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Interval-specific likelihood ratios for clinical interpretation of plasma pTau217 across assays

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Abbreviations: AD: Alzheimer's disease, PET: positron emission tomography, CSF: cerebrospinal fluid, NfL: neurofilament light chain, GFAP: glial fibrillary acidic protein; LR: likelihood ratio; CCUG: Cognitive Center UZ Ghent; CLEIA: chemiluminescence Enzyme Immunoassay; CLIA: chemiluminescence Immunoassay, ECLIA: electrochemiluminescence immunoassay; CI: confidence interval; PET: Positron Emission Tomografy

Abstract

Background

Plasma phosphorylated tau217 (pTau217) is a highly accurate blood-based biomarker for Alzheimer's disease (AD). However, absolute pTau217 concentrations differ substantially between assays, and results are commonly interpreted categorically using one or two assay-specific cut-offs, reducing a continuous biomarker into two or three diagnostic categories and limiting comparison across assays. We documented the performance of the HISCL™ pTau217 assay and valuated whether test result interval-specific likelihood ratios (LRs) could provide a common framework for interpreting plasma pTau217 across assays.

Methods

A total of 187 participants from the Cognitive Center UZ Ghent (CCUG) biobank were classified as AD (n=103) or non-AD cognitive disorders (n=84) using CSF biomarkers. Samples were analysed using 3 pTau217 assays: HISCL™ (Sysmex), Lumipulse G (Fujirebio) and Elecsys (Roche). Assay-specific concentration thresholds were derived from predefined sensitivity and specificity criteria, and interval-specific LRs were calculated for each assay.

Results

HISCL™ pTau217 showed a high diagnostic accuracy (AUC 0.952), comparable to Lumipulse (AUC 0.950, *p-value= 0.931*) and Elecsys (AUC 0.939, *p-value= 0.357*). Despite substantial differences in absolute concentrations, interval-specific LRs were closely aligned. Using 95% sensitivity and 95% specificity thresholds, LRs were 0.06-0.08 below the lower threshold, 0.40-0.85 for intermediate results, and 15.9-22.5 above the upper threshold. Subdivision by 90%, 95% and 99% specificity showed a stepwise increase, with LR from 0.10-0.13 up to 28.4-29.8, again with close agreement across assays.

Conclusion

Test result interval-specific LRs allow assay-specific pTau217 concentrations to be expressed on a common scale of diagnostic evidence, enabling comparison across assays while retaining information on result magnitude and allowing integration with the pre-test probability of AD. This proof-of-concept approach complements single- and two-cut-off strategies and warrants validation in larger, multicentre cohorts.

1. Introduction

Plasma phosphorylated tau 217 (pTau217) has shown high diagnostic accuracy for detecting Alzheimer's disease (AD) pathology across different clinical cohorts and analytical platforms, and is increasingly being implemented in specialized memory-care settings [1–3]. Its performance approaches that of established cerebrospinal fluid (CSF) and positron emission tomography (PET) biomarkers, while offering the advantages of a minimally invasive and readily accessible blood test. As a result, pTau217 is now being positioned as a clinically actionable biomarker within the diagnostic work-up of patients with cognitive impairment [1,4].

Current approaches to pTau217 interpretation rely on assay-specific cut-offs, using either a single threshold or, increasingly, a two-cut-off strategy. A single cut-off translates a continuous biomarker concentration into a positive or negative result, whereas two-cut-off strategies use thresholds with high sensitivity and specificity to define low-, intermediate-, and high-probability zones [1,4]. This approach reduces misclassification around a single threshold, but results falling between both thresholds remain indeterminate and generally require additional biomarker (such as CSF testing or PET) testing [4]. Furthermore, categorization into 3 broad zones does not quantify the diagnostic information associated with individual results within these zones.

Despite generally comparable diagnostic performance of the pTau217 assays, interpretation is further complicated by the lack of standardization between assays as there is no commutable reference material and standardized measurement scale [5]. Currently available assays use different analytical technologies, antibodies, and calibration procedures. This lack of standardization in absolute pTau217 concentrations across assays consequently necessitates the use of assay-specific cut-offs [2,6,7].

Test result interval-specific likelihood ratios (LRs) provide an alternative framework for expressing the diagnostic information contained in a continuous test result. Rather than assigning all results above or below a threshold the same interpretation, interval-specific LRs quantify how strongly results within different concentration ranges change the odds of disease [8,9]. Because LRs are independent of measurement units, assay-specific concentration intervals can be expressed on a common scale of diagnostic evidence. Moreover, LRs can be combined with the pre-test probability of AD to derive a corresponding post-test probability, allowing the diagnostic information provided by a pTau217 result to be integrated with the clinical context. This approach has previously been applied to autoantibody testing and, more recently, to CSF AD biomarkers across different analytical platforms [10], but has not yet been evaluated for plasma pTau217. In this study, interval-specific LRs were derived using two complementary interval definitions: a primary definition based on the

recommended 95% sensitivity and 95% specificity thresholds, and a secondary definition based on 90%, 95% and 99% specificity thresholds.

In this proof-of-concept study, we applied test result interval-specific LRs to 3 automated plasma pTau217 assays in a memory-clinic cohort and evaluated whether this approach could provide a common framework for interpreting assay-specific pTau217 concentrations. As a second objective, we evaluated the analytical and diagnostic performance of the HISCL™ pTau217 assay and compared it with the Lumipulse and Elecsys assays, for which diagnostic performance in this cohort was previously reported [3].

2. Materials and Methods

2.1. Patients and samples

The patient cohort was described previously [3]. Briefly, participants were selected from the ongoing Cognitive Center UZ Ghent (CCUG) Biobank, a prospective memory-clinic cohort collecting blood and CSF samples together with clinical, neuropsychological and imaging data from individuals referred for evaluation of cognitive complaints at Ghent University Hospital. Consecutively enrolled participants with available plasma samples were included.

Underlying AD pathology was defined by a concordant CSF biomarker profile based on the Aβ42/40 ratio, total tau and pTau181 measured using the LUMIPULSE G assays and established assay-specific cut-offs (n=180), or by amyloid-PET in a minority of participants (n=7), as described previously [3]. Based on this reference standard, participants were classified as having AD (n=103) or non-AD cognitive disorders (n=84). The non-AD group comprised patients with non-AD neurological disorders (n=54) and patients with psychiatric or psychological causes of cognitive complaints (n=30).

This study was approved by the local ethics committee of Ghent University Hospital (ONZ-2024-0422). All procedures were performed in accordance with the principles of the World Medical Association Declaration of Helsinki. Written informed consent was obtained from all participants prior to enrolment in the study.

2.2. Blood collection

Blood samples were collected in K2-EDTA tubes, centrifuged within 2h of collection, aliquoted, and stored at −80 °C in the CCUG Biobank at Ghent University Hospital according to standardized procedures. Before analysis, plasma samples were thawed to room temperature, centrifuged, and transferred to instrument cuvettes following the manufacturer's instructions.

2.3. Biomarker measurements

All samples were analysed with 3 plasma pTau217 assays. The analyses were performed at the Ghent University Hospital according to the manufacturer's instructions. Results were expressed in pg/mL.

Plasma concentrations of pTau217 were measured using the High-sensitivity chemiluminescence Enzyme Immunoassay (CLEIA) p-Tau217 (RUO) assay from Sysmex on the Automated Immunoassay System HISCL™ (Sysmex, Hamburg, Germany), the plasma pTau217 using the chemiluminescent enzyme immunoassay (CLEIA) on the LUMIPULSE G1200 platform (Fujirebio, Gent, Belgium) and the electrochemiluminescence immunoassay (ECLIA) Elecsys Phospho-Tau (217P) Plasma RUO assay on the Cobas pro e801 analyser (Roche Diagnostics, Mannheim, Germany). Plasma Aβ40 (HISCL™ β-

amyloid 1-40 assay) and Aβ42 (HISCL™ β-amyloid 1-42 assay) concentrations were additionally measured using CLEIA on the Automated Immunoassay System HISCL as well as on the Lumipulse G1200 analyser [3].

2.4. Statistical analysis

The analytical performance of the Fujirebio and Elecsys assays has been described previously [3,11]. The precision of the HISCL™ pTau217, Aβ40 and Aβ42 assays was determined using two levels of commercial quality control (QC) solutions (HISCL™ pTau217 Control for Research Use, and beta-amyloid control for Research Use) following the CLSI EP15-A3 protocol [12], with five measurements in a single run during five non-consecutive days. Linearity was measured by diluting a sample containing a high concentration of pTau217. Each dilution was determined in triplicate, and the mean value was considered the correct result. The lower limit of quantitation (LLOQ) was determined using a predefined CV specification of 20% [13]. Samples with a measured concentration below the LLOQ were interpreted using the LLOQ value.

Agreement between assays was evaluated by Passing-Bablok regression, Bland-Altman analysis of the mean relative bias, and Spearman rank correlation. Diagnostic accuracy for distinguishing AD from non-AD cognitive disorders was assessed by receiver operating characteristic (ROC) curve analysis, with areas under the curve (AUC) reported with 95% confidence intervals and compared between assays using the DeLong test. Optimal single cut-offs were determined by maximising the Youden index.

Assay-specific concentration thresholds for the interval-specific LR analysis were derived from the study cohort as the values corresponding to predefined sensitivities and specificities (95% sensitivity and 95% specificity for the primary analysis; 90%, 95% and 99% specificity for the secondary analysis). Test result interval-specific LRs were calculated as the proportion of AD patients within a given concentration interval divided by the proportion of non-AD patients within the same interval, with 95% confidence intervals [14]. Post-test probabilities were derived from the pre-test probability and the interval-specific LR using Bayes' theorem (post-test odds = pre-test odds × LR). All statistical analyses were conducted using MedCalc (version 15.6.1, Mariakerke, Belgium). A two-sided p-value < 0.05 was considered statistically significant.

3. Results

3.1. Study population

The study included 187 participants from the previously described CCUG memory-clinic cohort [3], including 103 patients with AD and 84 with non-AD cognitive disorders, corresponding to an AD prevalence of 55.1%. The non-AD group comprised 54 patients with non-AD neurological disorders and 30 with psychiatric or psychological causes of cognitive complaints. The median age was 72 years in the AD group and 70 years in the non-AD group. Patients with AD had lower baseline MoCA scores than those with non-AD disorders (median 19 vs. 22), whereas age and renal function were comparable between groups. Detailed demographic and clinical characteristics have been reported previously [3].

3.2. Analytical validation of the HISCL™ pTau217 assay

3.2.1. Imprecision, linearity and LoQ

Imprecision was assessed using commercially available quality control (QC) solutions. Within-laboratory imprecision ranged from 1.7% to 2.8% for pTau217, 2.8% to 2.9% for Aβ40, and 2.5% to 4.6% for Aβ42 (**Supplemental Table 1**). Linearity was maintained down to 0.15 pg/mL for pTau217 on the HISCL™ analyser (**Supplemental Figure 1**). The lower limit of quantitation (LLOQ) was estimated at 0.04 pg/mL for pTau217.

3.2.2. Method comparison

Passing-Bablok regression analysis demonstrated a proportional difference in pTau217 concentrations between the Lumipulse CLEIA and HISCL™ CLEIA assays [slope: 1.17 (95%CI: 1.13 – 1.22)] and between the Cobas ECLIA and HISCL™ CLEIA assays [Slope: 0.86 (95%CI: 0.82 – 0.91)]. Between the CLIA and ECLIA assays, both a proportional (slope = 1.363; 95% CI: 1.251–1.484) and systematic (intercept = 0.113; 95% CI: 0.098–0.133) difference was observed (**Supplemental Figure 2**). A statistically significant correlation was observed between the assays (range r = 0.88 – 0.94, *p-value <0.0001*). Furthermore, Bland-Altman analysis, demonstrated substantial inter-assay differences, with mean relative biases ranging from 11.7% to −58.4% (**Supplemental Figure 2**).

3.3. Diagnostic accuracy and assay-specific cut-offs

The HISCL™ pTau217 assay showed high diagnostic accuracy for distinguishing AD from non-AD cognitive disorders, with an AUC of 0.952 (95% CI 0.910–0.978) (Table 1). Diagnostic performance was comparable across the pTau217 assays, with AUCs of 0.952 for HISCL™, 0.950 for Lumipulse and 0.939 for Elecsys (DeLong test, *p-value >0.05*).

Using a two-cut-off approach targeting 95% sensitivity and 95% specificity [6], the HISCL™ lower and upper thresholds were 0.13 and 0.40 pg/mL, respectively. The corresponding thresholds were 0.13 and 0.25 pg/mL for Lumipulse and 0.27 and 0.50 pg/mL for Elecsys.

Within the HISCL™ and Lumipulse platform, combining pTau217 with Aβ42 or the Aβ42/Aβ40 ratio did not significantly improve diagnostic performance compared with pTau217 alone (AUC 0.952 vs. 0.958 and 0.960, respectively; DeLong test, *p-value* = 0.058 and *p-value* = 0.322, respectively, **Table 1**).

3.4. Test result interval-specific likelihood ratios across pTau217 assays

To quantify the diagnostic information associated with pTau217 results beyond binary classification using a single cut-off, test result interval-specific LRs were calculated for each assay. Using assay-specific thresholds corresponding to 95% sensitivity and 95% specificity, three concentration intervals were defined (**Table 2**). Results below the 95% sensitivity threshold yielded LRs ranging from 0.06 to 0.08, intermediate results LRs from 0.40 to 0.85, and results above the 95% specificity threshold LRs from 15.9 to 22.5 across the assays (**Figure 1**).

Interval-specific LRs allow the diagnostic information of a test result to be combined with the pre-test probability of AD. **Figure 1** shows the corresponding post-test probabilities across a range of pre-test probabilities. For illustration, at a pre-test probability of 50%, results below the 95% sensitivity threshold reduced the post-test probability to approximately 6–7%, whereas results above the 95% specificity threshold increased it to approximately 94–96%.

To further distinguish the diagnostic information associated with the magnitude of the pTau217 result, the concentration range was subdivided using assay-specific thresholds corresponding to 90%, 95%, and 99% specificity. LRs increased stepwise across the resulting intervals for all assays (**Table 2**; **Figure 2**). LRs ranged from 0.10 to 0.13 below the 90% specificity threshold and from 0.58 to 1.93 in the 90–95% interval. For higher pTau217 results, LRs increased to 9.04–9.9 in the 95–99% specificity interval and to 28.4–29.8 above the 99% specificity threshold. LR estimates in these two higher intervals were closely aligned across the assays despite substantial differences in the corresponding absolute pTau217 concentrations.

The separation between these higher intervals also translated into different post-test probabilities (**Figure 2**). For example, at a pre-test probability of 25%, an LR of approximately 9–10 corresponded to a post-test probability of approximately 75–77%, compared with approximately 90–91% for an LR of approximately 28–30. At a pre-test probability of 50%, the corresponding post-test probabilities were approximately 90–91% and 97%, respectively.

4. Discussion

Our findings provide proof-of-concept that interval-specific likelihood ratios (LRs) can offer a quantitative framework for interpreting plasma pTau217 results across different automated assays. Across assays, increasing pTau217 concentrations were associated with progressively higher LRs for AD, preserving diagnostic information that is partly lost when a continuous biomarker is reduced to a limited number of categories. Despite substantial differences in absolute pTau217 concentrations between assays, the interval-specific LRs were similar and allowed assay-specific results to be expressed on a common scale of diagnostic evidence. When combined with the pre-test probability of AD, these LRs could be translated into clinically meaningful post-test probabilities. In addition, the HISCL™ pTau217 assay showed high analytical and diagnostic performance comparable to the previously evaluated Lumipulse and Elecsys assays.

The increasing clinical use of plasma pTau217 creates challenges for both interpretation and comparability of results. A single cut-off reduces a continuous biomarker to a binary result and does not distinguish between concentrations just above the threshold and markedly elevated values. The recommended two-cut-off approach improves on this by defining low-, intermediate-, and high-probability zones and reducing misclassification around a single decision threshold [4,6,15]. However, intermediate results remain indeterminate and generally require additional biomarker testing, while results within each of the three zones are still interpreted categorically. At the same time, absolute pTau217 concentrations differ substantially between assays because of differences in assay design, antibodies, calibration, and analytical platforms [5–7]. Consequently, neither absolute concentrations nor cut-offs can currently be transferred directly between assays. Moreover, for a given assay, different cut-offs have been proposed depending on the intended use and care setting, with lower thresholds favouring sensitivity for screening in primary or secondary care and higher thresholds favouring specificity for confirmation in tertiary care, further complicating the interpretation and comparison of results. These limitations provide a rationale for an approach that retains more of the information contained in a continuous result while expressing its diagnostic significance on a scale that is independent of the assay measurement units.

Interval-specific LRs address these limitations by expressing the diagnostic evidence associated with different parts of the pTau217 concentration range. Applied to the recommended two-cut-off strategy, results below the 95% sensitivity threshold were associated with LRs of 0.06–0.08, whereas results above the 95% specificity threshold yielded LRs of 15.9–22.5. As an illustrative example from our cohort, the same sample yielded pTau217 concentrations of 0.20, 0.15, and 0.44 pg/mL with HISCL™, Lumipulse, and Elecsys, respectively. These values were close to their respective single cut-offs and resulted in discordant binary classifications, with the HISCL™ and Lumipulse results classified

as negative and the Elecsys result as positive. Using the two-cut-off approach, all results fell within the intermediate zone and would therefore require additional biomarker testing. Their corresponding LRs of 0.85, 0.56, and 0.40 provide an additional quantitative measure of the diagnostic evidence associated with each result (**Table 3**). This illustrates how LRs can complement, rather than replace, the established two-cut-off strategy by adding quantitative information to its categorical interpretation.

Further subdivision of the concentration range provided additional discrimination among results that would otherwise fall within the same broad category. LRs were approximately 9–10 for concentrations within the 95–99% specificity interval and 28–30 above the 99% specificity threshold. These estimates were similar across the assays despite substantial differences in the corresponding absolute concentrations. The similar interval-specific LRs observed across the assays support the potential of this approach to provide a common framework for interpreting assay-specific pTau217 results.

A further advantage of LRs is that their diagnostic impact can be interpreted in relation to the pre-test probability of AD. This is particularly relevant because pre-test probability reflects both patient characteristics and the stage of the diagnostic evaluation and may therefore vary considerably even within a memory-clinic population. Cognitive phenotype provides a clinically relevant example. In a large multicentre memory-clinic cohort, the prevalence of amyloid pathology among patients with MCI differed markedly according to cognitive phenotype, being 45.9% in those with amnestic MCI and 24.5% in those with non-amnestic MCI [16]. A comparable difference in amyloid positivity between amnestic and non-amnestic MCI has been reported previously [17]. Using these values illustratively, an LR of 10 would increase a pre-test probability of approximately 25% (non-amnestic MCI) to a post-test probability of approximately 77%, compared with an increase from approximately 46% (amnestic MCI) to 90%. An LR of approximately 30 would increase the corresponding post-test probabilities to approximately 91% and 96%, respectively. Thus, reporting the LR allows the same biomarker evidence to be interpreted differently according to the clinical probability of AD before testing. The similar interval-specific LRs observed across the assays would consequently yield comparable post-test probabilities across analytical platforms.

Beyond its diagnostic interpretation, the quantitative pTau217 concentration carries clinical information in its own right. pTau217 is measured on a continuous scale, and higher concentrations have been associated with more advanced AD pathology and with subsequent disease progression and cognitive decline [18–20]. The quantitative result may therefore provide information relevant to staging and prognosis beyond its diagnostic interpretation. This argues for a layered laboratory report

rather than a single categorical output: the quantitative pTau217 concentration, its assay-specific category, and the corresponding interval-specific LR each convey complementary information, while the LR can additionally be combined with the pre-test probability to derive a post-test probability of AD.

The second objective of this study was to evaluate the HISCL™ pTau217 assay. HISCL™ showed low imprecision and high diagnostic accuracy, with an AUC of 0.952, comparable to Lumipulse and Elecsys. Adding Aβ42 or the Aβ42/Aβ40 ratio did not significantly improve diagnostic performance. The added value of plasma Aβ biomarkers may depend on disease stage, with potential benefits reported in preclinical AD [21]. Substantial proportional differences in absolute pTau217 concentrations were observed between platforms, consistent with previous head-to-head comparisons [3,6,7]. Development of certified and commutable reference materials remains important to achieve analytical standardization. Interval-specific LRs should not be regarded as a substitute for such standardization: they do not harmonize measured concentrations but offer a potential means of harmonizing their clinical interpretation.

The present study evaluated interval-specific LRs in cognitively impaired patients attending a memory clinic, where pTau217 was interpreted in relation to underlying AD pathology as the cause of the clinical syndrome. AD status in this cohort was defined using CSF biomarkers, including CSF pTau181; the interval-specific LRs reported here therefore express the diagnostic evidence of plasma pTau217 with respect to a predominantly CSF-defined reference standard rather than amyloid PET, which should be considered when comparing LR estimates across studies using different reference standards. More broadly, the clinical use of pTau217 is likely to extend across the AD continuum and across healthcare settings. This includes primary-care populations with a lower prevalence and a broader spectrum of cognitive disorders, as well as cognitively unimpaired individuals, in whom pTau217 may detect AD pathology before the onset of clinical symptoms [1,22]. Evaluating the LR framework across these populations will be important to determine how interval-specific LRs vary with clinical setting and disease stage and, ultimately, to establish their generalizability.

Beyond these questions regarding the broader applicability of the LR framework, several limitations of the present study should be considered. The cohort included 187 participants, which was sufficient to explore the feasibility of interval-specific LR interpretation across the assays, but estimates were less precise for intervals containing relatively few participants, particularly at the extremes of the concentration range. In addition, this was a single-center proof-of-concept study, and interval-specific LRs were derived and evaluated within the same cohort; external validation is therefore required to establish the reproducibility and precision of the estimates. Although LRs are

independent of disease prevalence, they may be affected by spectrum effects and therefore vary with the characteristics of the populations in which they are derived. Finally, we did not develop or validate an approach for estimating individual pre-test probability, which will be important for translating LR-based interpretation into clinical practice.

In conclusion, interval-specific LRs provide a complementary framework for interpreting plasma pTau217 as a continuous biomarker. They retain information associated with the magnitude of a test result, can be integrated with pre-test probability to derive clinically meaningful post-test probabilities, and may allow assay-specific concentrations to be expressed on a common scale of diagnostic evidence. This approach does not replace analytical standardization or established two-cut-off strategies but may complement them by enabling a more quantitative and clinically contextualized interpretation of plasma pTau217 results. Larger multicenter studies are needed to externally validate the interval-specific LRs and determine whether their cross-assay consistency is maintained across populations and clinical settings.

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6. Declarations

6.1. Ethics approval and consent to participate

This study was approved by the local ethics committee of the Ghent University (ONZ-2024-0422). All procedures were conducted in accordance with the World Medical Association Declaration of Helsinki. Written informed consent was obtained from all participants before inclusion in the study.

6.2. Consent for publication

Not applicable.

6.3. Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

6.4. Clinical trial number

Not applicable

6.5. Competing interests

Tim Van Langenhove has received consulting fees from Eisai (paid to institution), and Eli Lilly paid to institution)..

6.6. Funding

Sysmex, Roche, Fujirebio provided the biomarker testing tools free of charge for this study. The companies had no role in study design, data collection, data analysis, interpretation, or manuscript preparation.

6.7. Author contributions

MO supervised and performed biomarker analyses, including sample processing and quality control of the assays, conceived the study-design, performed the main statistical analyses, data visualization, and figure preparation, and contributed to data interpretation and manuscript writing. JT contributed to patient selection, clinical assessment and co-wrote and revised the manuscript. MS contributed to patient selection, clinical assessment, and inclusion of participants in the Cognitive Centre Ghent University (CCUG) biobank and critically reviewed the manuscript. CB co-wrote and revised the manuscript. TVL conceived the study design, supervised the overall project, contributed to the statistical analyses and data interpretation, and co-wrote and revised the manuscript.

6.8. Acknowledgements

Not applicable.

6.9. Authors information (optional)

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FIGURES

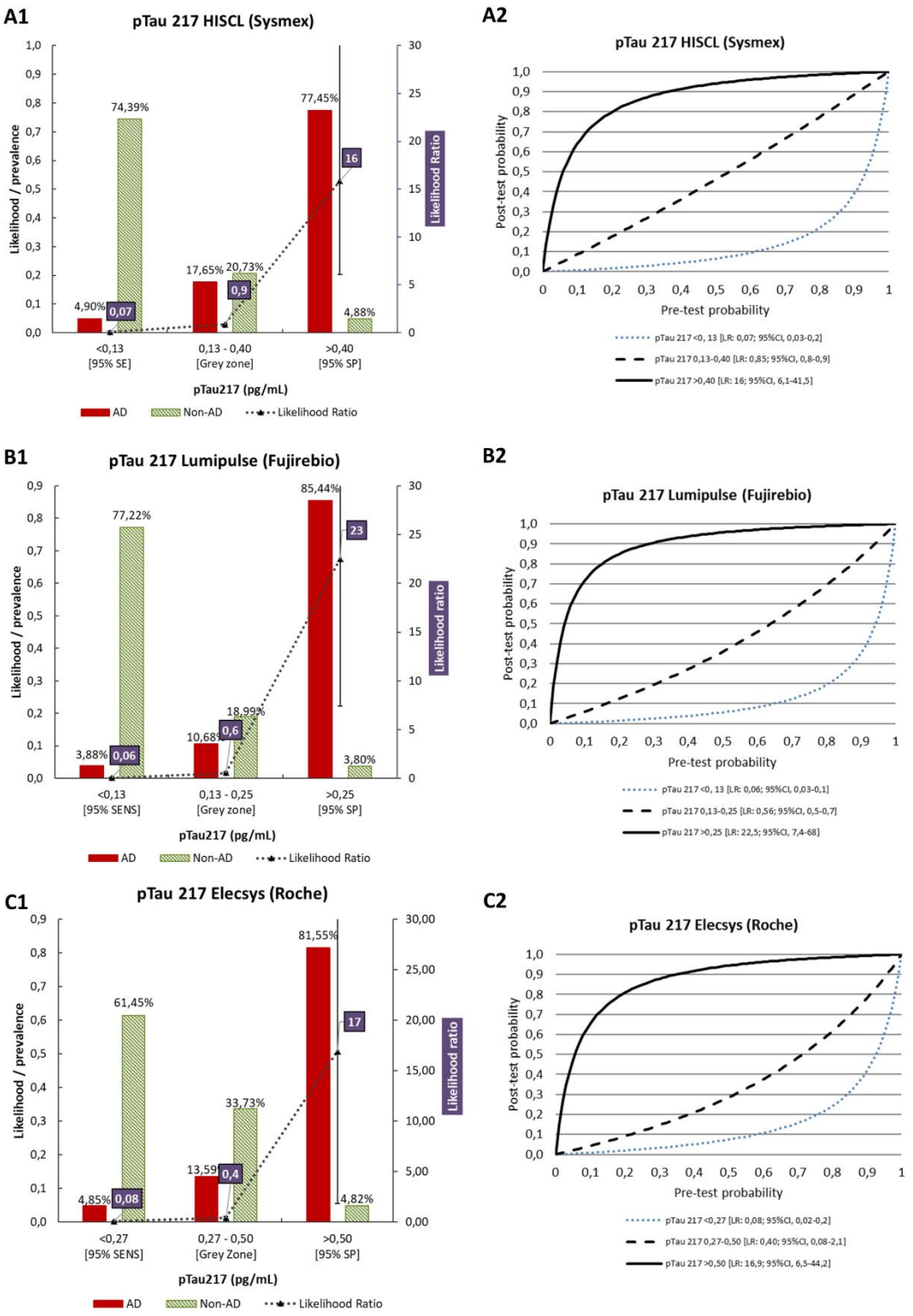


Figure 1. (**1**) Prevalence (likelihood) of AD and non-AD according to plasma pTau217 test result intervals, which are based on <95% sensitivity and >95% specificity levels for the HISCL™ (**A**),

Lumipulse (**B**) and Elecsys (**C**) assays. The corresponding likelihood ratios with 95% confidence intervals to the test result intervals are shown in the second y-axis. (**2**) Post-test probability for AD as a function of pre-test probability for different test result intervals (depicted as corresponding likelihood ratios including 95% confidence intervals) for the HISCL™ (**A**), Lumipulse (**B**) and Elecsys (**C**) assays.

Abbreviations: AD: Alzheimer's disease; SP, specificity; SE: sensitivity

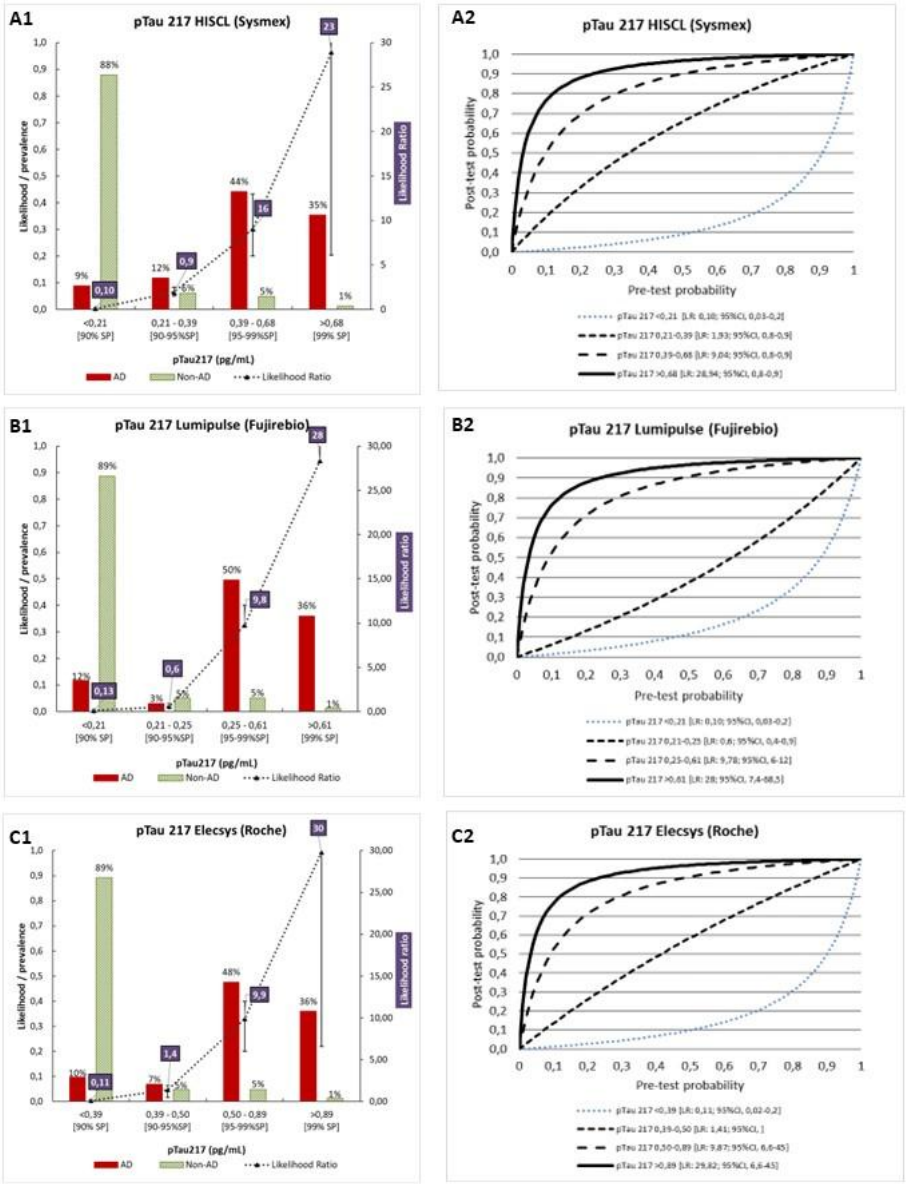
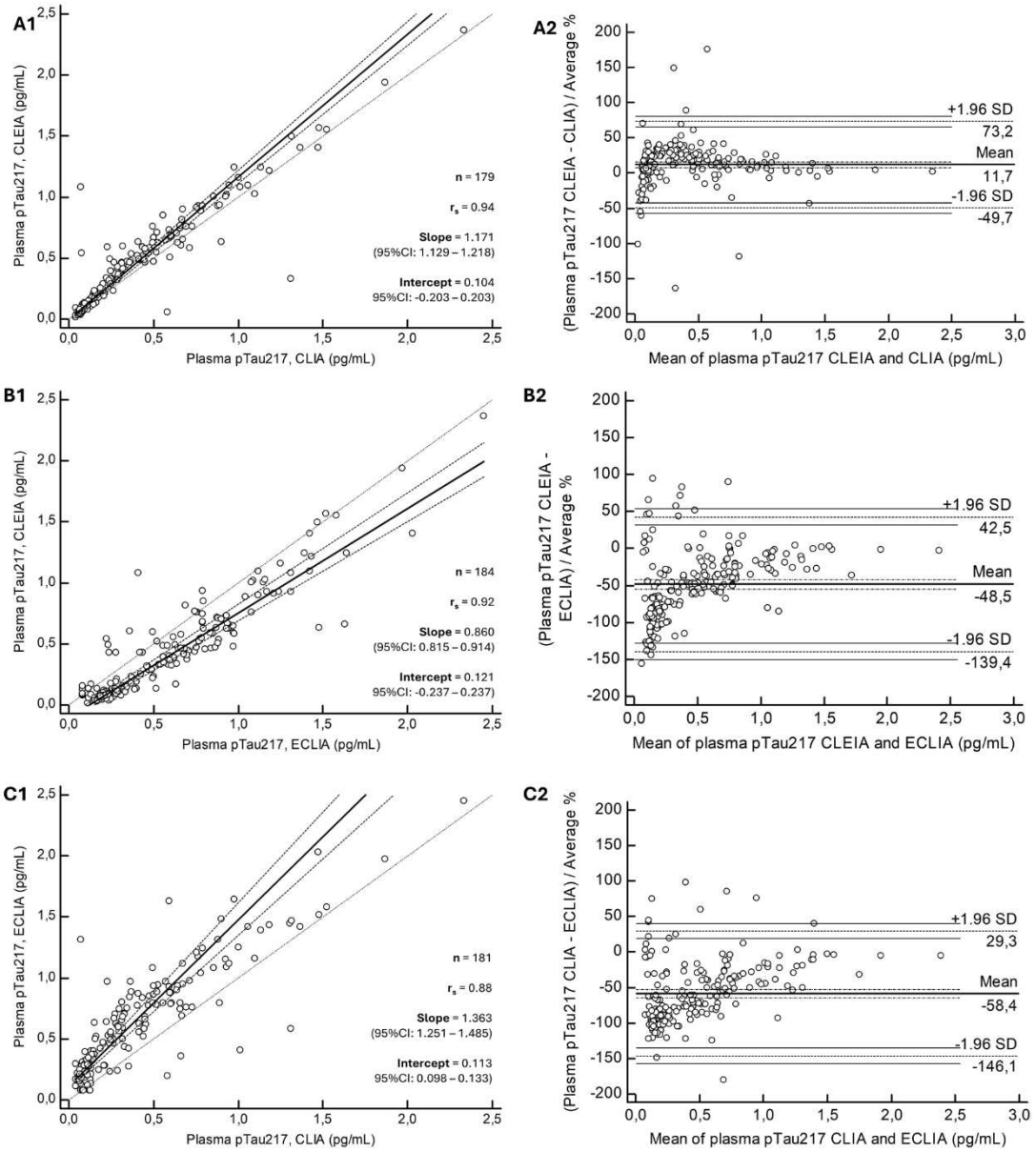


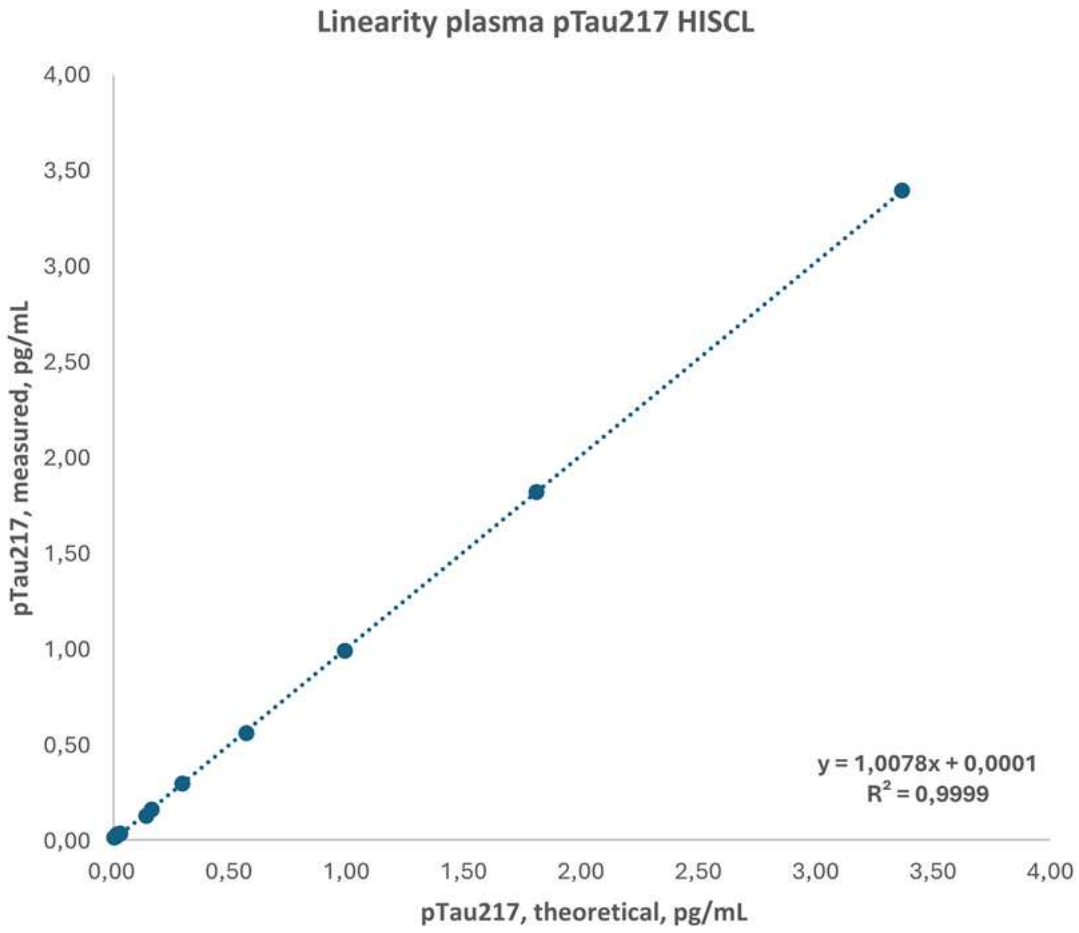
Figure 2. (**1**) Prevalence (likelihood) of AD and non-AD according to plasma pTau217 test result intervals, which are based on <90%, 90% to 95%, 95% to 99% and >99% specificity levels for the HISCL™ (**A**), Lumipulse (**B**) and Elecsys (**C**) assays. The corresponding likelihood ratios with 95% confidence intervals to the test result intervals are shown in the second y-axis. (**2**) Post-test probability for AD as a function of pre-test probability for different test result intervals (depicted as

corresponding likelihood ratios including 95% confidence intervals) for the HISCL™ (**A**), Lumipulse (**B**) and Elecsys (**C**) assays.

Abbreviations: AD: Alzheimer's disease; SP, specificity; SE: sensitivity



Supplemental Figure 1. Linearity of plasma pTau217 using a patient specimen with diluted buffered physiological saline. He dotted line represents the linear fitting line.



Supplemental figure 2. Comparison of plasma pTau217 between ECLIA (Roche; Elecsys), CLIA (Fujirebio; Lumipulse) and CLEIA (Sysmex, HISCL™). Passing-Bablok regression analysis (1). The bold line shows the fitted regression line. The 95%CI of the regression curve is shown as small, dashed lines. The regression equation (slope and intercept with corresponding 95%CI) and the Pearson correlation coefficient (r_s) are indicated. Bland-Altman plot demonstrating the relative difference obtained with ECLIA (Roche) against CLEIA (Fujirebio) (2). Horizontal lines are drawn at the mean difference between the two methods, and at the limits of agreement, which are defined as the mean difference plus and minus 1.96 times the standard deviation of the differences. The dotted horizontal lines present the 95% confidence intervals around the mean difference and the upper and lower limits of agreement.

Abbreviations: ECLIA: electrochemiluminescence immunoassays; CLEIA: Chemiluminescence enzyme immunoassay; CLIA: Chemiluminescence immunoassay

TABLES

Table 1. Diagnostic performance using single cut-off approach

Parameter	AUC (95%CI)	Optimal cut-off value (pg/mL)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
HISCL™ pTau 217	0.952 (0.910 – 0.978)	0.231	91.2% (93.9 – 95.9%)	91.5% (83.2 – 96.5%)	93.0 (86.1 – 97.1%)	89.3 (80.6 – 95.0%)
HISCL™ pTau 217 / (Aβ42/Aβ40)	0.960 (0.920 – 0.983)	2.156	93.1% (86.4 – 97.2%)	89.0% (80.2 – 94.9%)	91.3% (84.2 – 96.0%)	91.2% (82.8 – 96.4%)
HISCL™ pTau 217 / Aβ42	0.958 (0.918 – 0.982)	0.019	90.2% (82.7 – 95.2%)	93.9% (86.3 – 98.0%)	94.8% (88.4 – 98.3%)	88.5% (79.9 – 94.3%)
Lumipulse pTau217	0.950 (0.907 – 0.976)	0.246	85.4% (77.1 – 91.6%)	96.2% (89.3 – 99.2%)	96.7% (90.7 – 99.3%)	83.5% (74.3 – 90.5%)
Lumipulse pTau217/ (Aβ42/Aβ40)	0.957 (0.917 – 0.982)	3.891	85.4% (77.1 – 91.6%)	96.2% (89.3 – 99.2%)	96.7% (90.7 – 99.3%)	83.5% (74.3 – 90.5%)
Lumipulse pTau217/ Aβ42	0.957 (0.916 – 0.981)	0.0115	89.3% (81.7 – 94.5%)	94.9% (87.5 – 98.6%)	95.8% (89.7 – 98.9%)	87.2% (78.3 – 93.4%)
Elecsys p-Tau217	0.939 (0.895 – 0.969)	0.371	92.2% (85.3 – 96.6%)	89.2% (80.4 – 94.9%)	91.3% (84.2 – 96.0%)	90.2% (81.7 – 95.7%)

Abbreviations: AUC: Area under the ROC curve; CI: confidence interval, PPV: positive predictive value, NPV: negative predictive value.

Table 2. Plasma pTau217 test result specific LR, based on a sensitivity and specificity of 95% and a specificity of <90%, 90-95%, 95-99% and >99%

	Interval	Fraction of controls	Fraction of patients	LR	95%CI
Sensitivity/specificity 95%					
HISCL™	**<0.13**	0.744	0.049	0.07	0.03 – 0.17
	0.13-0.40	0.207	0.177	0.85	0.47 – 1.55
	>0.40	0.049	0.775	15.9	6.03 – 41.8
Lumipulse	**<0.13**	0.772	0.039	0.06	0.02 – 0.13
	0.13-0.25	0.190	0.107	0.56	0.27 – 1.17
	>0.25	0.038	0.854	22.5	7.3 – 69.6
Elecsys	**<0.27**	0.615	0.049	0.08	0.03 – 0.19
	0.27-0.50	0.337	0.136	0.40	0.09 – 1.01
	>0.50	0.048	0.816	16.9	6.5 – 44.3
<90%, 90-95%, 95-99% and >99% specificity					
HISCL™	**<0.21**	0.878	0.088	0.10	0.05 – 0.19
	0.21-0.39	0.061	0.118	1.93	0.71 – 5.3
	0.39-0.68	0.049	0.441	9.04	3.4 – 24.2
	>0.68	0.012	0.353	28.9	4.1 – 205.0
Lumipulse	**<0.21**	0.886	0.117	0.13	0.08 -0.23
	0.21-0.25	0.051	0.029	0.58	0.14 – 2.40
	0.25-0.61	0.051	0.495	9.8	3.8 – 25.4
	>0.61	0.013	0.359	28.4	4.0 – 200.7
Elecsys	**<0.39**	0.892	0.097	0.11	0.06 – 0.20
	0.39-0.50	0.048	0.068	1.41	0.43 – 4.7
	0.50-0.89	0.048	0.476	9.9	3.8 – 25.9
	>0.89	0.012	0.359	29.8	4.2 – 211.3

Abbreviations: LRs: likelihood ratios; CI: confidence interval

Table 3. A case example of communicating likelihood ratio values in a clinical laboratory report.

In the example below, laboratory A, using the HISCL™ assay, reported a pTau217 concentration of 0.20 pg/mL. Laboratory B, using the Lumipulse assay, measured 0.15 pg/mL, while laboratory C, using the Elecsys assay, reported 0.44 pg/mL. Based on the optimal assay-specific cut-offs derived from ROC analysis, the HISCL™ and Lumipulse results would be interpreted as negative, whereas the Elecsys result would be classified as positive. The patient was ultimately diagnosed with Alzheimer's disease.

When likelihood ratios are reported in addition to a single diagnostic cut-off, the interpretation is more informative. For this patient, the observed test results correspond to likelihood ratios whose 95% confidence intervals include 1, indicating that the results neither meaningfully increase nor decrease the probability of Alzheimer's disease. Consequently, despite the differences in absolute concentrations and binary classifications across assays, the likelihood ratio approach leads to a consistent interpretation of all three results: none provides sufficient evidence to confirm or exclude the diagnosis, and the patient should therefore be referred for further diagnostic evaluation.

Laboratory A – HISCL™	
pTau 217 (pg/mL)	0.20
>0.23 pg/mL: positive	
Likelihood ratio for Alzheimer's disease at diagnosis	0.85 (95%CI: 0.47 – 1.55)
Laboratory B - Lumipulse	
pTau 217 (pg/mL)	0.15
>0.25 pg/mL: positive	
Likelihood ratio for Alzheimer's disease at diagnosis	0.56 (95%CI: 0.27 – 1.17)
Laboratory C - Elecsys	
pTau 217 (pg/mL)	0.44
>0.37 pg/mL: positive	
Likelihood ratio for Alzheimer's disease at diagnosis	0.40 (95%CI: 0.09 – 1.01)

Abbreviation: CI: confidence interval

Supplementary Files

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- SupplementaltablesFINAAL.docx