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Associations of Fully Automated CSF and Novel Plasma Biomarkers With Alzheimer Disease Neuropathology at Autopsy

Running head: Neuropathologic correlates of fluid biomarkers

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Disclosure

H Zetterberg has served at scientific advisory boards for Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics and CogRx, has given lectures in symposia sponsored by Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program.

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Abstract

Objective: To study cerebrospinal fluid (CSF) biomarkers of Alzheimer's disease (AD) analyzed by fully automated Elecsys immunoassays in comparison to neuropathologic gold standards, and compare their accuracy to plasma phosphorylated tau (p-tau181) measured using a novel Simoa method.

Methods: We studied *ante-mortem* Elecsys-derived CSF biomarkers in 45 individuals who underwent standardized *post-mortem* assessments of AD and non-AD neuropathologic changes at autopsy. In a subset of 26 participants, we also analysed *ante-mortem* levels of plasma p-tau181 and neurofilament light (NfL). Reference biomarker values were obtained from 146 amyloid-PET-negative healthy controls (HC).

Results: All CSF biomarkers clearly distinguished pathology-confirmed AD dementia (N=27) from HC (AUCs=0.86-1.00). CSF total-tau (t-tau), p-tau181, and their ratios with A β ₁₋₄₂, also accurately distinguished pathology-confirmed AD from non-AD dementia (N=8; AUCs=0.94-0.97). In pathology-specific analyses, intermediate-to-high Thal amyloid phases were best detected by CSF A β ₁₋₄₂ (AUC[95% CI]=0.91[0.81-1]), while intermediate-to-high CERAD neuritic plaques and Braak tau stages were best detected by CSF p-tau181 (AUC=0.89[0.79-0.99] and 0.88[0.77-0.99], respectively). Optimal Elecsys biomarker cut-offs were derived at 1097/229/19 pg/ml for A β ₁₋₄₂, t-tau, and p-tau181. In the plasma subsample, both plasma p-tau181 (AUC=0.91[0.86-0.96]) and NfL (AUC=0.93[0.87-0.99]) accurately distinguished pathology-confirmed AD (N=14) from HC. However, only p-tau181 distinguished AD from non-AD dementia cases (N=4; AUC=0.96[0.88-1.00]), and showed a similar, though weaker, pathologic specificity for neuritic plaques (AUC=0.75[0.52-0.98]) and Braak stage (AUC=0.71[0.44-0.98]) as CSF p-tau181.

Conclusions: Elecsys-derived CSF biomarkers detect AD neuropathologic changes with very high discriminative accuracy *in-vivo*. Preliminary findings support the use of plasma p-tau181 as an easily accessible and scalable biomarker of AD pathology.

Classification of Evidence: This study provides Class II evidence that fully-automated CSF t-tau and p-tau181 measurements discriminate between autopsy-confirmed Alzheimer's disease and other dementias.

Keywords: plasma p-tau181, CSF, Elecsys, neuropathology, Autopsy, amyloid, tau, Thal phase, Braak stages, neuritic plaques, diffuse plaques, biomarkers

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Introduction

The recent guidelines of the NIA-AA Research Framework now define Alzheimer's Disease (AD) as a biological entity where an *in vivo* diagnosis of AD is no longer solely based on clinical diagnostic criteria but requires supporting evidence from positron emission tomography (PET) or fluid biomarkers of AD-typical amyloid- β (A β) and tau pathology.¹⁻³ In contrast to PET, bodily fluid-based measurements can provide different molecular biomarkers from a single assessment, are more cost effective, widely attainable, and are not limited by radiation exposure. Yet, the International quality control program (www.neurochem.gu.se/TheAlzAssQCprogram) has shown large variability (>15%) of the commonly used manual plate-based enzyme-linked immunosorbent assays (ELISAs) for AD biomarker quantification in cerebrospinal fluid (CSF) across several laboratories.⁴ A major step towards widespread clinical use of CSF biomarkers has been the development of standardized measurements through fully automated platforms with high test-retest reliability (<5%) and low laboratory- and kit-associated variability such as the Roche Elecsys® electrochemiluminescence immunoassays,⁵ which show excellent concordance with the manual ELISAs⁶ and have been well validated against A β PET.⁷⁻¹⁰

The recent development of assays to measure phosphorylated tau in blood offers an alternative opportunity to assess AD pathology in a cost-effective, highly accessible and scalable manner. Plasma concentrations of tau phosphorylated at threonine 181 (p-tau181) correlate highly with CSF measures of p-tau181 as well as with PET measures of A β and tau pathology,¹¹⁻¹⁵ and have been shown to distinguish between AD and other neurodegenerative disorders with high diagnostic accuracy comparable to CSF and PET-based measures of tau pathology.^{11-13, 16}

However, only relatively few studies have thus far aimed to validate the established CSF¹⁷⁻²¹ or the novel plasma p-tau181 biomarkers^{11, 22} against neuropathologic gold standards. Specifically, to date, there exists no neuropathologic validation of the fully automated Elecsys-derived A β and tau biomarker measurements, and currently recommended

cut-offs for these standardised measures are based on concordance studies with A β -PET or clinical criteria.^{7-10, 23}

In this study, we examined *ante-mortem* Elecsys-derived CSF biomarkers in relation to AD neuropathology assessed at autopsy in the same individuals. In preliminary analyses on a smaller subset of participants we also analysed *ante-mortem* levels of plasma p-tau181 and neurofilament light (NfL). Specifically, we first studied the diagnostic accuracy of the fluid biomarkers for distinguishing pathology-confirmed AD dementia cases from A β -PET-negative healthy controls and dementia cases without AD pathology at autopsy. We then assessed the specific associations of the different A β and tau biomarkers with distinct aspects of AD neuropathology, including established neuropathologic rating scales for regional extension of A β pathology (Thal phases), cortical density of diffuse and neuritic A β plaques (CERAD), and regional extension of neurofibrillary tangle (NFT) tau pathology (Braak stages). We derived pathology-specific biomarker cut-offs that best separated individuals with absent-to-low from those with intermediate-to-high levels of the respective AD neuropathologic correlate. Finally, we assessed the sensitivity of the biomarkers for the presence of common non-AD pathologies at autopsy, including cerebral amyloid angiopathy (CAA), Lewy body (LB) pathology, and limbic TDP-43 pathology.

Material and methods

Data source

Data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (<http://adni.loni.usc.edu/>). The ADNI is a public-private partnership that was launched in 2003 with the primary goal to test whether neuroimaging and other biological markers can be used to track disease progression in AD. For up-to-date information, see www.adni-info.org.

Standard protocol approvals, registrations, and patient consents

Data collection and sharing was approved by the Institutional Review Board of each participating institution in ADNI. All participants provided written informed consent in accordance with the Declaration of Helsinki and its later amendments.

Study participants

In the present study, we used data from the subsample of ADNI participants who had been followed up to autopsy for standardised neuropathological examinations (Neuropathology data freeze v.11; 12/04/2018). From this ADNI autopsy cohort, we identified 45 participants who had available *ante-mortem* CSF measurements, with an average time difference between lumbar puncture and death of 2.9 (SD 1.9, IQR 1.7-3.7, min-max=0.4-8.7) years (see Supplementary Figure S1 for a flowchart of patient selection; Data available from Dryad: <https://doi.org/10.5061/dryad.n2z34tmwr>). Participants were recruited between 2005 and 2013 and were followed-up to autopsy between 2008 and 2017. Thirty-five participants were diagnosed with AD dementia, six with mild cognitive impairment (MCI), and four as cognitively normal (CN) at their last clinical evaluation (on average 1.8 [SD 1.6, IQR 0.7-2.7] years before death) according to standard diagnostic criteria used in the ADNI study (<http://adni.loni.usc.edu/methods/>). A subsample of 26 participants (18 AD, 4 MCI, 4 CN) also had available plasma measurements which were used for a head-to-head

comparison of CSF and plasma biomarkers. Average time difference between CSF and plasma sampling was 1.2 (SD 1.2, IQR 0.8-1.2) years in this subsample.

In order to derive reference values for the biomarker measurements, we also included data from a control group of 146 CN individuals with a negative A β -PET scan who had both available CSF and plasma measurements. This A β -PET-negative control group was selected based on a global cortical [¹⁸F]Florbetapir-PET signal <12 Centiloids,²⁴ which was calculated from standardised uptake value ratios (SUVR) using equations derived by the ADNI PET Core (adni.loni.usc.edu/methods/pet-analysis).¹⁵

Neuropathologic examination

All neuropathologic assessments were performed by the same neuropathologist (Dr. Nigel Cairns) at the central laboratory of the ADNI neuropathology core at the Knight Alzheimer's Disease Research Center, Washington University School of Medicine, St. Louis (directed by Dr. John C. Morris), which provides uniform neuropathologic assessments of deceased ADNI participants (<http://adni.loni.usc.edu/about/#core-container>).²⁵ Neuropathologic evaluations assess a wide range of AD neuropathologic lesions and common non-AD pathologies following NIA-AA guidelines for the neuropathologic assessment of AD,²⁶ which are itemized in the Neuropathology Data Form Version 10 of the National Alzheimer Coordinating Center (NACC; <https://www.alz.washington.edu/NONMEMBER/NP/npguide10.pdf>).

The principal neuropathologic outcome measures of the present study were focused on established rating scales for different aspects of AD neuropathologic change (ADNC), including Thal phases of regional distribution of amyloid- β plaques ('A'), Braak stages of tau neurofibrillary tangle pathology ('B'), and CERAD scores for density of neuritic ('C') and diffuse ('D') plaques.²⁶ Following the NIA-AA guidelines, Thal phases (0-5) and Braak stages (0-6) were converted to 'A' and 'B' scores, so that all neuropathologic rating scales (A-D) are scored on a common semi-quantitative 4-point scale from absent (0), to low (1),

intermediate (2), and high (3). The A-B-C scores were further collapsed into a 4-point scale ADNC composite score according to NIA-AA neuropathologic criteria, where scores ≥ 2 correspond to a pathologic diagnosis of AD.^{26, 27} Thus, ADNC composite scores were used to classify patients with a clinical diagnosis of AD dementia (N=35) into “pathology-confirmed AD dementia” (ADNC ≥ 2 ; N=27) and “non-AD dementia” (ADNC ≤ 1 ; N=8) groups. Primary neuropathologic diagnoses in the non-AD dementia group included Lewy body disease (N=4), hippocampal sclerosis (N=2), argyrophilic grain disease (N=1), and frontotemporal lobar degeneration with TDP-43 inclusions (N=1). Although CERAD scores for density of diffuse plaques (D) are not used for calculating the ADNC composite score, they were included in pathology-specific analyses to allow assessment of biomarker-specific associations with neuritic vs diffuse amyloid- β plaques. Neuritic plaques are considered pathologically advanced forms of amyloid- β plaques and can be distinguished from diffuse plaques by the presence of dystrophic neurites, which typically also exhibit immunoreactivity for phosphorylated tau.^{26, 28}

In addition to AD-specific neuropathology, we also assessed common comorbid non-AD pathologies, including CAA, LB, and TDP-43 pathology. Presence of CAA was assessed in parenchymal and leptomeningeal vessels and scored on a semi-quantitative 4-point scale based on global brain area involvement (absent to widespread). Evidence of LB pathology was assessed according to modified McKeith criteria,^{26, 29} and assessment of TDP-43 pathology followed a regional evaluation of TDP-43-immunoreactive inclusions in the spinal cord, amygdala, hippocampus, entorhinal cortex/inferior temporal gyrus, and frontal neocortex.³⁰ For the purpose of the present study, all neuropathologic assessment scales/scores of non-AD pathologies were dichotomized into 0-absent and 1-present categories.

More detailed information on the implementation and operational definitions of the different neuropathologic rating scales are provided in the coding guidebook of the NACC

(<https://www.alz.washington.edu/NONMEMBER/NP/npguide10.pdf>).

CSF biomarkers

Available *ante-mortem* CSF samples were analysed for peptide levels of A β ₁₋₄₂, total tau (t-tau), and tau phosphorylated at threonine 181 (p-tau181) using the fully automated Roche Elecsys® electrochemiluminescence immunoassays on a cobas e601 instrument according to the kit manufacturer's instructions. The lower and upper technical limits for the biomarkers are 200 to 1700 pg/ml for A β ₁₋₄₂, 80 to 1300 pg/ml for t-tau, and 8 to 120 pg/ml for p-tau181.

In the present study we also included A β ₁₋₄₂ values beyond the upper technical limit, which are provided based on an extrapolation of the calibration curve. However, note that the use of these values is restricted to exploratory research purposes, and they should not be used for clinical decision making.

Plasma biomarkers

Blood samples were collected and processed according to the ADNI protocol³¹ and analysed at the Clinical Neurochemistry Laboratory, University of Gothenburg, Mölndal, Sweden. Plasma p-tau181 concentration was measured using a novel assay developed in-house on a Single molecule array (Simoa) HD-X (Quanterix, Billerica, MA, USA) instrument, as described previously.^{12, 14}

For comparison, we also included plasma neurofilament light (NfL) as a biomarker for general neurodegeneration, which is not specific for AD pathology.³² Plasma NfL was also measured using the Simoa platform as previously described.^{33, 34}

Statistical analysis

In a first analysis, we used Mann-Whitney U tests to study the difference in biomarker levels between pathology-confirmed AD dementia patients (ADNC \geq 2), A β -PET-negative control

participants, and patients with a clinical diagnosis of AD dementia but without neuropathologic evidence of AD pathology ($ADNC \leq 1$, “non-AD dementia”). The accuracy by which the biomarkers could discriminate between these groups was tested using the area under the curve (AUC) in receiver operating characteristic (ROC) curve analysis, and optimal biomarker cut-offs for group separation were derived based on the value that maximizes the Youden index (sensitivity + specificity - 1).

In a second set of analyses, we studied pathology-specific associations of the different biomarkers with distinct aspects of AD neuropathologic change. Given that biomarkers are supposed to reflect specific pathologic processes irrespective of their potential clinical consequences,³ these association analyses were carried out across pooled diagnostic groups to increase pathological variance in the sample. Associations between fluid biomarkers and neuropathologic measures were examined using two complementary analyses: first, Spearman partial correlations, adjusted for time interval between biomarker collection and death, were calculated for the association between each fluid biomarker and the different semi-quantitative neuropathologic rating scales. Second, the ability of the biomarkers to detect intermediate-to-high degrees of the different AD neuropathologic changes, as well as the presence of non-AD pathologies, was quantified using ROC curve analysis as described above. Similar to the ADNC composite score, the 4-point semi-quantitative rating scales (A-D) were dichotomized into high and low categories for this analysis based on a distinction of intermediate/high (2/3) vs absent/low (0/1) degrees of pathology.

All analyses were conducted separately for the full sample with available CSF data (N=45) and the subsample of 26 participants who additionally had plasma measurements. Statistical significance threshold was set at $p < 0.05$.

Data availability

Data used in this study has been made publicly available by the ADNI in the Laboratory of Neuro Imaging (LONI) database.

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Results

Sample characteristics

Demographic, clinical, and neuropathologic characteristics of the analysed sample are summarised in Table 1. Average time difference between biofluid collection and time of death was 2.9 years (SD 1.9, IQR 1.7-3.7, min-max=0.4-8.7). At the last assessment before death, a clinical diagnosis of AD dementia was given in the majority of individuals (78%). Intermediate-to-high neuropathologic change scores were considerably more frequent than absent-to-low scores for all neuropathologic rating scales, especially for Thal phases (A) and diffuse plaque (D) scores, and 71% of all cases had intermediate-to-high ADNC composite scores, qualifying for a neuropathologic diagnosis of AD.

Overall, the different neuropathological rating scales were highly interrelated, specifically A and D scores (Spearman's $\rho=0.92$) as well as B and C scores ($\rho=0.94$), whereas the associations between these neuropathologic categories were weaker (A-B: $\rho=0.86$; A-C: $\rho=0.84$; D-B: $\rho=0.77$; D-C: $\rho=0.76$; all $p<0.001$).

With regard to non-AD pathologies, 31% of all cases exhibited intermediate-to-high levels of CAA, which were associated with AD neuropathology scores, most notably A scores (CAA-A: $\rho=0.40$; $p=0.007$; CAA-B: $\rho=0.37$; $p=0.013$; CAA-C: $\rho=0.27$; $p=0.074$; CAA-D: $\rho=0.36$; $p=0.016$). About half of the sample (49%) had evidence of LB pathology, and 40% had evidence of TDP-43 pathology, but neither was associated with any AD neuropathology score (all $\rho<0.22$, $p>0.14$).

Demographic, clinical, and neuropathologic characteristics of the subsample with available plasma measurements were comparable to the full sample (Table 1).

Discriminative accuracy of fluid biomarkers for distinguishing pathology-confirmed AD dementia from healthy controls and non-AD dementia

In the full sample, all Elecsys CSF biomarkers were significantly different between pathology-confirmed AD dementia patients (N=27) and A β -PET-negative healthy controls

(N=146; all $p < 0.001$; Fig. 1) and differentiated between these groups with very high AUC values ranging from 0.86 (t-tau) to 1.00 (p-tau181/ $A\beta_{1-42}$ ratio) (Fig. 2). Optimal biomarker cut-offs for this differentiation were 838 pg/ml for $A\beta_{1-42}$, 211 pg/ml for t-tau, 19.3 pg/ml for p-tau181, 0.34 for t-tau/ $A\beta_{1-42}$, and 0.027 for p-tau/ $A\beta_{1-42}$. Elecsys t-tau and p-tau181 levels, as well as their ratios with $A\beta_{1-42}$, were also markedly higher in pathology-confirmed AD compared to non-AD dementia (N=8; all $p < 0.001$; Fig. 1) and separated these groups with very high accuracy (AUCs=0.94-0.97); differences in $A\beta_{1-42}$ levels, however, were only marginally significant ($p=0.07$, AUC [95% Confidence Interval]=0.71 [0.47-0.96])(Fig. 2).

In the subsample with available blood plasma measurements, plasma p-tau181 levels showed a similarly pronounced group difference and high discriminative accuracy for distinguishing pathology-confirmed AD (N=14) from $A\beta$ -PET-negative controls ($p < 0.001$, AUC=0.91 [0.86-0.96], optimal cut-off: 19.5 pg/ml) and from non-AD dementia cases (N=4; $p=0.003$, AUC=0.96 [0.88-1.00]; Fig. 2). Plasma NfL showed similarly good discrimination of pathology-confirmed AD from $A\beta$ -PET-negative controls ($p < 0.001$, AUC=0.93 [0.87-0.99], optimal cut-off: 45.7 pg/ml) but not from non-AD dementia cases ($p=0.33$, AUC=0.68 [0.37-0.99]).

Fluid biomarker associations with different AD neuropathologic rating scales and presence of non-AD pathologies

The distribution of biomarker values across the different AD neuropathologic rating scales is displayed in Fig. 3, and Table 2 lists the corresponding Spearman correlation coefficients. In the full sample, all individual Elecsys CSF biomarkers were significantly associated with the different AD neuropathologic rating scales, but neuropathologic correlations for $A\beta_{1-42}$ were strongest with diffuse plaque scores (D) and Thal phase (A), whereas those for t-tau and p-tau181 were strongest with neuritic plaques (C) and Braak stage (B). However, for all neuropathologic scores the strongest correlations were observed for the t-tau/ $A\beta_{1-42}$ and p-tau/ $A\beta_{1-42}$ ratios.

Correspondingly, ROC analyses indicated relatively high accuracy for all individual Elecsys CSF biomarkers to differentiate between high and low degrees of the different AD neuropathologic change scores (Fig. 4, Table 3). High and low degrees of Thal phase (A) and diffuse plaque scores (D) were best differentiated by $A\beta_{1-42}$ levels (AUC=0.91 [0.81-1] and 0.92 [0.83-1], respectively), yielding an optimal cut-off of 1097 pg/ml for both analyses. High and low degrees of Braak stage (B) and neuritic plaque scores (C) were best differentiated by p-tau181 levels (AUC=0.88 [0.77-0.99] and 0.89 [0.79-0.99], respectively), yielding an optimal cut-off of 19.1 pg/ml for both analyses. The t-tau cut-off that best differentiated between high and low Braak stage (B) was 229 pg/ml, and the same optimal cut-off was found for neuritic plaques (C), although lower cut-offs of 221 pg/ml and 210 pg/ml yielded identical Youden indices in this ROC analysis. For all neuropathologic rating scales, high and low degrees of pathology were best differentiated by the t-tau/ $A\beta_{1-42}$ and p-tau181/ $A\beta_{1-42}$ ratios (AUCs=0.95-0.98), where cut-offs of 0.27 for t-tau/ $A\beta_{1-42}$ and 0.016 for p-tau181/ $A\beta_{1-42}$ yielded best separation for Thal phases (A) and diffuse plaques (D), whereas higher cut-offs of 0.42 for t-tau/ $A\beta_{1-42}$ and 0.041 for p-tau181/ $A\beta_{1-42}$ yielded best separation for Braak stages (B) and neuritic plaques (C).

Among common non-AD pathologies, the presence of CAA was highly associated with CSF $A\beta_{1-42}$ levels (AUC=0.84 [0.73-0.96]) as well as with the t-tau/ $A\beta_{1-42}$ (AUC=0.88 [0.77-0.98]) and p-tau181/ $A\beta_{1-42}$ ratios (AUC=0.88 [0.78-0.98]), but not with t-tau or p-tau181 levels individually (Table 3, Fig. 4). No CSF biomarker detected the presence of LB or TDP-43 pathology.

In the subsample with available plasma measurements, plasma p-tau181 was only significantly associated with Braak stage (B) ($\rho=0.43$, $p=0.028$) and neuritic plaque scores (C) ($\rho=0.47$, $p=0.014$), with corresponding AUC values of 0.71 [0.44-0.98] and 0.75 [0.52-0.98] (optimal cut-off: 18.0 pg/ml), respectively (Fig. 3, Tables 2 and 3). Spearman correlations and AUC values of the associations between neuropathologic changes and Elecsys CSF biomarkers were comparable to the findings in the full CSF sample and were

consistently higher for CSF p-tau181 than for plasma p-tau181. Plasma NfL levels did not show any association with AD neuropathologic change scores or the presence of non-AD pathologies.

Classification of Evidence

The primary objective of this study was to study the accuracy of *ante-mortem* Elecsys-derived CSF biomarkers to detect AD neuropathology as assessed by neuropathological examination at autopsy. Our findings provide Class II evidence that the fully-automated Elecsys-derived CSF t-tau and p-tau181 measurements, as well as their ratios with $A\beta_{1-42}$ levels, discriminate between autopsy-confirmed Alzheimer's disease and other dementias with high diagnostic accuracy (AUCs=0.94-0.97).

Discussion

In this study, we examined the association of Elecsys-derived CSF biomarkers for AD pathology and plasma measures of p-tau181 and NfL with neuropathologic changes at autopsy. Our findings demonstrate that Elecsys CSF biomarkers separated pathology-confirmed AD dementia cases from healthy controls and non-AD dementia cases with very high discriminative accuracy *in-vivo*. In pathology-specific analyses, the individual Elecsys CSF A β and tau biomarkers showed strongest associations with the different AD neuropathologic measures that most closely reflect their pathologic target. Preliminary analysis of plasma p-tau181 in a smaller subset demonstrated comparable group separation accuracy and similar, albeit weaker, pathology-specific associations as CSF p-tau181. Taken together, our findings demonstrate, for the first time, the high neuropathologic validity and diagnostic accuracy of Elecsys CSF biomarkers and the potential of plasma p-tau181 as a cost-effective and scalable *in-vivo* measure of AD pathology.

Fully automated CSF biomarker assays have recently been developed to satisfy the unmet need for laboratory- and batch-independent absolute CSF measures that will enable the use of universal biomarker cut-offs in both research and clinical settings.⁵ However, validation studies for the fully automated Elecsys CSF assays have so far only covered the concordance with A β -PET measures or clinical diagnostic and prognostic variables,^{7-10, 23} leaving the neuropathological validity of these automated biomarker measurements unclear. Here, we provide first-time evidence of the very high diagnostic performance of Elecsys CSF biomarkers for discriminating between pathology-confirmed AD dementia and healthy controls as well as non-AD dementia. Notably, the diagnostic accuracy of the Elecsys-derived biomarkers was similar or even higher compared to previously reported results for non-automated CSF assays (see ³⁵ for a recent meta-analysis), suggesting that this automatization did not result in lowered performance. Similar to previous findings on the diagnostic accuracy of individual CSF biomarkers, we found that CSF A β ₁₋₄₂ discriminated better between AD and A β -PET-negative controls than CSF tau biomarkers,¹⁹ whereas CSF tau

biomarkers discriminated better between AD and non-AD dementia cases than CSF A β ₁₋₄₂.^{17,}
³⁶ The low CSF A β ₁₋₄₂ levels in the non-AD dementia group may partly be explained by comorbid A β pathology (three of four cases in this group with CSF A β ₁₋₄₂ levels below the threshold also had intermediate or high A scores) but may also be affected by other pathologic or physiologic factors known to influence CSF A β ₁₋₄₂ levels^{27, 37}. In both diagnostic contexts, the discriminative accuracy could be slightly improved by using the CSF tau-to-A β ₁₋₄₂ ratio.

Another key feature of our study was the evaluation of the relative sensitivity of Elecsys CSF biomarkers for different aspects of AD neuropathology. We found that although all Elecsys CSF biomarkers were strongly associated with the different AD neuropathologic change scores, these associations were generally strongest between each biomarker and its respective target pathology (i.e. CSF A β ₁₋₄₂ vs Thal phase and diffuse plaques, and CSF p-tau181 vs Braak stage and neuritic plaques). These results are congruent with a previous study examining pathology-specific associations of CSF biomarkers measured by standard non-automated assays, where pathologic measures of A β load were best correlated with CSF A β ₁₋₄₂ levels whereas pathologic measures of NFT load were best correlated with CSF p-tau181 levels.²⁰ However, in that study, the best neuropathologic correlate of both CSF biomarkers was CERAD neuritic plaque density. It also must be noted that CSF t-tau levels may be influenced by neurodegenerative processes such as neuronal death and axonal loss¹⁻³, which have not been assessed in the present study. Overall, the performance measures (correlation coefficients, AUC values) for the pathology-specific associations in that previous study were very similar to the ones observed here for the Elecsys CSF biomarkers, and highest performance for pathology detection was also observed for the tau-to-A β ₁₋₄₂ ratios. Regarding non-AD neuropathologic changes, we found that none of the Elecsys CSF biomarkers were associated with the presence of TDP-43 or LB pathology, but CSF A β ₁₋₄₂ levels were lower in cases with CAA. While this can be expected based on the pathologic substrate of CAA and has been reported previously for a non-automated CSF A β ₁₋₄₂ assay,¹⁸

the association between CSF A β ₁₋₄₂ levels and CAA pathology may also partly be explained by the high co-prevalence of CAA and A β plaque pathology. Disentangling the A β pathology-specificity of CSF A β ₁₋₄₂ levels would require larger and pathologically more heterogeneous study samples. Summarised, our results indicate high pathologic specificity of Elecsys CSF biomarkers for the different aspects of AD neuropathology and point to CAA as a potential confounder for the *in-vivo* assessment of A β plaque pathology using CSF A β ₁₋₄₂ levels.

While CSF biomarker estimates from different assays are largely in agreement and highly correlated,^{6, 38} they can show great differences in absolute quantifiable concentrations.⁴ This limits the development and application of universal abnormality thresholds for use across different laboratories and clinical settings. A key advantage of the Elecsys platform is its standardisation through full automation of the assay, which has been proven to provide stable cut-offs for detecting PET-measured A β -positivity and predicting clinical progression that generalise across cohorts.^{7-9, 23} Nevertheless, no prior study has yet derived cut-offs for the Elecsys platform using a neuropathology gold standard. This is particularly relevant since derivation of CSF cut-offs based on clinical diagnosis has been shown to result in biased estimates due to misdiagnosis and the presence of concomitant pathologies.³⁶ The pathology-based Elecsys cut-offs derived in this study are well within the range of previously established cut-offs based on correspondence to A β -PET or clinical endpoints. We found an optimal CSF A β ₁₋₄₂ cut-off of 1097 pg/ml for discriminating high and low Thal phases, whereas high and low Braak stages and neuritic plaque scores were best separated by cut-offs of 229 pg/ml and 19 pg/ml for t-tau and p-tau181, respectively (Supplementary Table S1; Data available from Dryad: <https://doi.org/10.5061/dryad.n2z34tmwr>). In comparison, optimal cut-offs for describing A β -PET-positivity have been reported in the range from 977-1100 pg/mL for A β ₁₋₄₂, 213-242 pg/ml for t-tau, and 19-21 pg/ml for p-tau181.⁷⁻⁹ The strong agreement between our neuropathology analysis and these *in-vivo* biomarker studies may be explained by the excellent accuracy of A β -PET for the detection of A β pathology,²⁴ further

supporting the generalisability of Elecsys CSF cut-offs across different research settings. ROC analyses for separating pathology-confirmed AD dementia patients from A β -PET-negative healthy controls in our study yielded very similar t-tau (211 pg/ml) and p-tau181 (19 pg/ml) cut-offs compared to the pathology-specific cut-offs, but indicated a considerably lower A β ₁₋₄₂ cut-off of 838 pg/ml (and thus higher cut-offs for the tau-to-A β ₁₋₄₂ ratios). This difference in the CSF A β ₁₋₄₂ cut-off can be expected due to the fact that pathological confirmation of AD requires the presence of both A β and tau pathologies and therefore implies more advanced disease stages that show lower average CSF A β ₁₋₄₂ levels.⁹

In preliminary findings from a smaller subsample analysis, we further compared the performance of Elecsys CSF biomarkers in the assessment of AD neuropathology with that of a novel plasma p-tau181 biomarker as well as with that of plasma NfL as a non-disease-specific neural injury marker. In line with recent neuropathologic studies of plasma p-tau181 and NfL biomarkers,^{11, 22, 39} both plasma p-tau181 and NfL demonstrated high accuracy for separating pathology-confirmed AD from healthy controls, but only p-tau181 demonstrated high accuracy in separating pathology-confirmed AD from non-AD dementia cases. The direct head-to-head comparison with CSF in our current study further indicated that the diagnostic accuracy of plasma p-tau181 was comparable to CSF p-tau181 in this differential diagnosis context. We also extended upon the existing neuropathologic studies on plasma p-tau181 measurements^{11, 22} by investigating the specific neuropathologic correlates of this novel biomarker. Our analysis indicated a pathologic specificity of plasma p-tau181 for Braak tau stage and neuritic plaque scores similar to that of CSF p-tau181, although these associations were notably weaker, suggesting superior performance of Elecsys CSF biomarkers in this context.

Our study does have limitations. Firstly, although relatively large for a combined *ante-mortem* CSF and *post-mortem* neuropathology examination, the sample size of our study was still limited, particularly for the *head-to-head* comparison between CSF and plasma biomarkers. Moreover, the ADNI cohort represents a rather selective research cohort that

may not be reflective of the general population, and the focus on autopsied individuals in this cohort introduces an additional selection bias, which is reflected in an older age, higher prevalence of males, and higher prevalence of an AD dementia diagnosis in our study sample (see Supplementary Figure S1; Data available from Dryad: <https://doi.org/10.5061/dryad.n2z34tmwr>). In addition, individuals with low levels of AD neuropathologic change and non-AD dementia cases were underrepresented in our study; the sensitivity of the examined CSF and plasma biomarkers for early AD neuropathologic change and their utility for differential dementia diagnosis thus remain to be established in more diverse cohorts. Cut-offs derived from small-sample analyses should be interpreted with caution, however, we are encouraged by the similarity between our cut-off values and those derived from larger studies using A β -PET or clinical outcomes as validation standards. Unfortunately, the CSF A β_{1-42} /A β_{1-40} ratio, which has been proposed to compensate for individual differences in physiological A β production,³⁷ could not be assessed in our study. Moreover, the combination of plasma p-tau181 and A β markers may increase the correspondence with neuropathologic measures similar to the CSF tau-to-A β_{1-42} ratio,^{11, 12} but no measures of plasma A β were available for this study sample.

Our neuropathologic association study demonstrates high neuropathologic validity of Elecsys-derived CSF biomarkers of AD and further provides pathology-derived concentration cut-offs for this standardised analysis platform, which will support harmonisation and interpretation of biomarker findings across different laboratories and clinical settings. In a smaller subset, our findings for plasma p-tau181 indicate similar, though weaker, pathology-specific associations with neuritic plaques and Braak tau stages as for CSF p-tau181. Its accuracy in discriminating between diagnostic groups adds strong support for the use of this easily accessible and scalable biomarker as a screening tool, particularly for the differential diagnosis of dementia. Performance of both Elecsys CSF and plasma p-tau181 measures as biomarkers of early stage AD neuropathology remains to be investigated in larger and

pathologically more diverse autopsy cohorts with available *ante-mortem* bodily fluid samples.

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Tables

Table 1. Cohort characteristics

	CSF sample	Plasma subsample
N	45	26
Age at DOD (years)	82.5 ± 7.6	82.0 ± 7.8
Sex (M/F)	36/9	20/6
CN/aMCI/ADD	4/6/35	4/4/18
A (0/1/2/3)	3/4/5/33	2/4/2/18
% intermediate-high (2/3)	84%	77%
B (0/1/2/3)	1/12/2/30	1/7/2/16
% intermediate-high (2/3)	71%	69%
C (0/1/2/3)	10/5/2/28	8/2/1/15
% intermediate-high (2/3)	67%	62%
D (0/1/2/3)	3/5/2/35	2/4/2/18
% intermediate-high (2/3)	82%	77%
ADNC (0/1/2/3)	3/10/2/30	2/6/2/16
% intermediate-high (2/3)	71%	69%
CAA (0/1)	31/14	17/9
% high	31%	35%
Lewy Bodies (0/1)	23/22	12/14
% pos	49%	54%
TDP-43 (0/1)	25/17	16/10
% pos	40%	39%

Degree of AD neuropathologic changes is reported using a semi-quantitative 4-point scale (0-absent, 1-low, 2-intermediate, and 3-high). Age is reported as mean ± standard deviation.

DOD: Date of death; CN: Cognitively normal; aMCI: amnesic mild cognitive impairment; ADD: Alzheimer's disease dementia; A: Thal phases of regional distribution of amyloid- β plaques; B: Braak stages of tau neurofibrillary tangle pathology; C: CERAD scores for density of neuritic plaques; D: CERAD scores for density of diffuse plaques; ADNC: Alzheimer's disease neuropathologic change summary score; CAA: Cerebral amyloid angiopathy.

Table 2. Spearman's rho for correlations of CSF and plasma biomarkers with AD neuropathology scores

	A	B	C	D
CSF sample (N=45)				
A β_{1-42}	-0.62**	-0.54**	-0.50**	-0.66**
t-tau	0.47**	0.58**	0.53**	0.40**
p-tau181	0.57**	0.66**	0.62**	0.50**
t-tau/A β_{1-42}	0.76**	0.79**	0.75**	0.70**
p-tau181/A β_{1-42}	0.75**	0.78**	0.75**	0.71**
Plasma subsample (N=26)				
Plasma p-tau181	0.17	0.43*	0.47*	0.17
Plasma NfL	-0.22	0.04	0.03	-0.22
CSF p-tau181	0.61**	0.69**	0.71**	0.61**

*/**, statistically significant at $p < 0.05/0.01$

Correlations of cerebrospinal fluid and plasma biomarkers with the different AD neuropathologic change scales were assessed using Spearman's rho correlations adjusted for time interval between biofluid collection and time of death. A: Thal phases of regional

distribution of amyloid- β plaques; B: Braak stages of tau neurofibrillary tangle pathology; C: CERAD scores for density of neuritic plaques; D: CERAD scores for density of diffuse plaques; CSF: cerebrospinal fluid.

Table 3. Area under the curve values of fluid biomarkers for detecting distinct AD neuropathologic changes and presence of non-AD pathologies

	A	B	C	D	CAA	LB	TDP43
CSF sample (N=45)							
A β_{1-42}	0.91** [0.81-1]	0.79** [0.62-0.97]	0.83** [0.68-0.99]	0.92** [0.83-1]	0.84** [0.73-0.96]	0.62 [0.45-0.78]	0.57 [0.39-0.75]
t-tau	0.79* [0.59-0.99]	0.83** [0.67-0.99]	0.84** [0.670-0.99]	0.83** [0.65-1]	0.65 [0.48-0.81]	0.51 [0.34-0.68]	0.52 [0.33-0.70]
p-tau181	0.84** [0.69-1]	0.88** [0.77-0.99]	0.89** [0.79-0.99]	0.87** [0.73-1]	0.67 [0.51-0.84]	0.52 [0.35-0.69]	0.50 [0.32-0.69]
t-tau/A β_{1-42}	0.96** [0.90-1]	0.95** [0.90-1]	0.98** [0.96-1]	0.97** [0.92-1]	0.88** [0.77-0.98]	0.59 [0.42-0.76]	0.58 [0.40-0.76]
p-tau181/A β_{1-42}	0.96** [0.90-1]	0.95** [0.89-1]	0.98** [0.94-1]	0.97** [0.92-1]	0.88** [0.78-0.98]	0.58 [0.41-0.75]	0.56 [0.38-0.74]
Plasma subsample (N=26)							
Plasma p-tau181	0.58 [0.27-0.90]	0.71 [0.44-0.98]	0.75* [0.52-0.98]	0.58 [0.27-0.90]	0.60 [0.35-0.84]	0.39 [0.16-0.63]	0.49 [0.27-0.72]
Plasma NfL	0.42 [0.11-0.72]	0.42 [0.13-0.71]	0.53 [0.26-0.80]	0.42 [0.11-0.72]	0.44 [0.21-0.67]	0.42 [0.18-0.66]	0.61 [0.39-0.83]
CSF p-tau181	0.88** [0.73-1]	0.87** [0.72-1]	0.88** [0.91-1]	0.88** [0.79-1]	0.62 [0.40-0.85]	0.57 [0.33-0.80]	0.54 [0.30-0.78]

*/**, statistically significant at $p < 0.05/0.01$

Areas under the receiver operating characteristic curve of Elecsys cerebrospinal fluid and plasma biomarkers for differentiating absent-to-low from moderate-to-high neuropathologic changes. 95% confidence intervals are reported in brackets.

A: Thal phases of regional distribution of amyloid- β plaques; B: Braak stages of tau neurofibrillary tangle pathology; C: CERAD scores for density of neuritic plaques; D: CERAD scores for density of diffuse plaques; CAA: Cerebral amyloid angiopathy; LB: Lewy bodies; CSF: cerebrospinal fluid; t-tau: total tau; p-tau181: tau phosphorylated at threonine 181; NfL: Neurofilament light.

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Figures and figure legends

Fig. 1. Fluid biomarker levels in pathology-confirmed AD dementia, non-AD dementia and A β -PET-negative healthy controls.

A) Cerebrospinal fluid levels of A β_{1-42} , t-tau, and p-tau181; B) Cerebrospinal fluid-based t-tau-to-A β_{1-42} and p-tau181-to-A β_{1-42} ratios; C) Plasma levels of p-tau181 and NfL. Dashed lines represent biomarker cut-offs corresponding to the optimal cut-offs determined in the ROC analysis of pathology-confirmed AD dementia vs amyloid-negative CN. AD: pathology-confirmed AD dementia patients (ADNC ≥ 2); non-AD: patients with a clinical diagnosis of AD dementia but without neuropathologic evidence of AD pathology (ADNC ≤ 1); A β - CN: cognitively normal individuals with a negative A β -PET scan; CSF: cerebrospinal fluid; t-tau: total tau; p-tau181: tau phosphorylated at threonine 181; NfL: Neurofilament light.

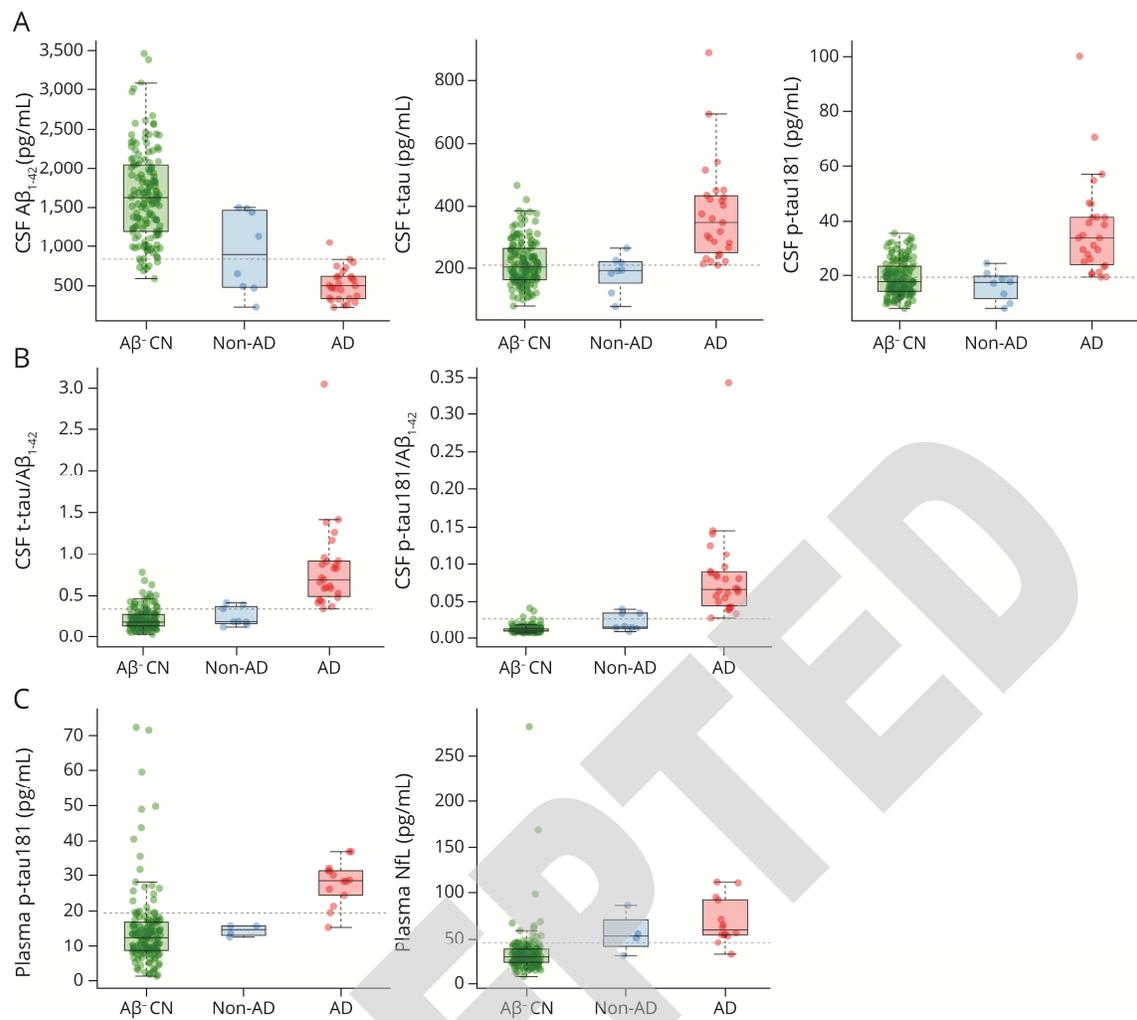


Fig. 2 Receiver operating characteristic curves for distinguishing pathology-confirmed AD dementia from non-AD dementia and A β -PET-negative healthy controls

Receiver operating characteristic curves showing the performance of Elecsys cerebrospinal fluid biomarkers (A) and plasma biomarkers in comparison to cerebrospinal fluid p-tau181 (B) for the discrimination of pathology-confirmed AD dementia from A β -PET-negative healthy controls (A.a and B.a) and non-AD dementia (A.b and B.b). Areas under the curve (AUC) and 95% CI are reported in the insert of each panel. CSF: cerebrospinal fluid; t-tau: total tau; p-tau181: tau phosphorylated at threonine 181; NfL: Neurofilament light.

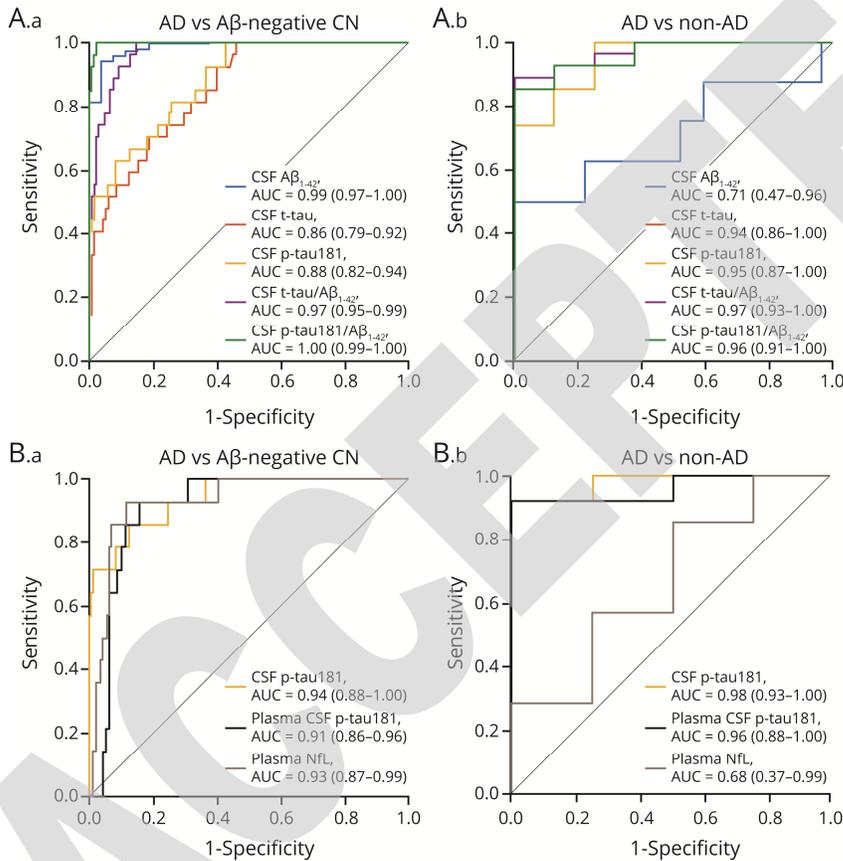


Fig. 3. Distribution of fluid biomarker levels across distinct AD neuropathologic change scores

A, B, C, and D represent 4-point semi-quantitative scales (0-absent, 1-low, 2-intermediate, and 3-high) describing Thal phases of regional distribution of amyloid- β plaques (A), Braak stages of tau neurofibrillary tangle pathology (B), CERAD scores for density of neuritic plaques (C), and CERAD scores for density of diffuse plaques (D). Solid black lines represent linear regression trends. Corresponding Spearman correlation coefficients are listed in Table 2. CSF: cerebrospinal fluid; t-tau: total tau; p-tau181: tau phosphorylated at threonine 181; NfL: Neurofilament light.

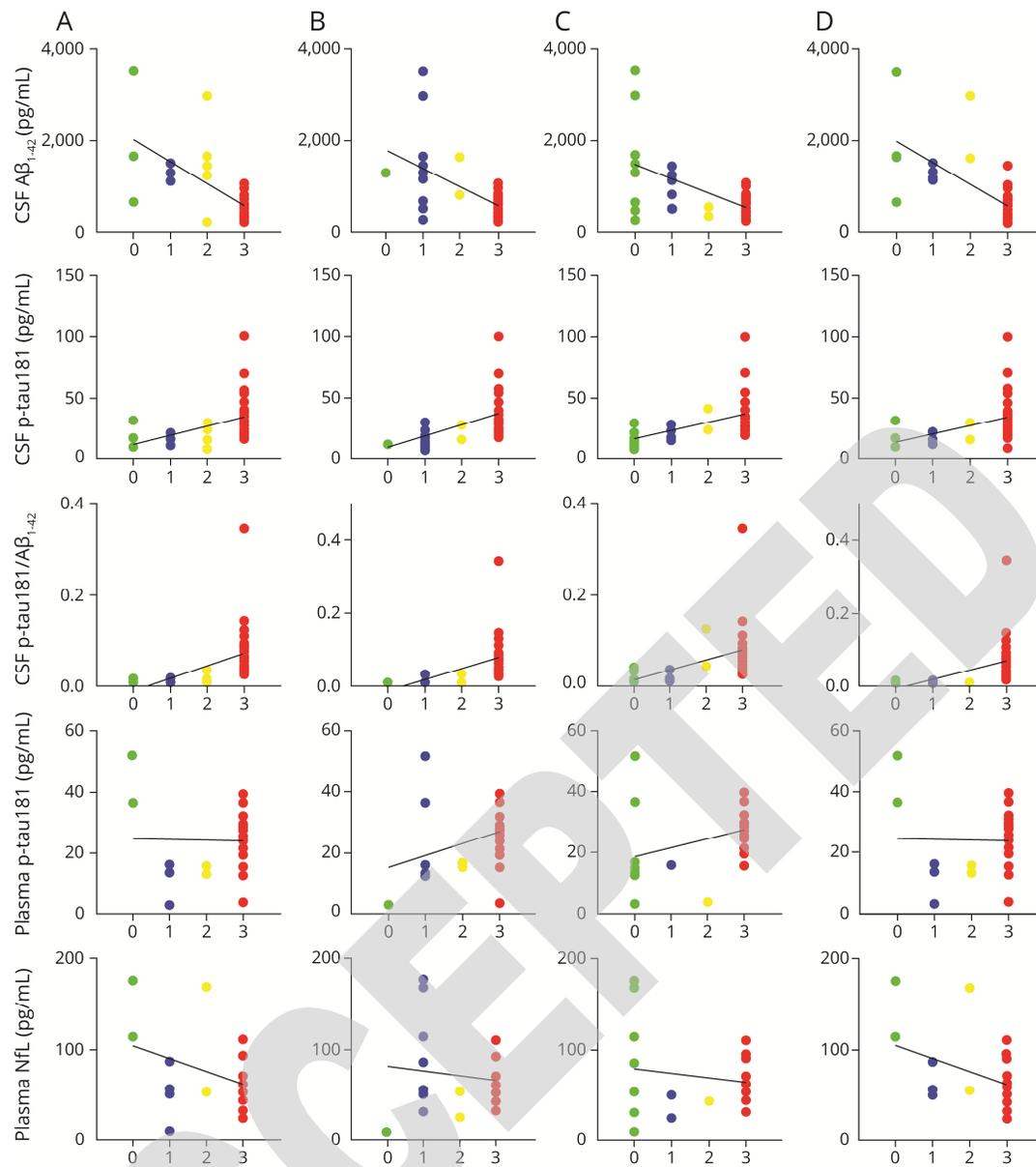
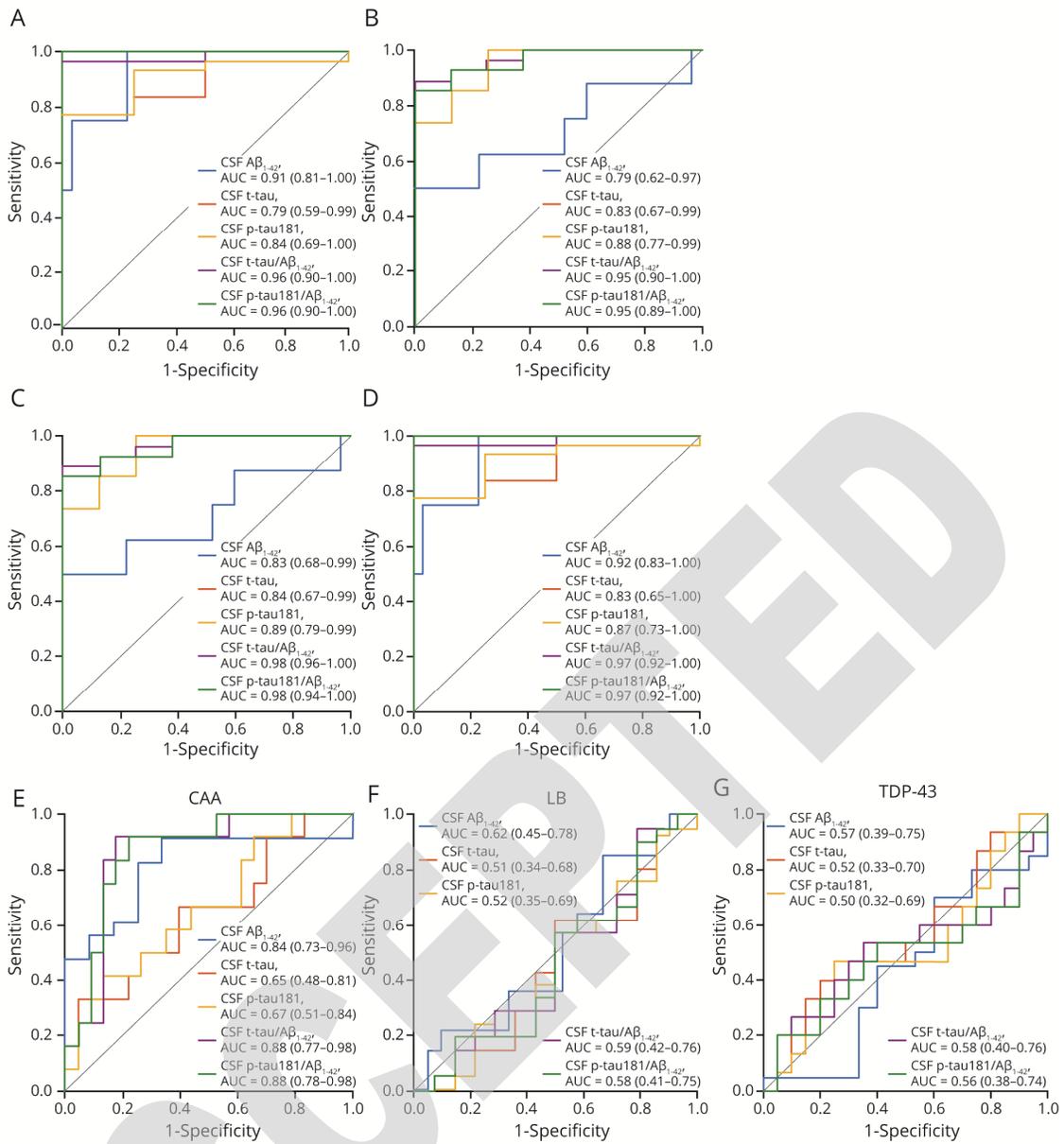


Fig. 4. Receiver operating characteristic curves of Elecsys cerebrospinal fluid biomarkers for detecting AD neuropathologic changes and presence of non-AD pathologies

Receiver operating characteristic curves showing the performance of Elecsys cerebrospinal fluid biomarkers for detecting intermediate-to-high degrees of different AD neuropathologic changes (A: Thal phases of regional distribution of amyloid- β plaques; B: Braak stages of tau neurofibrillary tangle pathology; C: CERAD scores for density of neuritic plaques; D: CERAD scores for density of diffuse plaques) and (E) presence of cerebral amyloid angiopathy (CAA), (F) Lewy body pathology (LB), (G) and TDP-43 pathology.



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