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Method comparison study of the Elecsys® β-Amyloid (1–42) CSF assay versus comparator assays and LC-MS/MS

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ABSTRACT

Background: Alzheimer's disease (AD) biomarkers, such as cerebrospinal fluid (CSF) amyloid-β (1–42; Aβ42), can provide high diagnostic accuracy. Several immunoassays are available for Aβ42 quantitation, but standardisation across assays remains an issue. We compared the Elecsys® β-Amyloid (1–42) CSF assay with three assays and two liquid chromatography tandem mass spectrometry (LC-MS/MS) methods.

Methods: Three method comparison studies evaluated the correlation between the Elecsys® β-Amyloid (1–42) CSF assay versus: INNOTEST® β-AMYLOID(1–42) (860 samples) and the Roche Diagnostics-developed LC-MS/MS method (250 samples); INNO-BIA AlzBio3 and the University of Pennsylvania (UPenn)-developed LC-MS/MS method (250 samples); and ADx-EUROIMMUN Beta-Amyloid (1–42) enzyme-linked immunosorbent assay (ELISA) (49 samples).

Results: High correlation was demonstrated between Elecsys® β-Amyloid (1–42) CSF and comparator assays: INNOTEST® β-AMYLOID(1–42) (Spearman's ρ, 0.954); INNO-BIA AlzBio3 (Spearman's ρ, 0.864); ADx-EUROIMMUN Beta-Amyloid (1–42) ELISA (Pearson's r, 0.925). Elecsys® assay and LC-MS/MS measurements were highly correlated: Pearson's r, 0.949 (Roche Diagnostics-developed method) and 0.943 (UPenn-developed method).

Conclusion: Findings from this multicentre evaluation further support use of the Elecsys® β-Amyloid (1–42) CSF assay to aid AD diagnosis. CSF-based certified reference materials should improve agreement across assays and mass spectrometry-based methods, which is essential to establish a global uniform CSF Aβ42 cut-off to detect amyloid pathology.

Abbreviations: Aβ, amyloid-β peptide; Aβ42, amyloid-β (1–42); AD, Alzheimer's disease; AIBL, Australian Imaging, Biomarkers and Lifestyle Study of Aging; CI, confidence interval; CRM, certified reference material; CSF, cerebrospinal fluid; CUSUM, cumulative sum; ELISA, enzyme-linked immunosorbent assay; JCTLM, Joint Committee for Traceability in Laboratory Medicine; LC-MS, liquid chromatography mass spectrometry; LC-MS/MS, liquid chromatography tandem mass spectrometry; PET, positron emission tomography; SD, standard deviation; UPenn, University of Pennsylvania

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1. Introduction

Alzheimer's disease (AD) is the most common cause of dementia, and is characterised by the progressive accumulation of extracellular amyloid-β peptide (Aβ) plaque deposits and intracellular tau protein neurofibrillary tangles [1–4]. The current AD diagnostic pathway relies on clinical symptoms and the ability to rule out other dementia causes [5]; this is a suboptimal approach, since the hallmarks of AD often begin years before the appearance of clinical symptoms [6]. In the novel National Institute on Aging and Alzheimer's Association Research Framework and International Working Group-2 criteria, biomarkers covering amyloid deposition, pathological tau and neurodegeneration are central to the diagnostic recommendations [7,8]. There is therefore a need for biomarkers for the diagnosis of AD that are more accessible and cost-effective than amyloid-positron emission tomography (PET) imaging [6]. Cerebrospinal fluid (CSF) biomarkers including Aβ(1–42) have shown clinical value in supporting AD diagnosis, and may aid the recruitment of appropriate patients into AD clinical trials.

Several immunoassays are currently available for the quantitation of Aβ(1–42) in CSF, and have previously shown high concordance with each other [9–11]. The most widely used are the INNOTEST® β-AMYLOID(1–42) assay (Fujirebio, Malvern, PA, USA) [12,13]; the INNO-BIA AlzBio3 assay (Fujirebio, Malvern, PA, USA) [14]; and the ADx-EUROIMMUN Beta-Amyloid (1–42) enzyme-linked immunosorbent assay (ELISA) (ADx Neurosciences, Gent, Belgium). The Elecsys® β-Amyloid (1–42) CSF immunoassay (Roche Diagnostics, Rotkreuz, Switzerland) is a novel assay for the in vitro quantitative determination of Aβ(1–42) in CSF and is the first assay to be standardised to a Joint Committee for Traceability in Laboratory Medicine (JCTLM)-approved reference measurement procedure [15,16]. The assay has also demonstrated excellent analytical performance [15], has been routinely assessed in the Alzheimer's Association Quality Control Programme since 2014 [17] and since 2016 has been available for use in countries accepting the CE mark [18]. Recent findings support the use of the Elecsys® β-Amyloid (1–42) CSF assay as a reliable alternative to PET imaging [3] to aid AD diagnosis [19].

Currently, commercially available assays are standardised using diverse in-house calibrator materials, which can lead to difficulties in the comparison of their results. Instead, the recommended procedure includes the use of certified reference material (CRM) with target values defined using the liquid chromatography mass spectrometry (LC-MS) method for reference standardisation [20]. Three such CRMs based on human CSF, with low, medium and high Aβ(1–42) levels, are available in Europe and the USA [21]. High correlation between measurements from different CSF Aβ(1–42) assays is an important prerequisite for standardisation by CRMs, to ensure that similar results are achieved between the assays and thereby enabling the comparison and interpretation of results across different laboratories.

Variation in CSF Aβ(1–42) measurements can also be introduced by differences in pre-analytical sample handling procedure. Correlation and systematic differences between Aβ(1–42) measurements may be influenced by various pre-analytical factors, such as the mixing procedure and tube filling volume used, emphasising a need for a unified pre-analytical protocol. Standardisation of pre-analytical procedures could reduce differences in the measurement of Aβ(1–42) concentrations across assays and research institutions, clinical practice and industry, and further enable cross-study comparisons [19,21,22].

We present the results of a method comparison evaluation of the Elecsys® β-Amyloid (1–42) CSF assay versus three comparator assays and two reference liquid chromatography tandem mass spectrometry (LC-MS/MS) methods [22,23].

2. Materials and methods

2.1. Immunoassays and LC-MS/MS

The Elecsys® β-Amyloid (1–42) CSF is an electrochemiluminescence sandwich immunoassay [15] that can be run on **cobas e** 601, **cobas e** 602 and MODULAR ANALYTICS E170 analysers and has a measuring range of 200–1700 pg/mL. The INNOTEST β-AMYLOID(1–42) is a solid-phase ELISA [12] with a measuring range of 125–2000 pg/mL. The INNO-BIA AlzBio3 is an xMAP® microbead-based multiplex sandwich immunoassay with a measuring range of 27–1575 pg/mL [14]. The ADx-EUROIMMUN Beta-Amyloid (1–42) ELISA has a measuring range of 126–2010 pg/mL. The LC-MS/MS reference methods developed by the University of Gothenburg and Roche Diagnostics and the University of Pennsylvania (UPenn; Philadelphia, PA, USA) have measuring ranges of 150–4000 and 100–3000 pg/mL, respectively [22,23]; both are JCTLM-approved reference measurement procedures (C11RMP9 and C12RMP1).

2.2. Study design

Three studies were performed to evaluate the correlation between the Elecsys® β-Amyloid (1–42) CSF assay versus three commercially available immunoassays (INNOTEST β-AMYLOID(1–42); INNO-BIA AlzBio3; and ADx-EUROIMMUN Beta-Amyloid (1–42) ELISA) and two LC-MS/MS methods [22,23].

2.2.1. Study 1: Elecsys® β-Amyloid (1–42) CSF assay versus INNOTEST β-AMYLOID(1–42) assay and LC-MS/MS

The method comparison of the Elecsys® β-Amyloid (1–42) CSF assay versus the INNOTEST β-AMYLOID(1–42) assay and LC-MS/MS reference method [22] was performed using de-identified leftover CSF samples that had been routinely collected at the Sahlgrenska University Hospital (Gothenburg, Sweden), following a procedure approved by the Ethics Committee at the University of Gothenburg (Gothenburg, Sweden; EPN 140811). Aliquots were prepared from the leftover CSF samples using 0.5 mL Sarstedt polypropylene tubes; the collected samples were frozen at −80 °C within 4 weeks and measurements were performed from October 2014 to September 2015 in small batches. Pre-analytical and quality control procedures, plate acceptance criteria and batch bridging for the INNOTEST analyses were conducted as described previously [11]. Samples were thawed at room temperature for 30 min and roller-mixed for 20 min before measurement.

Elecsys® and INNOTEST assay measurements were performed at the University of Gothenburg; 860 samples were measured in > 20 runs. INNOTEST assay measurements were performed in duplicate and mean values were used for the analyses. During the study, one lot change was reported for both assays; therefore, method comparison data include measurements of two different Elecsys® and INNOTEST assay lots. A subset of 250 samples was also measured using the LC-MS/MS procedure developed and validated by Roche Diagnostics GmbH (Penzberg, Germany) [22]. LC-MS/MS measurements were performed in-house for 6 days using three different calibrator sets.

2.2.2. Study 2: Elecsys® β-Amyloid (1–42) CSF assay versus INNO-BIA AlzBio3 assay and LC-MS/MS

The method comparison of the Elecsys® β-Amyloid (1–42) CSF assay versus the INNO-BIA AlzBio3 assay and LC-MS/MS reference method [23] was performed using 250 de-identified CSF samples from discarded routine clinic patients without clinical classification information. Four aliquots of ~500 μL were prepared from each sample and

Table 1
Descriptive statistics for the comparison between the Elecsys® β-Amyloid (1–42) CSF assay and comparator assays/LC-MS/MS reference methods.

	Elecsys®/INNOTEST (Study 1)	Elecsys®/AlzBio3 (Study 2)	Elecsys®/ADx-EUROIMMUN (Study 3)	Elecsys®/LC-MS/MS (Study 2)
N	788/788	299/229[a]	43[b]/43[b]	234/234[c]
Mean	711/487	895/316	909/564	889/915
SD	357/197	327/206	396/257	329/323
Median	633/460	863/311	889/517	862/888
Min–Max	201–1688/123–1062	232–1690/54–602	286–1683/158–1051	232–1690/204–1760

CSF, cerebrospinal fluid; LC-MS/MS, liquid chromatography tandem mass spectrometry; SD, standard deviation.
[a] 250 samples measured, but only 229 samples had valid measurements.
[b] A total of 49 samples were measured using both assays, but only 43 samples had valid results within the measurement range.
[c] 250 samples measured, but only 234 samples had valid measurements.

frozen at −80 °C until the day of analysis. Elecsys® assay, AlzBio3 assay and LC-MS/MS measurements were performed at UPenn from August 2015 to October 2015. AlzBio3 assay and LC-MS/MS measurements were performed in duplicate and mean values used for the analyses. The number of testing days for the Elecsys® assay, AlzBio3 assay and LC-MS/MS reference method was 6, 4 and 7, respectively. Samples were thawed at room temperature for 30 min and roller-mixed for 20 min before measurement. LC-MS/MS measurements were performed using the LC-MS/MS method developed and validated by UPenn [23].

2.2.3. Study 3: Elecsys® β-Amyloid (1–42) CSF assay versus ADx-EUROIMMUN Beta-Amyloid (1–42) ELISA

The method comparison of the Elecsys® β-Amyloid (1–42) CSF assay versus the ADx-EUROIMMUN Beta-Amyloid (1–42) ELISA was performed using a subset of 49 samples that were collected during the course of the Australian Imaging, Biomarkers and Lifestyle Study of Aging (AIBL) [16]. Aliquots were prepared, frozen and stored in a liquid nitrogen vapour tank at the Florey Institute of Neuroscience and Mental Health (Parkville, VIC, Australia), then transferred on dry ice to the University of Gothenburg where the Elecsys® CSF assay measurements were performed (three runs on 3 days). Samples were thawed for 30 min at room temperature and roller-mixed for 20 min. Nunc Cryobank 500 µL vials were used.

For the ADx-EUROIMMUN assay, 15 µL of calibrators, controls and undiluted CSF and 100 µL of biotinylated detector antibody were added in duplicates to coated wells; the plates were incubated for 180 min at room temperature. The wells were then washed five times with wash buffer and 100 µL of streptavidin–peroxidase (enzyme conjugate) was added; the plates were incubated for 30 min at room temperature. After a final wash, 100 µL of substrate was added and incubated for 30 min at room temperature, and then 100 µL of stop solution was added. Colour intensity was measured using a plate reader at a wavelength of 450 nm.

2.2.4. Statistical analyses

Descriptive statistics (mean, standard deviation [SD], median and range) were used to describe the distribution of Aβ(1–42) concentrations in each sample set. For the method comparisons, significance testing for non-linearity was conducted via cumulative sum (CUSUM) test [24]. Details of the CUSUM test and conversion of non-linear data to achieve linearity are described in the Supporting Information. Pairwise correlation coefficients were calculated: Pearson's correlation coefficients for linear comparisons and Spearman's correlation coefficients for non-linear comparisons. Weighted and unweighted Deming regression analyses [25] were performed to assess the systematic difference between the Elecsys® β-Amyloid (1–42) and comparator assays. Unweighted Deming regression was used in cases when visual inspection of scatter plots, difference plots and residual plots suggested constant variance of random errors across the complete range of values on the axes. Weighted Deming regression was used when heteroscedasticity of random errors was observed (variance of random errors increased proportionally to the corresponding mean values). The robust Passing–Bablok regression analyses [24] were performed for the comparison with the two LC-MS/MS methods since the slope values between LC-MS/MS and Elecsys® assay were expected to be close to 1. Concentrations within the measurement ranges of both assays (on X and Y axes) were used for the analyses. Confidence intervals (CIs) for intercept and slope of regression coefficients were calculated using the jackknife (for Deming regression) or bootstrap (for Passing–Bablok regression) methods. Method comparison analyses were performed using R-package mcr (version 1.2.1; R Foundation for Statistical Computing, Vienna, Austria).

3. Results

3.1. Elecsys® β-Amyloid (1–42) CSF assay versus INNOTEST β-AMYLOID (1–42) assay (study 1)

Aβ(1–42) was measured in 860 samples using both assays, of which 788 samples had valid measurements within the assays' measuring ranges. Mean (SD) Aβ(1–42) concentrations were 711 (357) pg/mL with the Elecsys® assay and 487 (197) pg/mL with the INNOTEST assay; corresponding median (range) concentrations were 633 (201–1688) and 460 (123–1062) pg/mL, respectively (Table 1).

Elecsys® and INNOTEST assay measurements were highly correlated (Spearman's ρ, 0.954), but demonstrated non-linear dependency; the CUSUM test statistic was 3.72 (higher than the critical value 1.22). Scatter and residual plots of the linear fit suggested non-linearity, and the regression line fitted the data poorly in the lower and higher concentration ranges (Fig. S1A, B). To achieve linearity, Elecsys® assay measurements were converted to the power of 0.7 (see Supporting Information for further details). Weighted Deming regression analysis applied to the transformed data demonstrated: slope, 0.164 (95% CI, 0.161–0.168) and intercept, 16.7 (95% CI, 15.2–18.1). The CUSUM test statistic was 1.01 (lower than the critical value of 1.22). Scatter and residual plots showed improvement of the non-linearity after the transformation (Fig. S1C, D). Back-transformation to the original scale yielded the curve y = (0.164× + 16.7)^(1/0.7), where x is the INNOTEST assay measurement and y is the Elecsys® assay measurement (Fig. 1).

3.2. Elecsys® β-Amyloid (1–42) CSF assay versus INNO-BIA AlzBio3 assay (study 2)

Aβ(1–42) was measured in 250 samples using both assays, of which 229 samples had valid assay measurements within the measuring ranges. Mean (SD) Aβ(1–42) concentrations were 895 (327) pg/mL with the Elecsys® assay and 316 (206) pg/mL with the AlzBio3 assay; corresponding median (range) concentrations were 863 (232–1690) and 311 (54–602) pg/mL, respectively (Table 1). Elecsys® and AlzBio3 assay measurements showed good correlation (Spearman's ρ, 0.864) and demonstrated a slight non-linear dependency; the CUSUM test statistic was 1.30 (higher than the critical value 1.22). The residual plot of the linear fit also suggested slight non-linearity, and the straight line fitted the data poorly in the lower concentration range (Fig. S2A, B). To

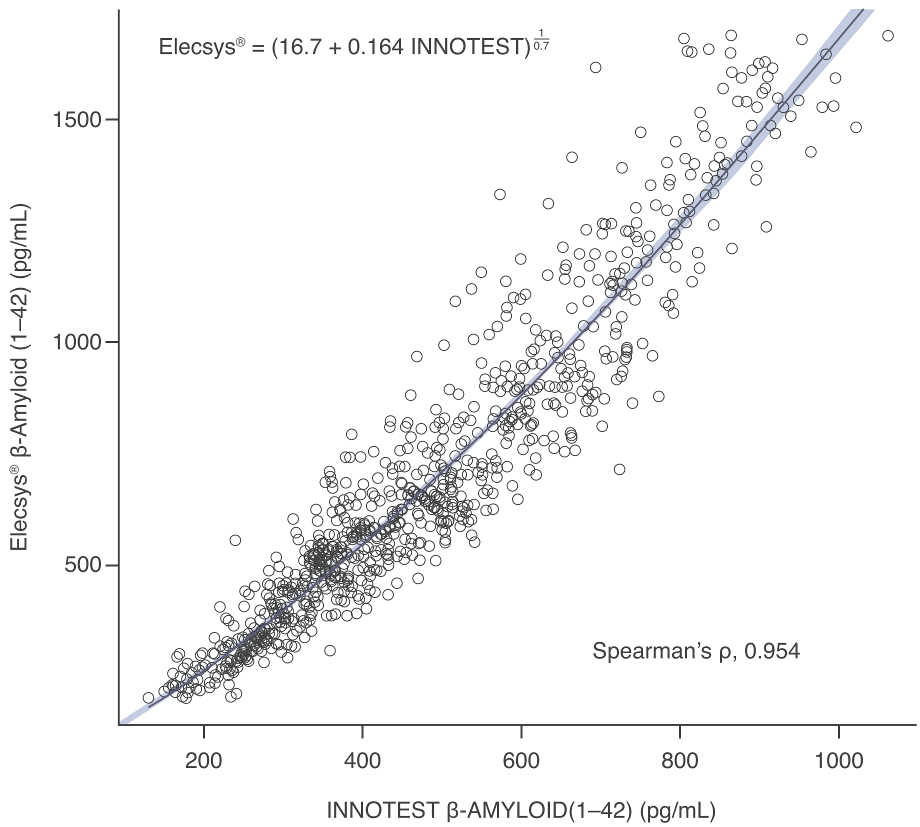

Fig. 1. Comparison of amyloid-β concentrations measured with the Elecsys® β-Amyloid (1–42) CSF assay (**cobas e** 601 analyser) and the INNOTEST β-AMYLOID(1–42) assay (Study 1). Curve fitted to data; the linear fit was performed based on the transformed data (see Fig. S1C) and then converted to the original scale. The shaded area represents the 95% confidence interval for the fitted curve. CSF, cerebrospinal fluid.

achieve linearity, Elecsys® assay measurements were converted to the power of 0.5. Deming regression analysis applied to the transformed data demonstrated: slope, 0.048 (95% CI, 0.044–0.052) and intercept, 14.6 (95% CI, 13.3–16.0). The CUSUM test statistic was 0.73 (lower than the critical value of 1.22). Residual plots showed improvement of the non-linearity after the transformation (Fig. S2C, D). Back-transformation to the original scale yielded the curve y = (0.048× + 14.6)^2, where x is the AlzBio3 assay measurement and y is the Elecsys® assay measurement (Fig. 2).

3.3. *Elecsys® β-Amyloid (1–42) CSF assay versus ADx-EUROIMMUN Beta-Amyloid (1–42) ELISA (study 3)*

Aβ(1–42) was measured in 49 samples using both assays, of which 43 samples had valid assay measurements within the measuring ranges. Mean (SD) Aβ(1–42) concentrations were 909 (396) pg/mL with the Elecsys® assay and 564 (257) pg/mL with the ADx-EUROIMMUN assay; corresponding median (range) concentrations were 889 (286–1683) and 517 (158–1051) pg/mL, respectively (Table 1). Elecsys® and ADx-EUROIMMUN assay measurements were highly correlated (Pearson's r, 0.925) and demonstrated a linear dependency (CUSUM test statistic, 0.70; lower than the critical value of 1.22; Fig. S3). Weighted Deming regression analysis demonstrated: slope, 1.51 (95% CI, 1.36–1.66) and intercept, 52.0 (95% CI, −3.20 to 107). The fitted line was y = 1.51× + 52, where x is the ADx-EUROIMMUN assay measurement and y is the Elecsys® assay measurement (Fig. 3).

3.4. *Elecsys® β-Amyloid (1–42) CSF assay versus LC-MS/MS (study 1 and 2)*

For the method comparison versus the LC-MS/MS method developed by the University of Gothenburg and Roche Diagnostics (Study 1), Aβ(1–42) was measured in 250 samples, of which 234 had valid measurements within the measuring ranges of both methods. Mean (SD) Aβ(1–42) concentrations were 720 (348) pg/mL with the Elecsys® assay and 577 (279) pg/mL with LC-MS/MS; corresponding median (range) concentrations were 671 (234–1682) and 542 (151–1493) pg/mL, respectively. Elecsys® assay and LC-MS/MS measurements were highly correlated (Pearson's r, 0.949) and demonstrated a linear dependency (CUSUM test statistic, 0.85; lower than the critical value of 1.22). Passing–Bablok regression analysis demonstrated: slope, 1.25 (95% CI, 1.19–1.32) and intercept, −1.36 (95% CI, −26.9 to 21.74; Fig. 4).

For the method comparison versus the LC-MS/MS method developed by UPenn (Study 2), Aβ(1–42) was measured in 250 samples, of which 234 had valid measurements within the measuring ranges of both methods. Mean (SD) Aβ(1–42) concentrations were 889 (329) pg/mL with the Elecsys® assay and 915 (323) pg/mL with LC-MS/MS; corresponding median (range) concentrations were 862 (232–1690) and 888 (204–1760) pg/mL, respectively. Elecsys® assay and LC-MS/MS measurements were highly correlated (Pearson's r, 0.943) and demonstrated a linear dependency. Passing–Bablok regression analysis demonstrated: slope, 0.97 (95% CI, 0.92–1.02) and intercept, 50.76 (95% CI, 8.59–90.96; Fig. 5).

4. Discussion

To address the need for a robust and sensitive biomarker approach to support AD diagnosis, a number of immunoassays have been developed for the measurement of Aβ42 in CSF, including the novel Elecsys® β-Amyloid (1–42) CSF assay. This assay has previously demonstrated an excellent analytical performance and is the first assay to be standardised to a JCTLM-approved reference measurement procedure [15]. We performed a method comparison between the Elecsys® β-Amyloid (1–42) CSF assay and three comparator assays – INNOTEST β-AMYLOID(1–42); INNO-BIA AlzBio3; and ADx-EUROIMMUN Beta-Amyloid (1–42) ELISA – and high correlation was demonstrated.

The strong bias in absolute levels observed between the immunoassays was expected, due to differences in reference

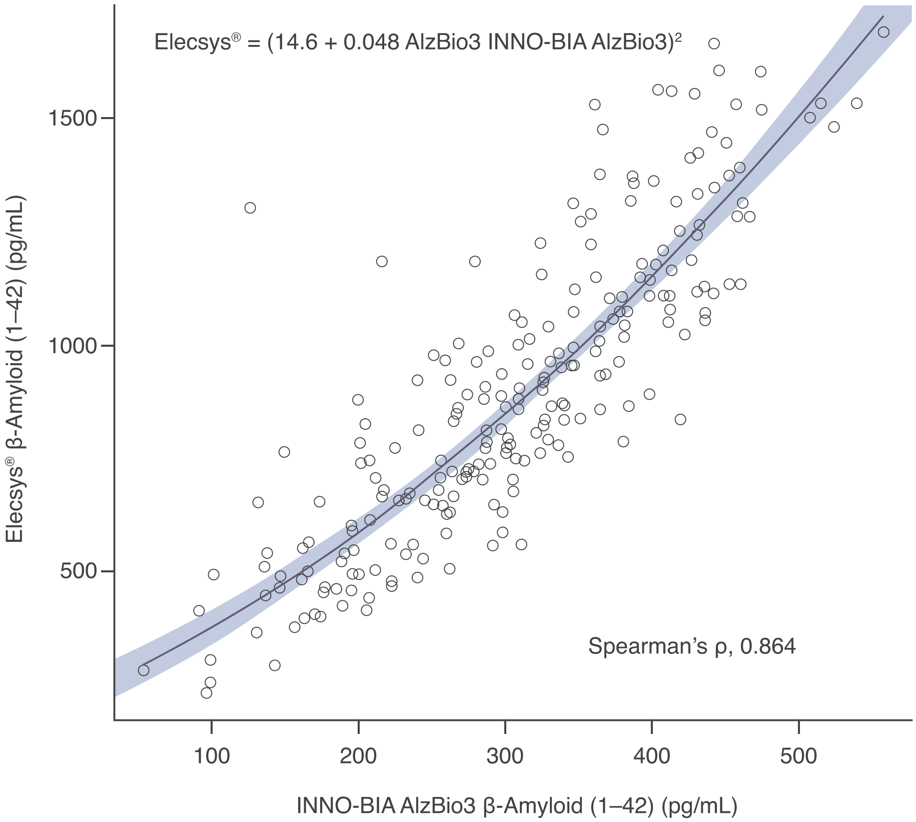

Fig. 2. Comparison of amyloid-β concentrations measured with the Elecsys® β-Amyloid (1–42) CSF assay (**cobas e** 601 analyser) and the INNO-BIA AlzBio3 assay (Study 2). Curve fitted to data; the linear fit was performed based on the transformed data (see Fig. S2C) and then converted to the original scale. The shaded area represents the 95% confidence interval for the fitted curve. CSF, cerebrospinal fluid.

standardisation, and the Elecsys® assay is a good premise for the standardisation of the assays using CRMs. Systematic differences between the assays may be reduced by using CRM samples for reference standardisation. Non-linearity was observed between the Elecsys® and INNOTEST assays in the lower concentration range, and between the Elecsys® and AlzBio3 assays, which aligns with previous studies [26].

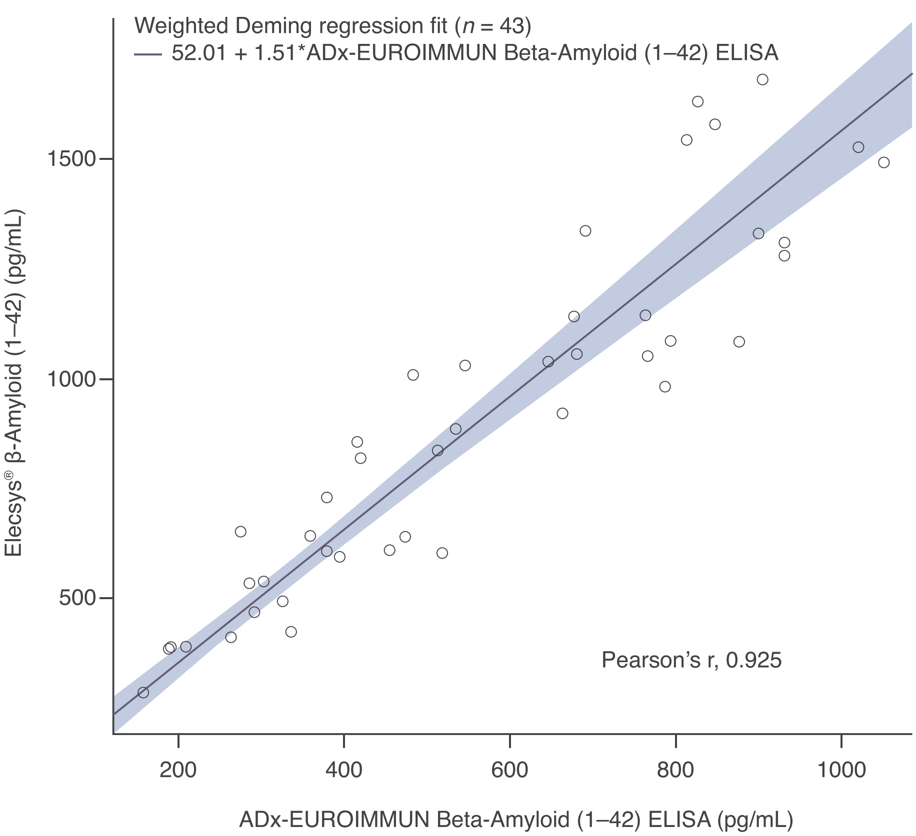

Fig. 3. Weighted Deming regression analysis for the comparison of amyloid-β concentrations measured with the Elecsys® β-Amyloid (1–42) CSF assay (**cobas e** 601 analyser) and ADx-EUROIMMUN Beta-Amyloid (1–42) ELISA (Study 3). The shaded area represents the 95% confidence interval for the fitted curve. CSF, cerebrospinal fluid; ELISA, enzyme-linked immunosorbent assay.

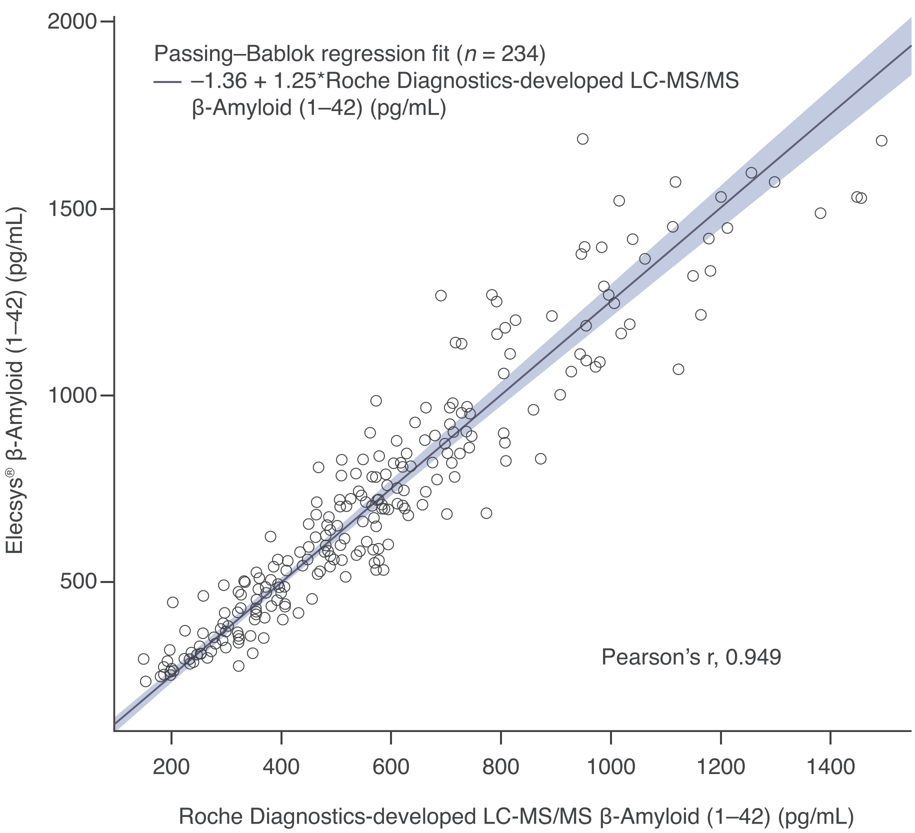

Fig. 4. Passing–Bablok regression fit for the comparison of amyloid-β concentrations measured with the Elecsys® β-Amyloid (1–42) CSF assay (**cobas e** 601 analyser) and the Roche Diagnostics-developed LC-MS/MS method (Study 1). The shaded area represents the 95% confidence interval for the fitted curve. CSF, cerebrospinal fluid; LC-MS/MS, liquid chromatography–tandem mass spectrometry.

This may be explained by potential interference of ELISA assays from Aβ40 and other Aβ peptide isoforms [27]. Linearity was observed between the Elecsys® and ADx-EUROIMMUN assays, but only a small number of samples were used for this comparison.

A method comparison study of the Elecsys® β-Amyloid (1–42) CSF assay compared with seven other CSF Aβ42 immunoassays also showed significant correlation between all assays (R2 range, 0.71–0.98; $p < .0001$) [28]. Good correlation was observed between Elecsys® β-Amyloid (1–42) CSF assay versus ADx-EUROIMMUN Beta-Amyloid (1–42) ELISA (R2, 0.98), INNO-BIA AlzBio3 assay (R2, 0.71) and INNOTEST β-AMYLOID(1–42) assay (R2, 0.84) [28]. Our study further adds to these findings by the use of larger sample sizes in each method comparison experiment.

The Elecsys® assay also demonstrated good correlation with linearity to the two JCTLM-approved LC-MS/MS methods developed by Roche Diagnostics and UPenn, using Institute for Reference Materials and Measurements CRM. We observed minimal systematic bias versus the UPenn method and a systematic bias of 25% to the Roche Diagnostics method, which is the reference method for the Elecsys® assay. In terms of linearity and correlation, our results are consistent with the findings from a previous study that compared the Elecsys® β-Amyloid (1–42) CSF assay with the JCTLM-approved LC-MS/MS method developed by Roche Diagnostics in 362 human CSF samples (Pearson's r, 0.929) [15]. The published bias between Elecsys® assay and LC-MS measurements ($y = 0.97\times + 7.58$) differs from the results described in the current paper. The inconsistencies between the LC-MS results from different laboratories (Roche and UPenn), and inconsistences within the Roche LC-MS procedure that led to the 25% bias to the Elecsys® assay, could be explained by the variability of the LC-MS calibration material and by the fact that no certified reference material was available as calibrator for the LC-MS/MS method at the time of the reference standardisation of the Elecsys® assay. This problem can now be solved by using Aβ(1–42) CRM samples comprising CSF pools with assigned target values, which were defined using LC-MS/MS methods in a large study that was performed in several laboratories. Reference standardisation of the immunoassays using CRMs may help to standardise their concentrations with the LC-MS/MS measurements. The adjustment of CSF Aβ(1–42) values based on CRMs is planned in samples from the Alzheimer's Disease Neuroimaging Initiative study; any systematic difference between LC-MS/MS and Elecsys® Aβ(1–42) CSF assay measurements are expected to be minimised after adjustment using CRMs [29].

Bias estimates between the methods should be carefully interpreted, since pre-analytical effects and site-to-site and lot-to-lot variability between the comparator assays can influence results. Furthermore, LC-MS/MS methods are subject to a number of potential sources of measurement variability, due to the complexity of the procedure [30].

The results reported here were obtained at multiple centres, using samples from three different clinical cohorts and two JCTLM-approved LC-MS/MS reference methods [22,23], thus improving the reliability of the results. However, the measurements within each method comparison study were performed at one site and in most cases using one reagent lot. Therefore, the bias estimates may be confounded by site and lot effects. The sample size of the AIBL subset used for the Elecsys® β-Amyloid (1–42) CSF versus ADx-EUROIMMUN Beta-Amyloid (1–42) ELISA comparison was relatively small, limiting interpretation of the findings from this comparison. Furthermore, our findings cannot be generalised to all laboratories due to differences in pre-analytical handling procedures.

The ability to reliably measure Aβ is important in the study of AD pathology and for monitoring the biological effect of AD drugs in clinical trials [31,32], and Aβ biomarkers are central to the research diagnostic recommendations in the new National Institute on Aging and Alzheimer's Association Research Framework and International Working Group-2 criteria [7,8]. CSF Aβ(1–42) has also shown promise as a biomarker for the prediction of clinical decline in non-dementia patients with mild cognitive impairment [19]. However, the interpretation of measurement results is limited by several factors. First,

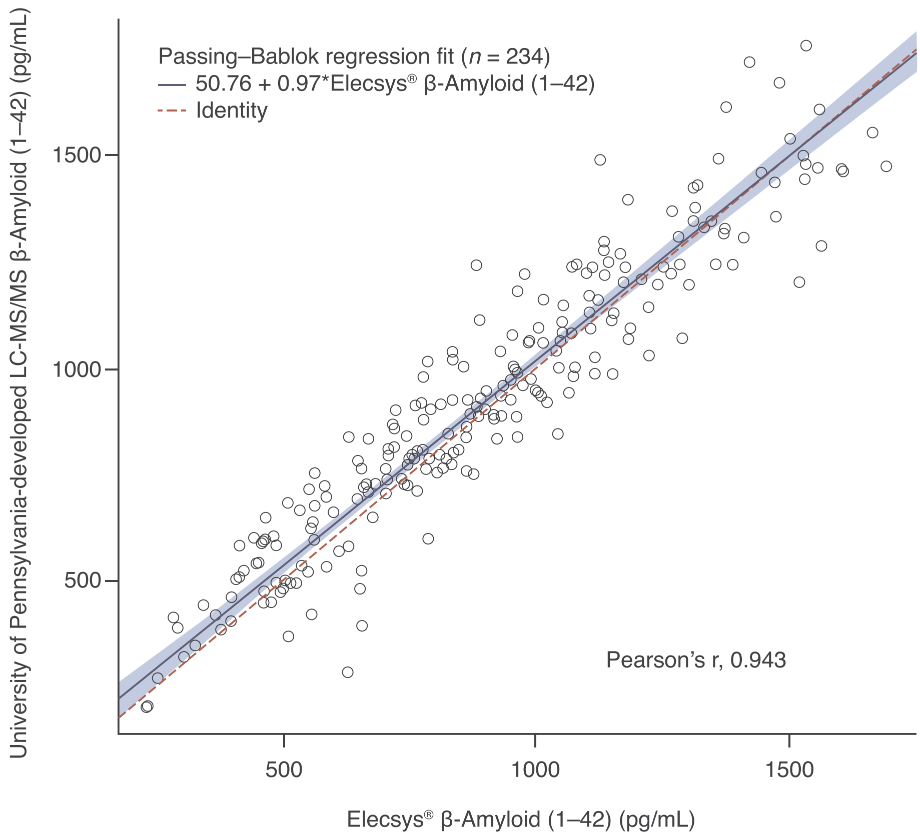

Fig. 5. Passing–Bablok regression fit for the comparison of amyloid-β concentrations measured with the Elecsys® β-Amyloid (1–42) CSF assay (**cobas e** 601 analyser) and the University of Pennsylvania-developed LC-MS/MS method (Study 2). The shaded area represents the 95% confidence interval for the fitted curve. CSF, cerebrospinal fluid; LC-MS/MS, liquid chromatography–tandem mass spectrometry.

systematic differences exist between assays due to different reference standardisation. Second, differences in pre-analytical procedures used for sample preparation at different centres can influence measured Aβ levels. Finally, analytical variability exists within measurement procedures (i.e. between-laboratory and lot-to-lot variability). These factors prevent the establishment of universal diagnostic cut-offs [20,33–39]. However, these limitations can be addressed. CRMs have recently been developed for distribution that can be used for reference standardisation and enable harmonisation between the measurement levels of different assays [20,26]. Several activities are ongoing with the objective of identifying a unified pre-analytical protocol that would guarantee comparability of Aβ(1–42) measurements in samples collected at different centres and measured at different sites. Finally, analytical variability can be reduced by implementation of enhanced quality control procedures. Together, these steps will facilitate the standardisation of different assays to a common reference and wider acceptance of CSF Aβ(1–42) as a reliable biomarker for AD.

5. Conclusion

These findings add to a growing body of evidence supporting the reliability and sensitivity of the novel Elecsys® β-Amyloid (1–42) CSF assay. Standardisation of the Elecsys® β-Amyloid (1–42) CSF assay to a JCTLM-approved reference method is a technical advantage and may result in improved clinical performance compared with established assays.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clinbiochem.2019.05.006.

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