal fluid (CSF) amyloid beta (Aβ1-42)-ratios improved the concordance with amyloid positron emission tomography (PET) positivity compared to Aβ1-42 alone.

Methods: We selected 288 individuals from the Amsterdam Dementia Cohort across the Alzheimer’s disease clinical spectrum when they had both CSF and amyloid PET visual read available, regardless of diagnosis. CSF Aβ1-42, phosphorylated tau (p-tau), and total tau (t-tau) were measured with Elecsys and Lumipulse assays, and Aβ1-40 with Lumipulse. CSF cut-points were defined using receiver operating characteristic (ROC) for amyloid PET positivity.

Results: For both Elecsys and Lumipulse the p-tau/Aβ1-42, Aβ1-42/Aβ1-40, and t-tau/Aβ1-42 ratios showed similarly good concordance with amyloid PET (Elecsys: 93, 90, 90%; Lumipulse: 94, 92, 90%) and were higher than Aβ1-42 alone (Elecsys 85%; Lumipulse 84%).

Discussion: Biomarker ratios p-tau/Aβ1-42, Aβ1-42/Aβ1-40, t-tau/Aβ1-42 on two automated platforms show similar optimal concordance with amyloid PET in a memory clinic cohort.

KEYWORDS
Alzheimer’s disease, amyloid-beta, amyloid positron emission tomography, biomarkers, cerebrospinal fluid, concordance cut-points, Elecsys, Lumipulse

1 INTRODUCTION

Cerebrospinal fluid (CSF) biomarkers for amyloid beta(1-42) (Aβ1-42), phosphorylated tau (p-tau), and total tau (t-tau) are part of recent research criteria to support a diagnosis of Alzheimer’s disease (AD).1 CSF Aβ1-42 concentrations decrease in the disease process when Aβ aggregates into plaques, while CSF p-tau concentrations increase along the formation of AD-specific tangle pathology and increases in CSF t-tau concentrations may in addition reflect other aspects of neurodegeneration.1,2 These biomarkers are altered in very early, pre-clinical stages of AD, when cognition is still normal.1 Therefore, CSF biomarkers have been proven to be useful tools for AD diagnostics. Still, their implementation in clinical practice has been a difficult trajectory marked by obstacles such as inter-laboratory and intra-laboratory variation.3,4 Efforts of collaborative initiatives such as BIOMARK-ADP,5 together with technological innovations, have led to the
development of standardized operating procedures for CSF collection and storage,\(^5\,^7\) a certified reference measurement procedure for A\(_{\beta1-42}\),\(^8\) and fully automated assays for the CSF biomarkers that have been calibrated against this reference method.\(^9\,^12\) These achievements greatly reduced the variation in CSF biomarker results across and within laboratories.\(^13\) The final goal of successful biomarker implementation is to establish global cut-off values that are independent of assay platform, cohort, or laboratory.

Because next generation automated assays seem to detect A\(_{\beta1-42}\) more accurately than the older manual immunoassays, that is, with less interference of A\(_{\beta40}\), and show different concentration ranges,\(^9\,^14\) re-establishment of biomarker cut-offs is essential. The current gold standard for cut-off determination of CSF biomarkers is amyloid positron emission tomography (PET), which is approved by the Food and Drug Administration (FDA) for in vivo amyloid pathology, or clinical diagnosis, because a definite diagnosis of AD can only be made at autopsy. Previous studies using either Elecsys or Lumipulse assays showed that CSF biomarker ratios improved the agreement with amyloid PET compared to single biomarker cut-points.\(^15\,^20\) For example, using the A\(_{\beta1-42}/A\beta40\) ratio compared to A\(_{\beta1-42}\) alone improved the concordance, which is hypothesized to be due to A\(_{\beta40}\) correcting for inter-individual biological variation in amyloid production and/or clearance, and/or A\(_{\beta40}\) correcting for artificial decrease of A\(_{\beta1-42}\) concentrations during the pre-analytical phase.\(^21\,^23\) Ratios of A\(_{\beta1-42}\) with p-tau or t-tau also improved the concordance with amyloid PET in research cohorts using either Elecsys or Lumipulse biomarkers.\(^15\,^20\) These findings call for a head-to-head validation of the performance of the different biomarker interpretation modalities on the different platforms in the clinical setting, such as the memory clinic. Also, it remains to be addressed whether similar cut-offs for the automated assays can be applied. Introduction of these automated biomarker assays in diagnostic practice calls for a re-evaluation of the use of application and optimal interpretation of biomarker results in a clinical setting.

We aimed to determine how the ratios of biomarkers, A\(_{\beta1-42}/A\beta40\), p-tau/A\(_{\beta1-42}\), and t-tau/A\(_{\beta1-42}\) improved discrimination of amyloid PET positivity compared to A\(_{\beta1-42}\) alone in a retrospective memory clinic cohort including AD and other types of dementia, to evaluate whether the improved performance was dependent on the automated platform used, and to define cut-off values for all biomarker combinations. Last, we compared our biomarker cut-offs to previously published cut-offs defined on the same platforms to evaluate the feasibility of a future universal cut-off.

## 2 METHODS

### 2.1 Study population

We selected CSF samples from patients from the Amsterdam Dementia Cohort\(^24\) that visited the memory clinic between 2006 and 2016 when they had an amyloid PET scan within one year of CSF collection available. All subjects underwent extensive neurological examination, neuropsychological testing, neuroimaging, and CSF biomarker testing as part of the diagnostic work-up. Clinical diagnoses were established by consensus during a multidisciplinary meeting according to consensus criteria.\(^25\,^28\) Diagnostic groups included in the current study were subjects presenting with subjective cognitive decline (SCD, n = 58), mild cognitive impairment (MCI, n = 42), possible/probable AD (n = 145), frontotemporal dementia (FTD, n = 23), dementia with Lewy bodies (DLB, n = 6), vascular dementia (VaD, n = 5), or other dementia (n = 9). All patients signed written informed consent to use medical and biomaterials for research purposes and the study was approved by the local ethical committee in accordance with the Declaration of Helsinki.

### 2.2 CSF biomarker measurements

#### 2.2.1 CSF collection and processing

CSF samples were obtained by lumbar puncture using a 25-gauge needle and syringe between the L3/L4, L4/L5, or L5/S1 intervertebral space, collected in polypropylene tubes and processed as previously described.\(^29\)

#### 2.2.2 Elecsys assays

A\(_{\beta1-42}\), p-tau (181P), and t-tau (Roche Diagnostics GmbH) were analyzed in CSF samples by board-certified technicians using the fully

### RESEARCH IN CONTEXT

1. **Systematic review**: Literature was reviewed using PubMed and meeting abstracts or presentations. The cerebrospinal fluid (CSF) Elecsys and Lumipulse biomarkers have separately been studied in a few recent publications, which are cited, but were not previously compared in a head-to-head comparison.

2. **Interpretation**: Our findings show that for both Elecsys and Lumipulse assays phosphorylated tau/amyloid beta (A\(_{\beta1-42}\)) ratios outperformed CSF A\(_{\beta1-42}\) alone in detecting positive amyloid positron emission tomography (PET) in a clinical diagnostic setting. Of note, concordance improved similarly for both the A\(_{\beta1-42}/A\beta40\) and total tau/A\(_{\beta1-42}\) ratios. Cut-offs were platform-specific, but biomarker concordance with amyloid PET positivity did not depend on the platform used in this head-to-head comparison.

3. **Future directions**: For clinical implementation, future studies should perform multicenter comparisons to further address the feasibility of determining universal cut-points for these ratios, independent of assay platform.
automated Elecsys biomarker assays.14 CSF of 17 samples (6%) needed transfer to a 0.5 mL Sarstedt tube as the original 2.0 mL Sarstedt tubes evoked an error on the Cobas e601 analyzer due to low sample volume (< 0.5 mL). No systematic effect in \( A_\beta_{1-42} \) results was observed between the transferred and the non-transferred samples (data not shown). The Elecsys \( A_\beta_{1-42} \) concentration exceeded the upper limit of detection of the assay at 1700 pg/mL in 42 cases (15%); these concentrations were included in all further analyses and graphs as 1700 pg/mL.

### 2.2.3 | Lumipulse assays

Next, pristine aliquots of the same samples were measured for \( A_\beta_{1-42} \), \( A_\beta_{1-40} \), p-tau (181P), and t-tau on the Lumipulse G 1200 system (Fujirebio Diagnostics, Inc.)19,20,30,31 by board-certified technicians according to manufacturer’s instructions. CSF of 16 samples (6%) needed transfer to a 1.7 mL polystyrene Hitachi tube as the original 2.0 mL Sarstedt tubes evoked an error on the Lumipulse analyzer. No systematic effect in \( A_\beta_{1-42} \) results was observed between the transferred and the non-transferred samples (data not shown).

### 2.3 | PET amyloid imaging

Amyloid PET imaging was conducted using \(^{11}\text{C}-\text{PiB} \) (n = 86), \(^{18}\text{F}-\text{florbetaben} \) (n = 133), \(^{18}\text{F}-\text{flutemetamol} \) (n = 64), and \(^{18}\text{F}-\text{florbetapir} \) (n = 5) tracers.32–35 PET scans were evaluated based on visual reading according to the manufacturers’ guidelines by an experienced nuclear medicine physician (BvB) and included as dichotomized scores (i.e., positive and negative).

### 2.4 | Statistical analyses

Groups were dichotomized for amyloid PET status, and pair-wise comparisons of demographic characteristics and biomarker concentrations were performed with chi-square (for categorical variables), Student’s t (for continuous variables with normal distribution), and Mann Whitney U (for continuous variables with non-normal distribution) tests. Biomarker cut-points were calculated based on optimal Youden’s index in receiver operating curve (ROC) analyses with amyloid PET result as gold standard. Areas under the curve (AUCs) were compared pairwise across \( A_\beta_{1-42} \), \( A_\beta_{1-42}/A_\beta_{1-40} \), p-tau/\( A_\beta_{1-42} \), and t-tau/\( A_\beta_{1-42} \) and were statistically compared per platform using 2000 bootstrapping iterations in the “roc.test” function of the “pROC” package (version 1.16.2) with D-statistic indicating the difference between the two AUCs.36 As the Elecsys assays did not include \( A_\beta_{1-40} \), the Elecsys \( A_\beta_{1-42}/A_\beta_{1-40} \) ratio was calculated with the Lumipulse \( A_\beta_{1-40} \) result. The AUC comparisons were corrected for multiple testing using Bonferroni correction: per assay six ratios were pair-wise compared, \( P \)-value threshold was 0.00833 (= 0.05/6); between assays (Elecsys versus Lumipulse) four ratios were pair-wise compared, \( P \)-value threshold was 0.0125 (= 0.05/4). Sensitivity, specificity, and overall percentage agreements (OPAs) were calculated for all biomarkers and biomarker ratios to detect positive amyloid PET status. Spearman correlations and Passing-Bablok regression analyses for direct comparison between Elecsys and Lumipulse biomarker concentrations were performed using the “mcr” package in R (version 1.2.1).37 Data analysis was performed using R statistical programming (version 3.6.1)38 and if not mentioned otherwise, \( P \)-values below 0.05 were considered statistically significant.

### 2.5 | Comparison of cut-points across global cohorts

We searched the literature for publications of CSF \( A_\beta \) cut-points to determine amyloid PET positivity in other cohorts using Elecsys or Lumipulse assays to evaluate the comparability of cut-points across these different settings and cohorts. We excluded the Elecsys \( A_\beta_{1-42}/A_\beta_{1-40} \) cut-point from our cohort in the comparison, because this ratio was calculated using the Lumipulse \( A_\beta_{1-40} \) result and no previous literature was available. Literature was selected by searching the PubMed database with combinations of terms “Elecsys” OR “Lumipulse,” AND “amyloid imaging” AND “concordance.” Papers that established cut-off values for Elecsys or Lumipulse assays to determine amyloid PET positivity, assessed by title and abstract screening, were included. We chose to report only one cut-point per cohort with preference for cut-points based on Youden’s index and preference for amyloid PET outcomes based on visual reads to align with the approach of the current study.

### 3 | RESULTS

#### 3.1 | Cohort characteristics

We included 288 individuals in the present study, who were on average 63 ± 7 years old; 131 (45%) were female and 179 (62%) had a positive amyloid PET read (Table 1). Compared to negative, amyloid PET-positive subjects had lower Mini-Mental State Examination (MMSE) scores, more often carried one or two apolipoprotein E (APOE) ε4 allele(s), and most often had AD-type dementia. Median time delay between CSF collection and PET imaging was 29 days and did not differ between the amyloid PET-positive and -negative groups. Compared to those with normal amyloid PET, patients with abnormal amyloid PET showed decreased CSF \( A_\beta_{1-42} \), increased CSF t-tau and p-tau concentrations (Table 1; Figure 1), but no significant difference in CSF \( A_\beta_{1-40} \) concentrations. In 42 cases (15%), the Elecsys \( A_\beta_{1-42} \) concentration exceeded the upper limit of detection of the assay at 1700 pg/mL, resulting in artificially skewed distributions. CSF \( A_\beta_{1-42} \) concentrations and the ratios of \( A_\beta_{1-42}/A_\beta_{1-40} \), p-tau/\( A_\beta_{1-42} \), and t-tau/\( A_\beta_{1-42} \) for both Elecsys and Lumipulse assays all showed different values for amyloid PET-positive cases compared to amyloid PET-negative cases (\( P < 0.001; \) Figure 1).
### Table 1: Cohort characteristics, stratified for amyloid PET visual read status

|                      | Total | Amyloid PET – | Amyloid PET + | \( P \)-value of pair-wise comparison |
|----------------------|-------|---------------|---------------|---------------------------------------|
| **N**                | 288   | 109           | 179           |                                       |
| **Sex = f (%)**      | 131 (45%) | 43 (39%)      | 88 (49%)      | 0.114                                 |
| **Age (mean [SD])**  | 63 (7) | 62 (8)        | 63 (6)        | 0.086                                 |
| **MMSE (mean [SD])** | 24 (4) | 26 (3)        | 23 (4)        | <0.001                                |
| **APOE \( \varepsilon \)4 carrier (%)** | | | | <0.001 |
| Unknown              | 8 (3) | 0 (0)         | 8 (5)         |                                       |
| Non-carrier          | 122 (42)| 67 (62)      | 55 (31)       |                                       |
| Carrier              | 158 (55)| 42 (39)      | 116 (65)      |                                       |
| **Days between CSF collection and PET imaging (median [IQR])** | 29 [15, 57] | 24 [14, 62] | 30 [16, 52] | 0.435 |
| **Diagnosis (%)**    |       |               |               | 2.044                                 |
| SCD                  | 58 (20)| 44 (40)      | 14 (8)        |                                       |
| MCI                  | 42 (15)| 17 (16)      | 25 (14)       |                                       |
| AD                   | 145 (50)| 10 (9)      | 135 (75)      |                                       |
| FTD                  | 23 (8) | 22 (20)      | 1 (1)         |                                       |
| DLB                  | 6 (2)  | 4 (4)        | 2 (1)         |                                       |
| VaD                  | 5 (2)  | 5 (5)        | 0 (0)         |                                       |
| Dementia other       | 9 (3)  | 7 (6)        | 2 (1)         |                                       |
| **Elecsys CSF A\( \beta \)1-42 (pg/mL, median [IQR])** | 852 [681, 1230] | 1522 [1097, 1700] | 742 [608, 872] | <0.001 |
| **Elecsys CSF p-tau (pg/mL, median [IQR])** | 27 [17, 39]| 15 [12, 20]| 35 [26, 44] | <0.001 |
| **Elecsys CSF t-tau (pg/mL, median [IQR])** | 282 [196, 368] | 195 [145, 262] | 336 [268, 405] | <0.001 |
| **Lumipulse CSF A\( \beta \)1-42 (pg/mL, median [IQR])** | 606 [478, 838] | 983 [692, 1312] | 529 [438, 616] | <0.001 |
| **Lumipulse CSF A\( \beta \)1-40 (pg/mL, median [IQR])** | 11770 [9874, 14064] | 11853 [8739, 14106] | 11744 [10090, 14048] | 0.247 |
| **Lumipulse CSF p-tau (pg/mL, median [IQR])** | 70 [38, 115]| 33 [25, 46]| 101 [74, 129] | <0.001 |
| **Lumipulse CSF t-tau (pg/mL, median [IQR])** | 520 [355, 755]| 355 [290, 442]| 656 [502, 852] | <0.001 |

Pair-wise comparisons were performed using chi-square tests for categorical variables, T-tests for continuous normally distributed variables and Mann-Whitney U test for non-normally distributed variables.

Abbreviations: A\( \beta \), amyloid beta; AD, Alzheimer’s disease; APOE, apolipoprotein E; CSF, cerebrospinal fluid; DLB, dementia with Lew bodies; FTLD, frontotemporal lobar dementia; IQR, interquartile ratio; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; PET, positron emission tomography; p-tau, phosphorylated tau; SCD, subjective cognitive decline; SD, standard deviation; t-tau, total tau; VaD, vascular dementia.

### 3.2 Biomarker ratios are better predictors of PET amyloid positivity than A\( \beta \)1-42 alone

We performed ROC analyses per assay with amyloid PET as reference and compared AUCs of single CSF biomarker A\( \beta \)1-42, with ratios of A\( \beta \)1-42 with A\( \beta \)1-40, p-tau, or t-tau (Figure 2, Tables 2 and 3). For both platforms, the p-tau/A\( \beta \)1-42 ratio resulted in the highest AUCs (95% confidence interval [CI]) and overall percentage agreements (95% CI): 0.95 (0.89–0.96) and 93 (90–96)% for Elecsys, 0.96 (0.93–0.99) and 94 (92–97)% for Lumipulse. AUCs and overall percentage agreements were also high for both A\( \beta \)1-42/A\( \beta \)1-40 (0.93 [0.89–0.96] and 90 [86–93]%) for Elecsys; 0.94 [0.91–0.98] and 92 [89–96]% for Lumipulse) and t-tau/A\( \beta \)1-42 (0.94 [0.91–0.98] and 90 [86–94]%) for Elecsys; 0.94 [0.90–0.97] and 90 [87–94]% for Lumipulse). For both Elecsys and Lumipulse assays, ratios with p-tau, t-tau, or A\( \beta \)1-40 performed better than A\( \beta \)1-42 alone (\( P < 0.01 \)). Sensitivity, specificity, and OPA percentages were largely overlapping across A\( \beta \)1-42 and the A\( \beta \)1-42 ratios, except for p-tau/A\( \beta \)1-42 versus A\( \beta \)1-42, for which the 95% CI of the OPA was higher and not overlapping with that of A\( \beta \)1-42 for either Elecsys or Lumipulse. For Lumipulse, additionally, the 95% CI of the OPA for the A\( \beta \)1-42/A\( \beta \)1-40 ratio (upper limit at 89%) did not overlap with that of A\( \beta \)1-42 (lower limit at 89%).

Sensitivity analyses including only patients with SCD, MCI, or AD-type dementia showed essentially similar results (Table S1 in supporting information). Biomarker cut-points and overall percentage agreements, and their 95% CIs, for A\( \beta \)1-42 and ratios were nearly identical. Again, for both Elecsys and Lumipulse, the p-tau:A\( \beta \)1-42 ratio had the highest overall percentage agreement with 95% CI not overlapping with that of A\( \beta \)1-42.
**FIGURE 1** Distribution of biomarkers and biomarker ratios between the amyloid positron emission tomography (PET)-positive and -negative groups. Boxplot with beeswarm for Elecsys (upper row) and Lumipulse (bottom row) biomarkers amyloid beta (Aβ) (A), Aβ/Aβ ratio (B), phosphorylated tau/Aβ ratio (C), and total tau/Aβ ratio (D) in relation to an amyloid PET-negative or -positive result. Dotted lines represent the cut-point obtained through receiver operating characteristic analysis (Table 2).

**FIGURE 2** Receiver operating characteristic curves of amyloid beta (Aβ) alone and as ratio of Aβ/Aβ, phosphorylated tau, or total tau to predict positron emission tomography (PET) amyloid positivity for the Elecsys (A) and Lumipulse (B) assays. See Table 2 for areas under the curves and concordance percentages. The gray line represents the identity line.
TABLE 2  Concordance of Elecsys and Lumipulse biomarker concentrations and ratios with amyloid PET result

| Biomarker | Method | AUC [95% CI] | Cut-point [95% CI] | Sensitivity [95% CI] | Specificity [95% CI] | OPA [95% CI] |
|-----------|--------|-------------|--------------------|---------------------|----------------------|--------------|
| Aβ1-42    | Elecsys| 0.88 [0.83–0.92] | 1089 [864–1120] pg/mL | 91 [77–95] % | 75 [69–89] % | 85 [80–89] % |
|           | Lumipulse| 0.88 [0.84–0.93] | 714 [606–798] pg/mL | 91 [75–98] % | 73 [65–91] % | 84 [79–89] % |
| Aβ1-42/Aβ1-40 | Elecsys Aβ1-42; Lumipulse Aβ1-40 | 0.93 [0.89–0.96] | 0.091 [0.080–0.10] | 96 [86–99] % | 80 [73–91] % | 90 [86–93] % |
|           | Lumipulse| 0.94 [0.91–0.98] | 0.071 [0.056–0.073] | 99 [89–100] % | 83 [79–94] % | 92 [89–96] % |
| p-tau/Aβ1-42 | Elecsys| 0.95 [0.92–0.98] | 0.020 [0.020–0.027] | 96 [90–98] % | 89 [84–96] % | 93 [90–96] % |
|           | Lumipulse| 0.96 [0.93–0.99] | 0.072 [0.052–0.095] | 97 [91–100] % | 91 [85–97] % | 94 [92–97] % |
| t-tau/Aβ1-42 | Elecsys| 0.94 [0.91–0.98] | 0.277 [0.194–0.313] | 89 [83–98] % | 90 [81–97] % | 90 [86–94] % |
|           | Lumipulse| 0.94 [0.90–0.97] | 0.688 [0.54–0.83] | 91 [83–96] % | 90 [84–97] % | 90 [87–94] % |

Abbreviations: Aβ, amyloid beta; AUC, area under the curve; CI, confidence interval; OPA, overall percentage agreement; PET, positron emission tomography; p-tau, phosphorylated tau; t-tau, total tau.

TABLE 3  Pair-wise statistical comparisons of AUCs from ROC analyses across biomarker concentrations and ratios

| Biomarker (ratio) comparison | Biomarker platform | D statistic | P-value |
|-----------------------------|--------------------|-------------|---------|
| Aβ1-42 vs. Aβ1-42/Aβ1-40    | Elecsys            | 3           | 0.005   |
| Aβ1-42 vs. p-tau/Aβ1-42     | Elecsys            | 4.27        | 0.00002 |
| Aβ1-42 vs. t-tau/Aβ1-42     | Elecsys            | 4           | 0.00006 |
| Aβ1-42/Aβ1-40 vs. p-tau/Aβ1-42 | Elecsys            | 2.42        | 0.02    |
| Aβ1-42/Aβ1-40 vs. t-tau/Aβ1-42 | Elecsys            | 2           | 0.1     |
| p-tau/Aβ1-42 vs. t-tau/Aβ1-42 | Elecsys            | 2.25        | 0.02    |
| Aβ1-42 vs. Aβ1-42/Aβ1-40    | Lumipulse          | 3           | 0.001   |
| Aβ1-42 vs. p-tau/Aβ1-42     | Lumipulse          | 4           | 0.00002 |
| Aβ1-42 vs. t-tau/Aβ1-42     | Lumipulse          | 3           | 0.002   |
| Aβ1-42/Aβ1-40 vs. p-tau/Aβ1-42 | Lumipulse          | 1           | 0.2     |
| Aβ1-42/Aβ1-40 vs. t-tau/Aβ1-42 | Lumipulse          | -0.6        | 0.6     |
| p-tau/Aβ1-42 vs. t-tau/Aβ1-42 | Lumipulse          | 2           | 0.02    |
| Aβ1-42 vs. Aβ1-42          | Elecsys vs. Lumipulse | -0.9       | 0.4     |
| Aβ1-42/Aβ1-40 vs. p-tau/Aβ1-40 | Elecsys vs. Lumipulse | -2.0       | 0.02    |
| p-tau/Aβ1-42 vs. t-tau/Aβ1-42 | Elecsys vs. Lumipulse | -1.4       | 0.2     |
| t-tau/Aβ1-42 vs. Aβ1-42 vs. Lumipulse | 0.9       | 0.4     |

Note: AUC distributions were compared using 2000 bootstrapping iterations. Bonferroni correction for multiple testing was applied; p-values that were below threshold are indicated in bold. Abbreviations: Aβ, amyloid beta; AUC, area under the curve; p-tau, phosphorylated tau; ROC, receiver operating characteristic; t-tau, total tau.

3.3 | Direct comparison Aβ1-42, p-tau, and t-tau between Elecsys and Lumipulse

Biomarkers Aβ1-42, p-tau, and t-tau correlated well between Elecsys and Lumipulse assays, with Spearman correlations of 0.97, 0.96, and 0.89, respectively (all P < 0.001). Conversion formulas to translate Elecsys to Lumipulse biomarker results obtained by Passing-Bablok regression analyses are presented in Figure S1 in supporting information.

3.4 | Comparison of cut-points with literature

Finally, Table 4 shows our cut-points listed together with those of previous studies. Five cohorts other than the current study reported cut-points for Elecsys biomarkers and three cohorts did so for Lumipulse biomarkers (Table 4). The majority of studies (four out of six studies; including in total 1392 patients from five independent cohorts) have used Elecsys, and only two other studies used Lumipulse (two out of six studies, including in total 411 patients from three independent cohorts). Previous determined cut-points used the optimized Youden’s index, except for the BioFinder and Alzheimer’s Disease Neuroimaging Initiative cohorts, which were calculated based on optimized performance (positive predictive agreement [PPA] and negative predictive agreement [NPA]) and stability of PPA and NPA when varying cut-offs slightly. For Elecsys, cut-offs showed comparable values for different markers, except for Aβ1-42 in the Expedition cohorts that had a much higher cut-point. For Lumipulse, cut-offs for biomarker ratios were very comparable, but that of Aβ1-42 was similar to Knight’s Alzheimer’s Disease Research Center cohort, but lower than the Sant Pau Initiative on Neurodegeneration (SPIN) and Eisai cohorts, probably due to differences in cohort composition.

4 | DISCUSSION

In this large clinical sample set with CSF and PET measures for amyloid, we found that next generation fully automated Elecsys and Lumipulse assays showed similar high concordance with amyloid PET (OPA: 90%–94%) when using biomarker ratios with either Aβ1-40 or t-tau or p-tau, and improved concordance compared to CSF Aβ1-42 alone (OPA: 84%–85%). Cut-points for Elecsys and Lumipulse biomarkers were largely...
### TABLE 4  
Cut-points for $A\beta_{1-42}$ and $A\beta_{1-40}$ ratios using Elecsys and Lumipulse assays in comparison to amyloid PET imaging across global cohorts

| Cohort               | Cohort composition  | N (%) PET | Biomarker method | Cut-point method | Cut-point $A\beta_{1-42}$ (pg/mL) | Cut-point $A\beta_{1-42}/A\beta_{1-40}$ | Cut-point p-tau/A$\beta_{1-42}$ | Cut-point t-tau/A$\beta_{1-42}$ | Amyloid PET method |
|----------------------|---------------------|-----------|------------------|-----------------|----------------------------------|--------------------------------------|-------------------------------|-------------------------------|------------------|
| ADC                  | n = 58 SCD; n = 42 MCI; n = 145 AD; n = 23 FTD; n = 6 DLB; n = 5 VaD; n = 9 other dementia | 179 (62%)  | Elecsys          | Youden’s        | 1089 [95% CI: 864–1120]          | 0.02 [95% CI: 0.020–0.028]           | 0.277 [95% CI: 0.194–0.313] | Visual reads |
| AIBL<sup>15</sup>    | n = 140 CN; n = 33 MCI; n = 27 AD; n = 2 FTD | 84 (42%)   | Elecsys          | Youden’s        | 1054 0.064 0.0183 0.258 | Quantitative SUVR, dichotomized on tracer-specific threshold |
| BioFINDER<sup>16</sup> | n = 120 SCD; n = 153 MCI | 110 (40%)  | Elecsys          | Optimized for (1) performance (PPA and NPA) and (2) stability of PPA and NPA when varying cut-offs slightly | 1100 n/a 0.022 0.26 | Visual reads |
| ADNI<sup>16</sup>    | n = 94 SMC (significant memory concern); n = 272 EMC; n = 152 LMCI; n = 128 AD | 347 (54%)  | Elecsys          | Optimized for (1) performance (PPA and NPA) and (2) stability of PPA and NPA when varying cut-offs slightly | 977 n/a 0.025 0.27 | Visual reads |
| Knight’s ADRC<sup>17</sup> | Community-dwelling volunteers involved in normal aging and dementia studies; CDR 0/0.5/1/2/3; n = 176/183/1/0 | 50 (25%)   | Elecsys          | Youden’s        | 1098 0.075 0.0198 0.211 | Quantitative SUVR, dichotomized on tracer-specific threshold |
| EXPEDITION and EXPEDITION2<sup>18</sup> | n = 55 mild AD; n = 20 moderate AD, participating in phase 3, double-blind, placebo-controlled international trials of solanezumab | Not reported for visual reads | Elecsys          | Youden’s        | 1198 0.0233 0.289 | Visual reads |

(Continues)
| Cohort          | Cohort composition                                                                 | N (%) PET+ | Biomarker method | Cut-point method | Cut-point Aβ1-42 | Cut-point Aβ1-42/Aβ1-40 | Cut-point p-tau/Aβ1-42 | Cut-point t-tau/Aβ1-42 | Amyloid PET method |
|-----------------|------------------------------------------------------------------------------------|------------|------------------|------------------|-----------------|------------------------|-----------------------|----------------------|---------------------|
| ADC             | n = 58 SCD; n = 42 MCI; n = 145 AD; n = 23 FTD; n = 6 DLB; n = 5 VaD; n = 9 other dementia | 179 (62%)  | Luminpulse       | Youden’s         | 714 [95% CI: 606–798] | 0.071 [95% CI: 0.056–0.073] | 0.072 [95% CI: 0.052–0.095] | 0.688 [95% CI: 0.54–0.83] | Visual reads         |
| SPIN            | n = 6 CN; n = 35 MCI; n = 12 AD; n = 30 DLB; n = 9 FTD; n = 2 other diagnoses       | 59 (63%)   | Luminpulse       | Youden’s         | 916             | 0.062                  | 0.068                  | 0.62                 | Visual reads         |
| Eisai           | Subjects with early AD included in the BAN2401-201 and MISSION AD E2609-301/302 clinical trials; CDR 0/0.5/1/2: n = 0/120/10/0 | 81 (62%)   | Luminpulse       | Youden’s         | 818             | n/a                    | n/a                   | 0.53                 | Visual reads         |
| Knight’s ADRC   | Community-dwelling volunteers involved in normal aging and dementia studies; CDR 0/0.5/1/2: n = 165/18/3/1 | 49 (26%)   | Luminpulse       | Youden’s         | 732             | n/a                    | n/a                   | 0.54                 | Quantitative SUVR, dichotomized on tracer-specific threshold |

Note: The ADC cohort (current study) is taken as the reference; cut-points of other cohorts that exceed the 95% CI range of the ADC cohort are underlined.

Abbreviation: Aβ, amyloid beta; AD, Alzheimer’s disease; ADC, Amsterdam Dementia Cohort; ADNI, Alzheimer’s Disease Neuroimaging Initiative; ADRC, Alzheimer’s Disease Research Center; CDR, Clinical Dementia Rating; CI, confidence interval; CN, cognitively normal; DLB, dementia with Lewy bodies; FTD, frontotemporal dementia; MCI, mild cognitive impairment; NPA, negative predictive agreement; PET, positron emission tomography; PPA, positive predictive agreement; p-tau, phosphorylated tau; SCD, subjective cognitive decline; SPIN, Sant Pau Initiative on Neurodegeneration; SUVR, standardized uptake value ratio; t-tau, total tau; VaD, vascular dementia.
Our finding that CSF biomarker ratios of Aβ1-42 show better concordance with the amyloid PET outcome than Aβ1-42 alone for Elecsys and Lumipulse assays is in line with previous findings. This suggests that method calibration of the next-generation assays has indeed increased the consistency in biomarker results reported across studies. It is supposed that decreases in CSF Aβ1-42 reflect aggregation of soluble Aβ1-42 into plaques. Our, and other, results suggest that apparently the improved measurements of the soluble part of Aβ1-42 makes it more difficult to measure aggregation. The concordance of CSF Aβ1-42 with amyloid PET results was ±93% with the older generation Innoltest assay, but decreased to ±85% using the next-generation assays (current study and Janelidze et al.5 and Doecke et al.41). Direct comparison studies of soluble Aβ1-42 measured with older versus newer generation assays showed an r² of 0.8 to 0.9, which was lower than Aβ1-42 correlations between next-generation assays only.5,14,42 suggesting that older and newer assays may not identically reflect Aβ1-42. We and others collectively show that ratios with Aβ1-42 for the next-generation assays strongly improve concordance with amyloid PET to 90% to 95%,15-20 For Aβ1-42/Aβ1-40 an explanation might be that this ratio better reflects the rate of amyloid precursor protein metabolism and as such correct for physiological Aβ1-42 effects. Aβ1-42 as ratio of p-tau or t-tau might give a better reflection of aggregation likely due to the correlation of high p-tau and t-tau levels with amyloid plaques.43 It might seem less intuitive to combine CSF p-tau with Aβ1-42 for prediction of amyloid PET, although for clinical use combining two hallmark pathologies instead of only the amyloid pathology contributes to a more accurate risk prediction of developing AD in preclinical stages.44

We achieved a concordance of 90% to 94% for CSF Aβ1-42 biomarkers and ratios compared to PET. The small number of cases with discordant CSF and PET results could be explained by either changes in CSF Aβ1-42 preceding those in amyloid PET15 or amyloid PET changes preceding those in CSF.46 Longitudinal studies showed that patients with CSF+/PET− amyloid status seem to be in the earliest stages of AD development, as they turned amyloid positive on PET within the next years.45,47,48 Patients with CSF-/PET+ discordant amyloid status did not develop amyloid or tau accumulation on PET in the next five years,48 but did deteriorate on cognition,47 suggesting that CSF and PET amyloid reflect different aspects of amyloid pathology.

Comparison of biomarker cut-points across assays and cohorts (Table 4) suggests similar performance of Elecsys and Lumipulse assays; these assays can thus be used interchangeably to detect amyloid positivity, provided that assay-specific cut-offs are used. For multicenter studies, we recommend using one type of assay or using dichotomized biomarker results based on assay-specific cut-points. It is important to mention that cut-points and corresponding sensitivity and specificity percentages when based on Youden’s index will naturally show variation across cohorts that is inherent to differences in cohort compositions (i.e., diagnoses, disease severity, and age). For Lumipulse in particular, larger cohorts are required to assess the across-cohort stability of biomarker cut-points. Also, cut-points will depend on pre-analytical conditions. Pre-analytics were the same for the analyses within the current study, but not completely similar compared to the other studies presented in Table 4 nor to the situation deemed ideal in routine diagnostics, which is direct biomarker analysis without sample freezing. The latter would, however, be hard to implement in view of analyses of samples that are shipped, for example, from smaller memory clinics without biomarker lab facilities or for centralized biomarker analyses performed in clinical trials. Lumipulse assay standards were recalibrated against the certified reference material at time of biomarker analysis in this study,9 but the Elecsys assays were not. Recent recalibration of the Elecsys assay standards compared to Lumipulse and another assay showed the promising result of < 9% between-assay bias in Aβ1-42 concentrations measured in the certified reference materials.50 Assay comparison studies in clinical cohorts should further examine the feasibility of using global cut-points for CSF biomarker interpretation with these recalibrated assays.

Our study was performed in a real-world memory clinic setting as we did not only include patients in the AD dementia spectrum, but also other dementias such as FTD, DLB, and VaD, which do not typically show amyloid pathology. The agreement of the CSF amyloid and amyloid PET results was however not different in our cohort when we excluded diagnoses other than AD, MCI, or SCD (15% of the original cohort), suggesting that CSF biomarkers perform well for amyloid PET positivity regardless of clinical diagnosis. This supports the use of CSF biomarkers in clinical diagnostic settings.

Because the Aβ1-40 assay is not commercially available for Elecsys for use in clinical practice, we here combined the Lumipulse Aβ1-40 result with the Aβ1-42 result from Elecsys. This resulted in an AUC of 0.93, similar to AUCs reported in studies that measured the amyloid peptides on the same platform.15,17 Potential noise due to differences in reagents and protocols between platforms was thus not reflected in the performance of this combined ratio. To enable use of amyloid ratios in clinical practice, we therefore suggest that the Elecsys biomarkers can be combined with the Aβ1-40 result from another platform, such as Lumipulse, to obtain a ratio of Aβ1-42/Aβ1-40.

The major strength of this study is that we compared CSF biomarker results between two next-generation (Elecsys and Lumipulse) assays in the same dataset. As both assays are applied in a clinical setting and for clinical trial analyses, such head-to-head comparison is important for future alignment of biomarker results interpretation. Our results show a strong agreement between biomarker ratios and amyloid PET for both platforms, meaning that biomarker outcomes from either platform reliably reflect the presence of amyloid pathology, as long as the platform-specific cut-points are applied.

A limitation of the current study is that the Elecsys Aβ1-42 assay has its upper limit of detection at 1700 pg/mL. Although for diagnostic purposes (when biomarker status is determined for dichotomized values) this is not an issue, it hampers research on better understanding continuous CSF concentrations.51 The performance of the Elecsys ratios might be slightly underestimated by including these values as 1700 pg/mL instead of their actual, higher concentration, because for two to five cases the resulting biomarker ratio was classified as pathological (which was not the case when entering a hypothetical value,
e.g., 2500 pg/mL). Another limitation could be that quantitative analyses for the PET scans were not available in this study. Visual reads, however, are the FDA-approved method of identifying amyloid positivity; moreover, scans were read by one experienced nuclear medicine physician (BvB) and according to standardized procedures, which increases robustness of the reading results. Furthermore, different tracers were used for amyloid PET scoring, but any potential variation was minimized by using visual reads of PET results and the intra-rater reliability of different tracers applied within one subject was 100% (BvB).

Altogether, based on the data here presented we recommend using the p-tau/Aβ1-42, Aβ1-42/Aβ1-40, or t-tau/Aβ1-42 ratio for AD pathology when using the automated assays Elecsys or Lumipulse, as these most accurately reflect the amyloid PET result. These ratios can be used for CSF biomarker interpretation in routine clinical settings or for clinical trial evaluation.

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CONFLICTS OF INTEREST
Eline A.J. Willemse, Betty M. Tijms, Bart N.M. van Berckel, Wiesje M. van der Flier, Philip Scheltens, and Charlotte E. Teunissen have no competing interests to declare. Nathalie Le Bastard is a full-time employee of Fujirebio Europe NV, Gent, Belgium.

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Study supervision: Philip Scheltens and Charlotte E. Teunissen. Study conception and design: Eline A.J. Willemse, Betty M. Tijms, Nathalie Le Bastard, Wiesje M. van der Flier, Philip Scheltens, and Charlotte E. Teunissen. Data acquisition: Eline A.J. Willemse, Bart N.M. van Berckel, Wiesje M. van der Flier, Philip Scheltens. Analysis and interpretation of data: Eline A.J. Willemse, Betty M. Tijms, Wiesje M. van der Flier, Philip Scheltens, and Charlotte E. Teunissen. Drafting of the manuscript: Eline A.J. Willemse. Obtaining funding: Bart N.M. van Berckel, Wiesje M. van der Flier, Philip Scheltens, Nathalie Le Bastard, Charlotte E. Teunissen. All authors critically revised the manuscript for its intellectual content.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION
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