Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring 10 (2018) 563-572

CSF Biomarkers

Diagnostic performance of Elecsys immunoassays for cerebrospinal fluid Alzheimer's disease biomarkers in a nonacademic, multicenter memory clinic cohort: The ABIDE project

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Abstract

Introduction: We compared the automated Elecsys and manual Innotest immunoassays for cerebrospinal fluid (CSF) Alzheimer's disease biomarkers in a multicenter diagnostic setting.
Methods: We collected CSF samples from 137 participants in eight local memory clinics. Amyloid β(1–42) (Aβ42), total tau (t-tau), and phosphorylated tau (p-tau) were centrally analyzed with Innotest and Elecsys assays. Concordances between methods were assessed.
Results: Biomarker results strongly correlated between assays with Spearman's ρ 0.94 for Aβ42, 0.98 for t-tau, and 0.98 for p-tau. Using Gaussian mixture modeling, cohort-specific cut-points were estimated at 1092 pg/mL for Aβ42, 235 pg/mL for t-tau, and 24 pg/mL for p-tau. We found an excellent concordance of biomarker abnormality between assays of 97% for Aβ42 and 96% for both t-tau and p-tau.
Discussion: The high concordances between Elecsys and Innotest in this nonacademic, multicenter cohort support the use of Elecsys for CSF Alzheimer's disease diagnostics and allow conversion of results between methods.

Conflicts of interest: E.W., I.M., B.T., F.B., I.H., L.B., J.C., E.K., R.M., G.R., N.S., N.V., and M.Z. report no conflicts of interest. A.F. is a contractor of Roche, and S.W. is employed by Roche. Research programs of W.F. have been funded by ZonMW, NWO, EU-FP7, Alzheimer Nederland, Cardiovascular Onderzoek Nederland, stichting Dioraphte, Gieskes-Strijbis fonds, Pasman stichting, Biogen MA Inc, Boehringer Ingelheim, Piramal Neuroimaging, Roche BV, Janssen Stellar, Combinostics. W.F. has performed contract research for Biogen MA Inc and Boehringer Ingelheim. W.F. has been an invited speaker at Boehringer Ingelheim and Biogen MA Inc. W.F. holds the Pasman chair. All funding is paid to her institution. C.T. has functioned in advisory boards of Fujirebio and Roche, received nonfinancial support in the form of research consumables from ADx Neurosciences and Euroimmun, performed contract research, or received grants from Probiodrug, Janssen prevention center, Boehringer, Brainsonline, Axon Neurosciences, EIP Pharma, Roche.

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https://doi.org/10.1016/j.dadm.2018.08.006

Keywords: Elecsys; Innotest; Immunoassay; Cerebrospinal fluid; Biomarkers; Alzheimer's disease; Method comparison; Gaussian mixture modeling; Multicenter study; Nonacademic cohort; Clinical setting; Conversion formula

1. Introduction

Alzheimer's disease (AD) pathology is reflected in cerebrospinal fluid (CSF) by decreased levels of amyloid β(1–42) (Aβ42) and increased levels of phosphorylated tau (p-tau) and total tau (t-tau) [1,2]. These biomarkers are part of the diagnostic research criteria for AD [3–6]. However, use in clinical practice is hampered by high measurement variability, low diagnostic specificity toward non-AD neurocognitive diseases at the mild cognitive impairment (MCI) stage, and limited understanding of the role of CSF biomarkers versus other biomarker modalities in clinical practice and in design of clinical trials [7]. To achieve implementation of the CSF biomarkers in clinical practice, it is important to reduce the large variation observed in the current methods and results for CSF biomarker measurements. Major sources of intralaboratory and interlaboratory variations that currently hamper the establishment of universal biomarker cut-points are preanalytical handling of the CSF samples and analytical variation [8–12].

The Innotest enzyme-linked immunosorbent assay (ELISA) is widely used for routine CSF biomarker analysis and involves several steps of manual pipetting, which can cause variation in biomarker values. The interlaboratory variation of Innotest results has been reported to be >15% in an international quality control program (www.neurochem.gu.se/TheAlzAssQCprogram) [8]. Another complication is that for the Innotest an upward drift of Aβ42 values over time has been shown [8,13–15]. To reduce variation in manual immunoassays, multiplex assays or (semi)automated platforms are being developed [16–19]. The fully automated Elecsys assays for CSF Aβ42, t-tau CSF, and p-tau (181P) run on the *cobas e* 601 analyzer (Roche Diagnostics GmbH, Penzberg, Germany), an instrument commonly used in academic hospital laboratories [20]. The Elecsys assays are reported to have very good intralaboratory and interlaboratory variations of 2% to 5% [7]. The Aβ42 CSF assay has been validated technically in artificial or quality control CSF (www.neurochem.gu.se/TheAlzAssQCprogram) [7,20], and clinically in research cohorts [21,22]. The Elecsys assays have not yet been tested in a real-life setting, with a more diverse patient cohort and less standardized (pre)processing of samples compared with a research setting, that is, when CSF is collected in multiple, nonacademic memory clinics and sent to a central laboratory for biomarker analysis.

The aim of this study was to compare CSF biomarker results of the novel Elecsys assays with results obtained from the manual Innotest ELISAs. CSF samples were collected from nonacademic hospitals, reflecting variation in real-life diagnostic settings, and preanalytical protocol deviations were closely monitored.

2. Methods

2.1. Patients

This study analyzes data acquired as part of the AD biomarkers in daily practice (ABIDE) project that focuses on the optimal use of diagnostics test, including CSF, in daily clinical practice [23]. For the present study, we included patients that were screened for suspected AD in eight nonacademic local memory clinics. The prospective design and real-life setting of the project is representative for the variation in routine diagnostics.

We collected 137 CSF samples from patients attending eight different local memory clinics throughout the Netherlands. Inclusion criteria were presentation with suspected cognitive dysfunction at memory clinic, for which the doctor requested CSF analysis, and a Mini-Mental State Examination score ≥18. Patients were included between May 2015 and January 2017. The syndrome diagnosis was staged as "cognitively normal (CN)," "MCI," or "dementia," and was based on the severity of cognitive impairment and impairment in activities of daily living. All patients signed informed consent, and the study was approved by the institutional ethical committee.

2.2. Sample collection

CSF withdrawal and processing was performed according to the international consensus guidelines [24,25]. To allow for comparison of processing and analyses within the same CSF sample, the same volume of CSF (ranging from 0.5 to 7 mL; on average 2.5 mL) was collected in two tubes (Sarstedt 10 mL polypropylene, 62.610.018 [Nümbrecht, Germany]) per patient, which were processed according to an identical preanalytical protocol. Processing included centrifugation (1800*g*, 10 min, 4°C) and transfer of the supernatant to novel tubes of the same type, then the CSF sample was stored at 4°C or −20°C until transport. Clinicians and laboratory employees were personally instructed (E.W., I.M.) on use of these particular Sarstedt tubes for CSF. We closely monitored whether sample collection was per protocol and provided feedback if needed. Both tubes were sent to the

Neurochemistry Laboratory of the VU University Medical Center (since June 2018 Amsterdam UMC), cooled or frozen, and were kept at −80°C pending analysis of the biomarkers Aβ42, t-tau, and p-tau. The storage time at −80°C before biomarker analysis was 1 to 4 days for the Innotest, and 4 to 23 months for Elecsys. No differences in biomarker results were observed between different storage times (Supplementary Fig. 1).

2.3. CSF Aβ42, t-tau, and p-tau measurements

The tube analyzed with Innotest was tested in routine biomarker procedures within 2 weeks after arrival over the period May 2015 to January 2017 (Innotest Aβ42, Innotest htau-Ag, and Innotest p-tau (181P) assays, Fujirebio, Ghent, Belgium) according to the manufacturer's instructions. Aβ42 and p-tau were measured over two different lots and t-tau over three. No differences in biomarker results between lots were observed, based on the internal quality controls (data not shown). Innotest calibrator concentrations ranged from 63 to 4000 pg/mL for Aβ42, 40 to 2300 pg/mL for t-tau, and 16 to 1000 pg/mL for p-tau. Cut-points at our center were determined at 813 pg/mL for Aβ42 [15], 375 pg/mL for t-tau, and 52 pg/mL for p-tau [26]. The tubes analyzed by Elecsys were kept at −80°C until measurement within three consecutive days in May 2017 using the Elecsys Aβ42 CSF, Elecsys t-tau CSF, and Elecsys p-tau (181P) CSF assays (Roche Diagnostics GmbH) run on the *cobas e* 601 analyzer (Roche Diagnostics). Elecsys assays use a similar sandwich immunoassay principle as the Innotest assays, but are fully automated, and have been extensively validated in our laboratory [20]. Elecsys measuring ranges were as follows: 200 to 1700 pg/mL for Aβ42, 80 to 1300 pg/mL for t-tau, and 8 to 120 pg/mL for p-tau. For Elecsys Aβ42 levels above the upper limit of the measuring range (1700 pg/mL), values were extrapolated for data analysis (see Supplementary Material).

2.4. Statistical analyses

Statistical analyses were done in R version 3.4.0 [27]. Group comparisons for syndrome diagnosis were performed: age was statistically tested using an analysis of variance and a potential influence of collection center or gender was tested using the Fisher exact test. Mini-Mental State Examination and the CSF biomarker concentrations were tested with Kruskal-Wallis tests, followed by Dunn's post hoc multiple group comparisons using Bonferroni's *P* value correction. Innotest and Elecsys Aβ42, t-tau, and p-tau levels were compared using Spearman correlation and Passing–Bablok regression analysis, first including all samples, and then with samples stratified for preanalytical protocol compliance. Biomarker cut-points for Elecsys results were determined based on Gaussian mixture modeling [28,29]. For each biomarker, we first determined the number of distributions present in the data using a bootstrapping. Next, we defined a cut-point based on the point of intersection between the lines of distributions present in the sample as determined with Gaussian mixture modeling. Ninety-five percent confidence intervals (CIs) of the cut-point estimates were determined with bootstrapping using 999 bootstrap samples. We assessed concordance of biomarker abnormalities and a combined CSF AD profile between assays using clinical cut-points for Innotest results (Section 2.3) and mixture modeling-derived cut-points for Elecsys. Subjects were appointed a CSF AD profile when they showed a decreased concentration of Aβ42 in combination with an increased p-tau concentration, in accordance with the National Institute on Aging and Alzheimer's Association criteria [6].

3. Results

3.1. Baseline characteristics

Patient characteristics are presented in Table 1. Eight nonacademic hospitals sent between 1 and 43 CSF samples to the central laboratory. The population was relatively young—with average age of 66 years (range = 47–83) and the female-to-male ratio was 40:60. Syndrome diagnoses in the cohort were distributed as 25 (18%) subjects as CN, 36 (26%) subjects with MCI, and 75 (55%) subjects with dementia. Innotest biomarker values ranged for Aβ42 between 321 and 1693 pg/mL, for t-tau between 119 and 2200, and for p-tau between 23 and 216. For Elecsys Aβ42 concentrations that were above the upper limit of detection, extrapolated values were used for subsequent analysis (n = 27 (20%) samples). All CSF biomarker concentrations (both Innotest and Elecsys results) were different in the total dementia group compared with CN. Aβ42 concentrations (both Innotest and Elecsys results) were lower in MCI compared with CN.

3.2. Preanalytical variability

Compliance to the predefined preanalytical protocol in this multicenter study was actively monitored (Fig. 1). A small majority of the samples (78, 57%) were collected, processed, and transported exactly conform protocol. Despite repeated personal feedback to adjust when necessary, protocol deviations were observed in 43% of the CSF samples during collection, processing, and transport to VU University Medical Center, illustrating a considerable variation in relevant preanalytical steps.

3.3. Comparison of Innotest and Elecsys immunoassay results

Innotest and Elecsys measurements showed strong correlations, with Spearman's ρ [95% CI] of 0.94 [0.91; 0.96] for Aβ42, 0.98 [0.97; 0.99] for t-tau, and 0.98 [0.96; 0.99] for p-tau. Fig. 2 illustrates that for all three biomarkers, the two methods showed a systematic difference as indicated

Table 1
Demographic and clinical characteristics of the cohort

Characteristic	Total	Syndrome diagnosis		
		CN	MCI	Dementia
N (%)	137 (100%)	25	36	75
Center (%)				
A	6 (4%)	2 (8%)	0 (0%)	4 (5%)
B	43 (31%)	7 (28%)	11 (31%)	25 (33%)
C	12 (9%)	2 (8%)	1 (3%)	9 (12%)
D	30 (22%)	5 (20%)	2 (6%)	23 (31%)
E	23 (17%)	6 (24%)	11 (31%)	6 (8%)
F	17 (12%)	1 (4%)	11 (31%)	4 (5%)
G	1 (1%)	1 (4%)	0 (0%)	0 (0%)
H	5 (4%)	1 (4%)	0 (0%)	4 (5%)
Gender = *V* (%)	54 (40%)	9 (36%)	11 (31%)	34 (45%)
Age, mean (SD)	67 (7)	66 (9)	66 (7)	68 (6)
MMSE, median [IQR]	26 [24, 29]	29 [28, 30]	27 [25, 29]†	25 [22, 27]‡
Innotest Aβ42 (pg/mL), median [IQR]	644 [525, 1032]	1124 [907, 1269]	747 [557, 1207]*	570 [495, 702]‡
Elecsys Aβ42 (pg/mL), median [IQR]	850 [620, 1403]	1536 [1203, 1971]	973 [641, 1662]*	748 [569, 977]‡
Elecsys Aβ42 above ULOD (%)	27 (20%)	12 (48%)	9 (25%)	5 (7%)
Innotest t-tau (pg/mL), median [IQR]	451 [285, 729]	290 [227, 352]	351 [250, 463]	635 [384, 915]‡
Elecsys t-tau (pg/mL), median [IQR]	256 [178, 359]	179 [146, 227]	203 [156, 258]	308 [211, 430]‡
Innotest p-tau (pg/mL), median [IQR]	62 [45, 89]	48 [40, 58]	54 [44, 63]	79 [56, 105]‡
Elecsys p-tau (pg/mL), median [IQR]	23 [16, 35]	16 [12,19]	19 [14,24]	30 [20, 43]‡

Abbreviations: Aβ42, amyloid β(1–42); CN, cognitively normal; CSF, cerebrospinal fluid; IQR, interquartile range; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; p-tau, phosphorylated tau; SD, standard deviation; t-tau, total tau; ULOD, upper limit of detection.

NOTE. Data are represented as *n* (%), mean (SD), or median [IQR]. Twenty-seven Elecsys CSF Aβ42 concentrations were above the ULOD; for these, extrapolated values were used for the analysis. For one subject the syndrome diagnosis was not known. Statistical significance of MCI and dementia against CN tested with Dunn's post hoc multiple group comparisons using Bonferroni's *P* value correction is indicated.

*$P < .05$.
†$P < .01$.
‡$P < .001$.

by the 95% CIs of the intercepts that did not include 0: the intercept for Aβ42 was −365 [−452; −293], for t-tau was 52 [45; 59], and for p-tau was −3.9 [−5.2; −2.9]. Also, a proportional difference was found between the two methods for all three biomarkers indicated by the 95% CIs of slopes that did not include 1: the slope for Aβ42 was 1.87 [1.74; 2.02], for t-tau was 0.42 [0.42; 0.43], and for p-tau was 0.44 [0.42; 0.46].

Next, we examined whether preanalytical protocol deviations influenced the method comparison for Aβ42. When we stratified the analysis for samples that were collected conform preanalytical protocol versus deviating from protocol, results for Passing–Bablok regression analyses were comparable (Fig. 3). Specific protocol deviations, for example, an extra CSF transfer or extra freeze/thaw cycle for measurement of Elecsys, did not seem to decrease the association between methods, although these groups were too small to perform statistical testing. For t-tau and p-tau, neither an effect of protocol deviations on the association between the methods was observed (Supplementary Fig. 2).

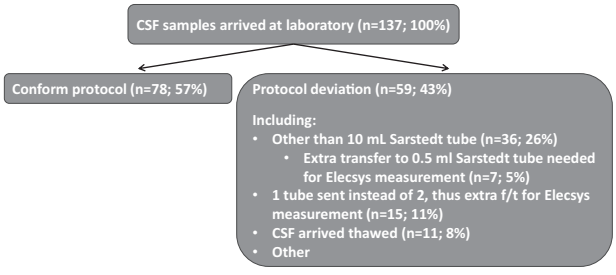


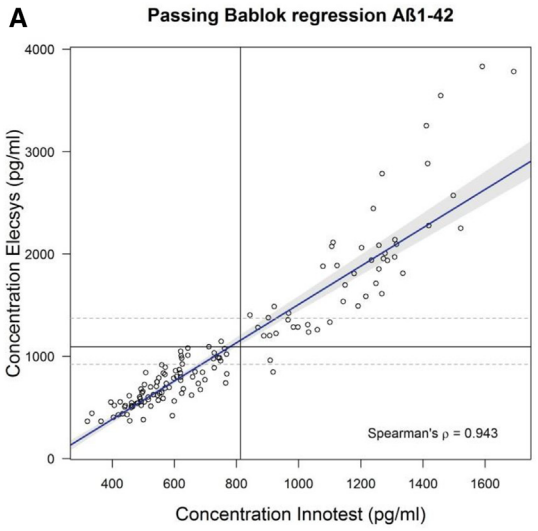
Fig. 1. Schematic overview of preanalytical deviations during CSF sample collection for biomarker measurement. Please note that one sample could be subject to more than one protocol deviation. Percentages relate to the total sample size. Abbreviations: CSF, cerebrospinal fluid; f/t, freeze/thaw cycle.

3.4. Elecsys biomarker cut-point calculation using Gaussian mixture modeling

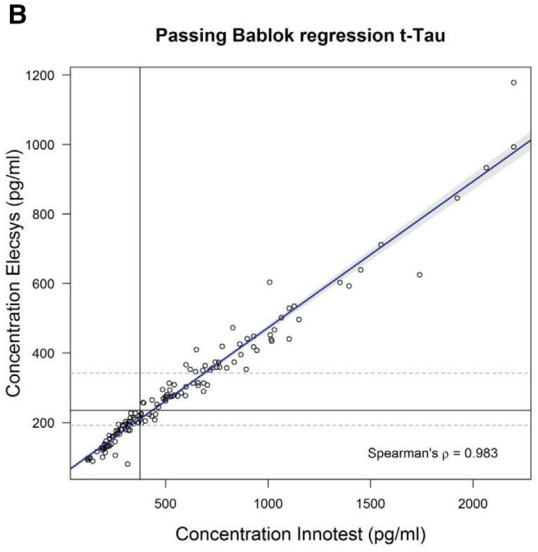
As no cut-points for the Elecsys biomarkers were available at the start of the study, we determined these using Gaussian mixture modeling (Fig. 4). Two distributions were found in the Elecsys results for Aβ42, resulting in a cut-point of 1092 [921; 1371] pg/mL. For both t-tau and p-tau, three distributions best fitted the data and the cut-point between the first two distributions was used for further analyses, which was 235 [192; 342] pg/mL for t-tau and 24 [14; 31] pg/mL for p-tau.

3.5. AD classification concordance between Innotest and Elecsys

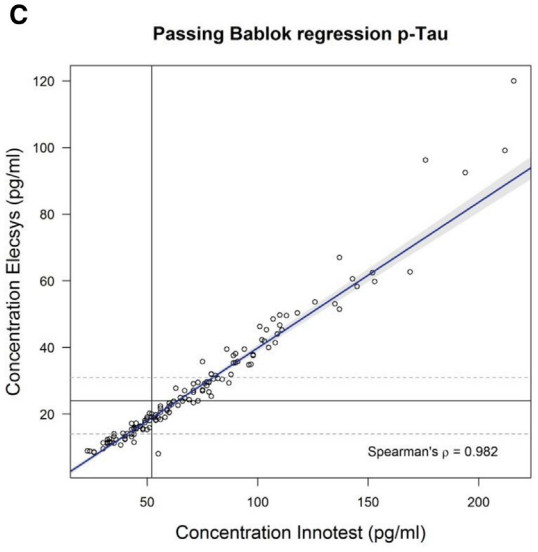
Concordance of biomarker results for the two assays was assessed for the three biomarkers separately, as well as for



Elecsys Aβ42 (pg/ml) =
-365 + 1.87 * Innotest Aβ42 (pg/ml)



Elecsys t-Tau (pg/ml) =
52 + 0.42 * Innotest t-Tau (pg/ml)



Elecsys p-Tau (pg/ml) =
-3.9 + 0.44 * Innotest p-Tau (pg/ml)

Fig. 2. Passing–Bablok regression analyses comparing Innotest and Elecsys results and conversion formulas for Aβ42 (A), t-tau (B), and p-tau (C). For Elecsys Aβ42 values >1700 pg/mL, extrapolated values were used for the analysis (see Supplementary Material). Cut-points with 95% confidence interval for Elecsys (horizontal solid and dotted lines) were determined through Gaussian mixture modeling analyses. Abbreviations: Aβ42, amyloid β(1–42); p-tau, phosphorylated tau; t-tau, total tau.

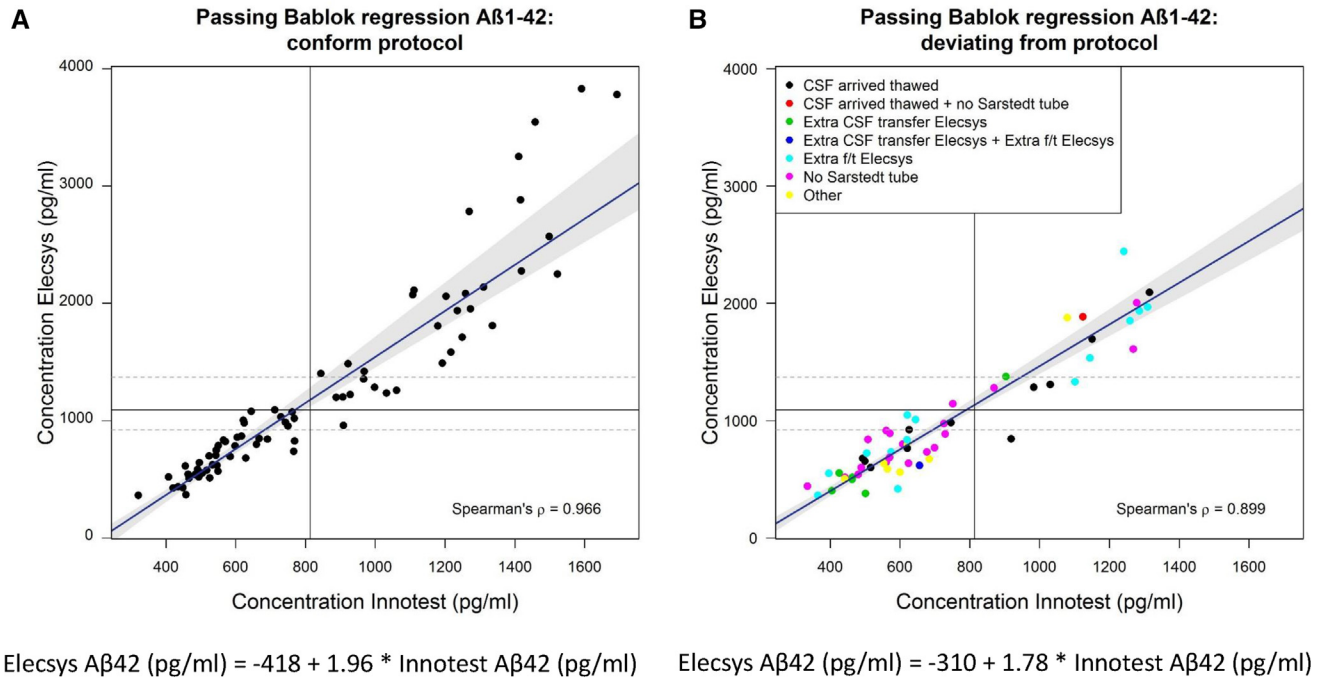


Fig. 3. Spearman correlation, Passing–Bablok regression analysis, and conversion formulas for Aβ42 results in samples with identical preanalytical protocols (A; $n = 78$) and samples with differences in preanalytical protocols (B; $n = 59$). The solid diagonal represents the regression line and the gray area represents the 95% CI. Solid horizontal and vertical lines indicate the biomarker cut-points, and dashed lines indicate 95% CI. Abbreviations: Aβ42, amyloid β(1–42); CI, confidence interval; CSF, cerebrospinal fluid; p-tau, phosphorylated tau; t-tau, total tau.

amyloid and p-tau biomarkers combined to determine an AD biomarker profile (Tables 2 and 3). For Aβ42, 97% of the samples were concordant for biomarker abnormality between Innotest and Elecsys. For both t-tau and p-tau the concordances were 96%. When combining biomarkers into AD biomarker profile (i.e., decreased Aβ42 combined with increased p-tau [6]), concordance was 89%. Fifteen cases (11%) were discrepant and for most of these cases Elecsys p-tau values were slightly below the cut-point, within the 95% CI (Supplementary Table 1).

4. Discussion

In this diagnostic nonacademic dementia multicenter cohort, we observed 96% to 97% concordance between the routinely used manual Innotest and the automated Elecsys biomarker results for CSF biomarkers Aβ42, t-tau, and p-tau. For the combined AD biomarker profile (i.e., decreased Aβ42 with increased p-tau) concordance was 89% and discordant cases showed concentrations close to the biomarker cut-points. Our results suggest that the results from both methods are well interchangeable in a clinical diagnostic setting.

At this point the CSF biomarkers are used in research criteria for AD, but are not used in clinical diagnostic routine yet, partly because of high intralaboratory and interlaboratory variations. Manual assays are usually associated with analytical variation, which is supposed to be solved by automation. This notion is supported by the observation in previous studies of lower interassay and interlaboratory variation percentages of Elecsys compared with the manual ELISA tests: a mean of 5% coefficient of variation for Elecsys compared with >15% coefficient of variation for Innotest in biomarker results measured in the Alzheimer's Association Quality Control program since 2014 (www.neurochem.gu.se/TheAlzAssQCprogram). However, these studies were performed in selected research settings, which may not be comparable to a real-life situation. In the present study, samples were collected in a multicenter setting, which increases the variation in the preanalytical protocol. To reduce this variation, extra efforts were made to harmonize the sample collection at the eight secondary memory clinics, including by the use of a standardized protocol [24], by close contact with the local personnel involved, by constant monitoring and feedback on protocol deviations to avoid repetition, and by supply of the 10 mL Sarstedt tubes. Still, deviations in the preanalytical protocol were reported in 43% of the samples, which could have weakened the correlation between the two methods. No effect of preanalytical protocol deviations, however, was found on the strength of the correlation between the two methods, indicating that no method-specific preanalytical effects have occurred. However, the potential influence of preanalytical effects on both Innotest and Elecsys measurements was not formally assessed in this study and protocol deviations affecting both measurements could have influenced biomarker results.

The strong correlation between the biomarker results from the two methods in our study is in line with previous results that showed high correlations of Elecsys Aβ42

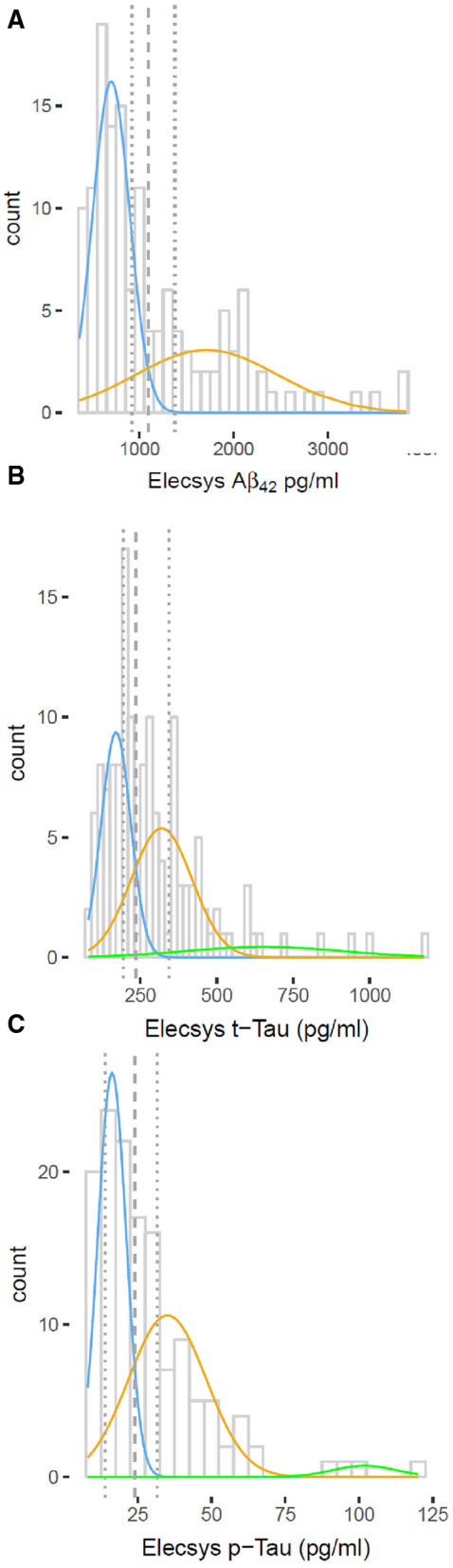


Fig. 4. Gaussian mixture modeling analysis for Elecsys Aβ42 (A), t-tau (B), and p-tau (C) results. Cut-points (striped lines) with 95% CI (dotted lines) were determined through bootstrapping based on the estimated distributions. Bars indicate the observed data. Abbreviations: Aβ42, amyloid β(1–42); CI, confidence interval; p-tau, phosphorylated tau; t-tau, total tau.

concentrations with the Aβ42 liquid-chromatography mass-spectrometry reference method and with amyloid imaging [20–22]. Because of these strong linear correlations, we obtained conversion formulas to translate between Innotest and Elecsys results from Passing–Bablok regression analysis, which are needed to enable use of both types of results for clinical research and follow-up. We would like to emphasize, however, that these conversion formulas are preliminary and should be sustained with more data. In addition, these conversion formulas are specific to the present data set and are not suitable for other cohorts that use different preanalytical protocols.

Because cut-points for the Elecsys assays were not yet available at the start of this study, we derived cut-points based on a Gaussian mixture modeling, which is a data-driven approach that allows discovering distributions in data, which may reflect pathologic and normal ranges of biomarker values [28,29]. Cut-points for the Aβ42 Elecsys measurements have been previously reported in three cohorts: 1100 pg/mL in the Biomarkers for Identifying Neurodegenerative Disorders Early and Reliably cohort, 977 pg/mL in the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort [21], and 1098 pg/mL in the Knight Alzheimer's Disease Research Center cohort [22]. The close similarity of the Biomarkers for Identifying Neurodegenerative Disorders Early and Reliably and Alzheimer's Disease Research Center cut-points that were obtained in research settings with our cut-point obtained in real-life diagnostic setting (1092 pg/mL) suggests that the automated Elecsys assay provides robust measurements. The Aβ42 cut-point of the ADNI cohort was lower compared with the cut-points in other cohorts, probably because of the more extensive preanalytical protocol of ADNI or because of other (patient-related) variation factors in the cohort [21]. Although correlation analyses facilitate comparison of two methods on a group level, concordance analyses of biomarker abnormality based on cut-points are relevant to allow method comparisons on an individual level. Of the 11% discordant cases in our comparison, most had biomarker values near the cut-point. On a general note, biomarker values near the cut-point need cautious interpretation, as imprecision of (one of) the assays, or biological variation, can play a role in the exact absolute values. As such, cut-points should never be considered as absolute measure, but always in the context of other biomarkers and clinical history. Clinicians could interpret 95% CIs surrounding cut-points as a "gray zone" of at risk for abnormality rather than a conclusive positive or negative biomarker outcome. Also, the borders of this gray zone could facilitate patient stratification for clinical intervention studies. To illustrate, using the value at the lower gray border of the Aβ42 cut-point could help to select a group with high certainty of amyloid pathology, whereas using the value at the upper gray zone border could help to select patients in early phases of amyloid pathology development.

A potential limitation of the present study is that for 20% of our samples, the Elecsys measurements were above the

Table 2
Concordance analyses of Innotest and Elecsys biomarker results based on biomarker cut-points. (A) AD classification based on Aβ42, t-tau, or p-tau alone

		Elecsys				Elecsys				Elecsys	
Aβ42 pathologic?		False	True	t-tau pathologic?		False	True	p-tau pathologic?		False	True
Innotest	False	47 (34%)	2 (1%)	Innotest	False	62 (45%)	2 (1%)	Innotest	False	67 (49%)	0
	True	2 (1%)	86 (63%)		True	4 (3%)	69 (50%)		True	6 (4%)	64 (47%)

Abbreviations: AD, Alzheimer's disease; Aβ42, amyloid β(1–42); p-tau, phosphorylated tau; t-tau, total tau.

NOTE. Applied cut-points for Innotest were 813 for Aβ42, 375 for t-tau, and 52 for p-tau, and for Elecsys these were 1092 for Aβ42, 235 for t-tau, and 24 for p-tau.

upper limit of the measuring range at 1700 pg/mL, which was also reported in other studies [21,22]. For the present study, Aβ42 concentrations >1700 pg/mL were calculated by extrapolation of the calibration curve. This estimation of the high Elecsys Aβ42 concentrations could have biased the correlation between the methods in the higher range. However, as these values concern the upper limit of the normal range of Aβ42 concentrations, these are not clinically problematic as such individuals are clearly in the normal range. Furthermore, the sample size of our cohort was relatively small, and so determining cut-points in a data-driven way with Gaussian mixture modeling might be less accurate, which is reflected by the relatively large CIs for the cut-points. Still, our cut-point for Elecsys Aβ42 showed very close correspondence to those determined in other cohorts [21,22], suggesting that Gaussian mixture modeling robustly detects the normal and pathologic distributions of Aβ42, even in a relatively small population. Another potential limitation is that the time lag of analysis of CSF pairs between Innotest and Elecsys spanned between 4 and 23 months, which may have increased discordant biomarker results. However, we did not see an effect of storage time on biomarker concentrations (Supplementary Fig. 1) and observed very high concordance, so it is unlikely that an additional 4 to 23 months of storage influenced the results. Furthermore, our previous study showed stable CSF Aβ42, t-tau, and p-tau concentrations over 12 years of biobank storage [14].

In conclusion, the fully automated Elecsys assays demonstrated closely corresponding outcomes compared with the manual Innotest with regard to biomarker abnormality of CSF Aβ42, t-tau, and p-tau in nonacademic memory clinics, reflecting daily practice diagnostic settings. Because the Elecsys assays are automated and allow direct sample measurement cost-effectively, these assays seem promising to succeed in reducing the biomarker variability. Altogether, this study provides the basis to introduce these automated assays in clinical practice, hopefully leading to more reproducible biomarker results on a global scale.

Table 3
Concordance analyses of Innotest and Elecsys biomarker results based on biomarker cut-points. According to the NIA-AA criteria that combine decreased Aβ42 with increased p-tau

		Elecsys	
AD according to NIA-AA?		False	True
Innotest	False	67 (49%)	0
	True	15 (11%)	55 (40%)

Abbreviations: AD, Alzheimer's disease; NIA-AA, National Institute on Aging and Alzheimer's Association.

NOTE. Applied cut-points for Innotest were 813 for Aβ42, and 52 for p-tau, and for Elecsys these were 1092 for Aβ42, and 24 for p-tau.

Acknowledgments

The authors thank Kees van Uffelen, Annemarie Stam, and Joop Nijhof for excellent technical and logistic assistance and Udo Eichenlaub and Richard Batrla-Utermann from Roche for in-kind contribution of the Elecsys assays and biostatistical assistance to extrapolate the amyloid β(1–42) results detected outside the measurement range. This study was funded by ZonMW-Memorabel (ABIDE; Project No. 733050201), a project in the context of the Dutch Deltaplan Dementie.

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Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.dadm.2018.08.006.

RESEARCH IN CONTEXT

1 Systematic review: The authors reviewed the literature using Pubmed and meeting abstracts and presentations. Although the fully automated Elecsys assays for the cerebrospinal fluid Alzheimer's disease biomarkers amyloid β(1–42), total tau, and phosphorylated tau are novel in the field, there have been several recent publications describing Elecsys results compared with amyloid imaging in research cohorts. These relevant citations are appropriately cited.

2 Interpretation: Our findings show that Elecsys results compare well to the traditionally used Innotest results in a multicenter diagnostic cohort of nonacademic memory clinic patients.

3 Future directions: This article provides evidence for method translation between Innotest and Elecsys results in a real-life diagnostic setting. Replication of Elecsys results in other central biomarker core facilities are needed to reveal whether the establishment of global cut-points for cerebrospinal fluid biomarkers using the Elecsys assays will be feasible in the near future.

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