Received: 8 May 2020 | Revised: 30 July 2020 | Accepted: 2 September 2020

DOI: 10.1002/alz.12204

Alzheimer's & Dementia®
THE JOURNAL OF THE ALZHEIMER'S ASSOCIATION

FEATURED ARTICLE

An examination of a novel multipanel of CSF biomarkers in the Alzheimer's disease clinical and pathological continuum

Carol Van Hulle[1,2] | Erin M. Jonaitis[1,3] | Tobey J. Betthauser[1,2] | Richard Batrla[4,#] | Norbert Wild[5] | Gwendlyn Kollmorgen[5] | Ulf Andreasson[6] | Ozioma Okonkwo[1,2,3] | Barbara B. Bendlin[1,2,3] | Sanjay Asthana[1,2,7] | Cynthia M. Carlsson[1,2,3,7] | Sterling C. Johnson[1,2,3,7] | Henrik Zetterberg[6,8,9,10] | Kaj Blennow[6,8]

[1] Wisconsin Alzheimer's Disease Research Center, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, Wisconsin, USA

[2] Department of Medicine, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, Wisconsin, USA

[3] Wisconsin Alzheimer's Institute, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, Wisconsin, USA

[4] Roche Diagnostics International AG, Rotkreuz, Switzerland

[5] Roche Diagnostics GmbH, Penzberg, Germany

[6] Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, the Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden

[7] Geriatric Research, Education and Clinical Center at the William S. Middleton Memorial Veterans Hospital, Madison, Wisconsin, USA

[8] Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden

[9] Department of Neurodegenerative Disease, UCL Institute of Neurology, London, UK

[10] UK Dementia Research Institute at UCL, London, UK

Correspondence
Carol Van Hulle, University of Wisconsin-Madison, School of Medicine and Public Health, 600 Highland Ave, Madison, WI 53792.
Email: vanhulle@medicine.wisc.edu

[#]Richard Batrla was an employee of Roche Diagnostics International AG at the time of this work; current affiliation: Novartis Pharmaceuticals, Basel, Switzerland.

Funding information
NIH National Institute of Aging, Grant/Award Numbers: R01 AG027161, R01 AG031790, R01 AG037639, R01 AG021155, R01AG054059, R01 AG062167, UF1AG051216, P50 AG033514, P30 AG062715; NIH National Center for Advancing Translational Sciences (NCATS), Grant/Award Number: UL1TR000427; Swedish Research Council, Grant/Award Number: #2017-00915; Alzheimer Drug Discovery Foundation (ADDF), USA, Grant/Award Number: (#RDAPB-201809-2016615; Swedish

Abstract

Introduction: This study examines the utility of a multipanel of cerebrospinal fluid (CSF) biomarkers complementing Alzheimer's disease (AD) biomarkers in a clinical research sample. We compared biomarkers across groups defined by clinical diagnosis and $pTau_{181}/A\beta_{42}$ status (+/−) and explored their value in predicting cognition.

Methods: CSF biomarkers amyloid beta $(A\beta)_{42}$, $pTau_{181}$, tTau, $A\beta_{40}$, neurogranin, neurofilament light (NfL), α-synuclein, glial fibrillary acidic protein (GFAP), chitinase-3-like protein 1 (YKL-40), soluble triggering receptor expressed on myeloid cells 2 (sTREM2), S100 calcium binding protein B (S100B), and interleukin 6 (IL6), were measured with the NeuroToolKit (NTK) for 720 adults ages 40 to 93 years (mean age = 63.9 years, standard deviation [SD] = 9.0; 50 with dementia; 54 with mild cognitive impairment [MCI], 616 unimpaired).

Results: Neurodegeneration and glial activation biomarkers were elevated in $pTau_{181}/A\beta_{42}$+ MCI/dementia participants relative to all $pTau_{181}/A\beta_{42}$- participants. Neurodegeneration biomarkers increased with clinical severity among $pTau_{181}/A\beta_{42}$+ participants and predicted worse cognitive performance. Glial activation biomarkers were unrelated to cognitive performance.

Alzheimer Foundation, Grant/Award Number: #AF-742881; Hjärnfonden, Sweden, Grant/Award Number: #FO2017-0243; Swedish government and the County Councils, the ALF-agreement, Grant/Award Number: #ALFGBG-715986; European Union Joint Program for Neurodegenerative Disorders, Grant/Award Number: JPND2019-466-236

Discussion: The NTK contains promising markers that improve the pathophysiological characterization of AD. Neurodegeneration biomarkers beyond tTau improved statistical prediction of cognition and disease stages.

KEYWORDS
Alzheimer's disease, amyloid positron emission tomography imaging, biomarker validation, cerebrospinal fluid biomarkers, glial activation, inflammation, neurodegeneration

1 | INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disease with an extended preclinical phase wherein pathologic amyloid β (Aβ) and tau proteins aggregate before onset of cognitive impairment.[1–3] Over the past two decades, tremendous progress has been made in detecting abnormal forms of Aβ and tau proteins in cerebrospinal fluid (CSF) and positron emission tomography (PET) imaging.[4–7] In particular, CSF in vitro diagnostic (IVD) immunoassays for Aβ_{42}, tau phosphorylated at serine-181 (pTau$_{181}$), and total tau protein (tTau) concentrations have demonstrated excellent diagnostic precision in AD.[1,8] However, there is still heterogeneity in progression to symptomatic AD that may be explained by other pathophysiologies. The NeuroToolKit (NTK) is a panel of automated CSF immunoassays developed to complement established core AD biomarkers Aβ_{42}, pTau$_{181}$, and tTau[9–12] with markers for glial activation and inflammation, synaptic degeneration, and damage to long axons, to provide new tools to explore disease pathogenesis (see Wild et al. [2020], this issue.

Our objectives were to (1) confirm the utility of core AD biomarker positivity derived from CSF measured using automated Elecsys CSF NTK immunoassays in clinical research sample and (2) explore associations with biomarkers in the NTK that are not specific to AD, but may indicate the presence and severity of neurodegeneration and glial activation and thereby account for variability in clinical diagnosis and cognitive performance.

2 | METHODS

2.1 | Participants

Using a uniform preanalytic protocol across included longitudinal studies, CSF was obtained from N = 720 adults ages 45 to 93 years (M = 63.9, standard deviation [SD] = 9.0; 51.6% female) participating in the Wisconsin Registry for Alzheimer's Prevention study (WRAP, n = 205),[13] the Wisconsin Alzheimer's Disease Research Center (WADRC, n = 411), or affiliated studies (Statins in Healthy, At-Risk Adults: Impact on Amyloid and Regional Perfusion [SHARP, n = 63; NCT00939822]; the Alzheimer's Disease Connectome Project [ADCP, n = 9]; Fitness Aging in the Brain, [FAB, n = 40]). Enrollment criteria varied across studies (see supporting information). The combined sample includes cognitively unimpaired (CU) individuals, participants with mild cognitive impairment (MCI) or dementia due to suspected AD, and is enriched for parental history of AD dementia (determined through review of parental medical records, autopsy reports, results of a dementia questionnaire, or participant self-report). All participants had decisional capacity and completed an informed consent process before undergoing study procedures. Lumbar punctures (LPs) were performed within 1 year of cognitive testing. If participants completed multiple LPs, their most recent LP was selected for analysis.

2.2 | Clinical diagnosis

WRAP, WADRC, FAB, and ADCP participants' cognitive performance and functional status were adjudicated by consensus conference at each visit. Diagnoses of MCI or dementia due to suspected AD were assigned based on National Institute on Aging-Alzheimer's Association (NIA-AA) criteria,[14,15] without reference to biomarkers; n = 50 participants were diagnosed with dementia (49 suspected AD and 1 dementia-other cause), n = 54 had MCI (47 MCI presumed due to AD and 7 MCI-other cause), and n = 616 CU. SHARP participants' cognition was assessed formally at pre-study, and those with signs of cognitive impairment were excluded from enrollment.

2.3 | CSF collection

CSF samples were acquired with a uniform preanalytical protocol between 2010 and 2018. Samples were collected in the morning after an 8- to 12-hour fast using a Sprotte 24- or 25-gauge atraumatic spinal needle and 22 mL of fluid was collected via gentle extraction into polypropylene syringes and combined into a single 30 mL polypropylene tube. After gently mixing, samples were centrifuged to remove red blood cells or other debris; 0.5 mL CSF was aliquoted into 1.5-mL polypropylene tubes and stored at −80°C within 30 minutes of collection (see supporting information for details).

2.4 | CSF assays

All CSF samples were re-assayed at the Clinical Neurochemistry Laboratory, University of Gothenburg, using the same batch of reagents, under strict quality control procedures. The following immunoassays were performed on a cobas e 601 analyzer: Elecsys β-amyloid(1-42) CSF, Elecsys Phospho-Tau (181P) CSF and Elecsys Total-Tau CSF, S100 calcium binding protein B (S100B), and interleukin-6 (IL6). The remaining NTK panel was assayed on a cobas e 411 analyzer including Aβ(1-40) CSF, markers of synaptic damage and neuronal degeneration

(neurogranin, neurofilament light protein [NfL], and α-synuclein), and markers of glial activation (glial fibrillary acidic protein [GFAP], chitinase-3-like protein 1[YKL-40], and soluble triggering receptor expressed on myeloid cells 2 [sTREM2]).

2.5 | Amyloid PET imaging

A subset of 185 participants also underwent dynamic [C-11]Pittsburgh compound B (PiB) PET imaging (0–70 minutes post-injection) within 2 years of their most recent LP (mean time between PiB and LP was 0.35 ± 0.71 years). Imaging methods and PiB quantification have been previously described.[16] PiB(+/–) status was determined by visual inspection inter-rater reliability = 0.95, intra-rater reliability = 0.96.[16]

2.6 | Biomarker positivity

We used receiver operating characteristic (ROC) analyses to derive positivity thresholds for AD biomarkers (ADB) using PiB(+/–) as the standard of comparison. ROC analyses were conducted using the MatLab perfcurve function (The Mathworks, Natick, Massachusetts, USA). The optimal threshold for $A\beta_{42/40}$ and $pTau_{181}/A\beta_{42}$ discrimination was based on equally weighted cost functions for positive and negative agreement.[17] Due to the greater availability of Elecsys® IVD immunoassays in clinical settings, $pTau_{181}/A\beta_{42}$ was used in analyses requiring continuous measures of ADB, and $pTau_{181}/A\beta_{42}$ positivity status was used for analyses with dichotomous ADB status (ADB[+/–]).

Thresholds for $pTau_{181}$, tTau, NfL, neurogranin, and α-synuclein status were determined by establishing a reference group of 223 CSF amyloid ($A\beta_{42/40}$) negative, cognitively unimpaired younger participants (ages 40–60 years). Biomarker positivity thresholds for these analytes were set at +2SD above the mean of this reference group.[18]

2.7 | Cognitive outcomes

The primary cognitive outcome was clinical diagnosis. As a secondary cognitive outcome, we examined the cross-sectional relationship between biomarkers and cognitive performance, using a three-test Preclinical Alzheimer Cognitive Composite (PACC3) described by Jonaitis et al.[19] and based on the work of Donohue et al.[20] Due to variations in cognitive batteries across cohorts, Trail-Making Test B replaced Digit Symbol as the executive function measure and Craft Story Delayed Recall was used to impute Logical Memory II-A based on a published crosswalk.[21] Continuous cognitive outcomes were matched to the nearest LP visit. Only matches less than a year apart were included, and no cognitive visit was matched more than once.

2.8 | Statistical analysis

Statistical analyses were conducted in R.[22] Sample characteristics were compared across clinical diagnosis using analysis of variance for continuous measures and chi-square for categorical measures.

HIGHLIGHTS

- Alzheimer's disease (AD) biomarker positive (pTau/Aβ42) participants had higher levels of neurodegeneration biomarkers across levels of clinical severity.
- Biomarkers for glial activation were differentiated in cognitively impaired, but not cognitively unimpaired, participants.
- Biomarkers of neurodegeneration beyond tau accounted for additional variation in cognitive performance over time.
- An expanded panel of cerebrospinal fluid biomarkers that include neurodegeneration and neuroinflammatory markers represents an important array of tools that may play a role in staging AD and other neurodegenerative diseases.

RESEARCH IN CONTEXT

1. Systematic review: The authors reviewed the extant literature using PubMed and Google Scholar. A small number of studies have been published using the same NeuroToolKit (NTK) automated assay for core Alzheimer's disease (AD) biomarkers. However, this study examines the extended NTK assay, which includes additional markers for neurodegeneration and glial activation.
2. Interpretation: Our results indicate that the NTK panel of neurodegeneration and neuroinflammatory markers represents an important array of tools that may play a role in staging AD and confer new insights into the pathogenesis of AD and its clinical manifestation.
3. Future directions: A number of hypotheses are generated from these results. For example, focusing on developing meaningful thresholds for neurofilament light may enhance detection of subjects with neurodegeneration (N+). Also, studies with a more clinically diverse sample are required to establish the contexts under which glial markers signify or contribute to risk for AD.

Associations between CSF values and clinical severity were tested with linear regressions. The R package emmeans 1.4.3.01[23] was used to compare mean differences among groups defined by ADB status and clinical diagnosis.

We evaluated the potential added explanatory value of exploratory NTK biomarkers when modeling cognitive outcomes using categorical (clinical diagnosis) and continuous (PACC3) measures of cognition. Observations were excluded if they were missing ADB or NTK biomarkers, or any covariates (n = 47). SHARP participants (n = 66) received a different cognitive battery and were excluded from analyses of continuous cognitive performance. Logistic regression was used

TABLE 1 Sample demographics at most recent LP by clinical diagnosis

	Total	Dementia	MCI	CU	*P*
***n* (% Female)**	720 (63.3%)	50 (36.0%)[a]	54 (42.6%)[b]	616 (67.4%)	<.001
non-Hispanic, White, *n* (%)	676 (93.9%)	49 (100%)	44 (93.6%)	576 (94.3%)	.17
Age, *m* (SD)	63.9 (±9.0)	72.6 (±8.5)[a]	72.4 (±8.4)[b]	62.4 (±8.1)	<.001
***APOE4*+, *n* (%)**	251 (34.8%)	33 (67.3%)[a]	27 (50.0%)	220 (35.7%)	.02
Parental AD+, *n* (%)	501 (69.6%)	31 (63.3%)	29 (53.7%)[b]	441 (71.6%)	.02
Education, *m* (SD)	16 (±2.6)	14.4 (±2.6)[a]	16.1 (±2.6)	16.2 (±2.4)	<.001
MMSE, *m* (SD)	28.5 (± 2.5)	21.6 (±3.7)[a]	27.4 (±2.0)[b]	29.4 (±0.9)	<.001
CDR Sum of Boxes, *m* (SD)	0.67 (±1.5)	4.5 (±1.6)[a]	1.7 (±1.3)[b]	0.08 (±0.27)	<.001
ASCVD ≥ 7.5, n %	323 (55.4%)	33 (89.2%)[a]	29 (85.2%)[b]	261 (50.9%)	<.001
Hypertension, n (%)	162 (25.2%)	28 (56.0%)[a]	21 (42%)[b]	113 (20.8%)	<.001
Diabetes, n %	44 (6.7%)	6 (12%)	5 (9.8%)	33 (6.1%)	.19
MDD, n %	202 (31.2%)	19 (38.0%)	19 (38.0%)	164 (31.0%)	.39
Number LPs, 1/2/3/4+	–	48/2/0/0	47/6/0/1	362/81/115/58	–
LP interval in years, *m* (SD)	–	–	3.7 (2.8)	2.0 (1.5)	–
PiB PET, *n*	185	2	16	167	
PiB(+), *n* (%)	47 (25%)	2 (100%)	10 (63%[b]	35 (21%)	<.001
Age at PiB, *m* (SD)	67.0 (±7.6)	71.0 (±4.5)	71.9 (±8.4)[b]	66.5 (±7.5)	.02
Years Δt(PiB – LP), *m* (SD)	0.4 (±0.7)	0.3 (±0.5)	0.2 (±0.6)	0.4 (±0.7)	.17

Abbreviations: AD, Alzheimer's disease; *APOE*4+, apolipoprotein E4 carrier; ASCVD, atherosclerotic cardiovascular disease 10 year risk percent (≥7.5 is high risk); CDR, Clinical Dementia Rating; CU, cognitively unimpaired; Dementia, dementia due to suspected AD or other causes; LP, lumbar puncture; MCI, mild cognitive impairment due to suspected AD or other causes; MDD, major depressive disorder; MMSE, Mini-Mental State Examination; PiB PET, [C-11] Pittsburgh compound B positron emission tomography; SD, standard deviation.

Notes: Clinical status (MCI/Dementia) was determined based on National Institute on Aging-Alzheimer's Association (NIA-AA) criteria, without reference to biomarkers. Each LP visit was matched to the participant's nearest consensus conference (average Δtime [age Diagnosis-age LP] = .25 ± .30 years). Six participants were missing MMSE (5 CU, 1 MCI), *n* = 79 CU participants were missing CDR Sum of Boxes due to variations in cognitive testing across cohorts (see supporting information). Parental history of AD was determined through parent medical records, autopsy reports, results of a dementia questionnaire, or participant self-report. ASCVD 10-year risk was calculated using the 2013 American College of Cardiology/American Heart Association algorithm (*n* = 145 CU participants were missing data). Diagnosis of hypertension, diabetes, and MDD was obtained at study entry (3 MCI and 72-87 CU participants were missing data).

[a]Dementia vs CU, $P < .05$.

[b]MCI vs CU, $P < .05$.

to model the relationship between clinical diagnosis (pooled MCI and dementia vs CU), continuous ADB, and additional NTK biomarkers for neurodegeneration or glial activation. Linear mixed effects regression was used to model the relationship between continuous cognitive performance (PACC3), continuous ADB, and additional NTK markers. Models were fit via maximum likelihood estimation. A likelihood ratio test (LRT) was used to compare larger models containing neurodegeneration and gliosis markers, respectively, with a reduced model including only continuous ADB and key covariates, age at LP, sex, apolipoprotein *ε4* (*APOE4*) carrier status, and years of education. Due to the nature of the study, we did not correct *p*-values for multiple testing.

3 | RESULTS

3.1 | Sample characteristics and CSF analytes

Sample characteristics are shown by clinical diagnosis in Table 1. Participants were aged 40 to 93 years ($M = 63.9$, $SD = 9.0$), mostly white, and highly educated. Cognitively unimpaired participants were younger, more educated, and less likely to carry the *APOE4* risk allele compared to impaired groups. Performance on the Mini-Mental State Examination (MMSE) and the Clinical Dementia Rating Scale Sum of Boxes (CDR-SB) tracked with diagnostic category, as expected (Table S3 in supporting information).

3.2 | Biomarker positivity thresholds

3.2.1 | CSF amyloid and ADB ratios

ROC analyses indicated high diagnostic consistency between PiB visual positivity and $A\beta_{42/40}$ and $pTau_{181}/A\beta_{42}$. Area under the curve was 97% for both ratios. ROC derived thresholds for biomarker positivity were 0.046 for $A\beta_{42/40}$ (96% negative agreement, 92% positive agreement) and 0.038 for $pTau_{181}/A\beta_{42}$ (98% negative agreement, 83% positive agreement). Applying these thresholds to

the full study sample resulted in 46/50 (92%) dementia, 31/54 (61%) MCI, and 98/604 (16%) of CU participants identified as $A\beta_{42/40}$(+), and 46/50 (92%) dementia, 31/54 (61%) MCI, and 66/606 (11%) CU participants identified as pTau/$A\beta_{42}$ positive (ie, ADB[+]). $A\beta_{42/40}$ and $pTau_{181}/A\beta_{42}$ positivity agreed in 669/708 (94%) of cases with disagreement observed for 36 cases classified as $A\beta_{42/40}$(+)/pTau/$A\beta_{42}$(-), and 3 cases classified as $A\beta_{42/40}$(-)/pTau/$A\beta_{42}$(+).

3.2.2 | Tau and neurodegeneration positivity

The average $pTau_{181}$ concentration among the reference group of cognitively unimpaired, amyloid negative adults aged 40 to 60 years was 15.1 (SD = 4.8) pg/mL resulting in a $pTau_{181}$ positivity threshold of 24.8 pg/mL. Applying this threshold to the non-reference sample indicated 38/49 (78%) dementia, 25/47 (53%) MCI, 68/385 (18%) of the CU participants were $pTau_{181}$ positive. Similarly derived positivity thresholds for other CSF neurodegeneration analytes are reported in Table S2a in supporting information. Of these neurodegeneration markers (NfL, neurogranin, and alpha-synuclein), neurogranin was the only analyte that did not indicate stepwise increases in the proportion of positive cases with increasing clinical severity. The proportion of NfL and α-synuclein positivity within each diagnostic group was highest in dementia cases (20/50 [40%] NfL[+]; 18/50 [36%] α-synuclein[+]), followed by MCI cases (12/54 [22%] NfL[+]; 11/54 [20%] α-synuclein[+]), and then CU cases (15/401 [4%] NfL[+]; 35/401 [9%] α-synuclein[+]). However, biomarkers varied in agreement for neurodegeneration positivity (Cohen's kappa ranged 0.36–0.51; Table S2b in supporting information).

3.3 | CSF analyte observations by ADB status

Scatterplots and correlations between CSF analytes for biomarker groups (AD, neurodegeneration, and glial activation) are shown by ADB status in Figure 1A-C (See Figure S1 in supporting information for correlations between all CSF analytes). Correlations between $A\beta_{42}$, $A\beta_{40}$, $pTau_{181}$, and tTau were typical of those observed in AD (Figure 1A).[24] Due to the high correlation between $pTau_{181}$ and tTau (r = .98), tTau was excluded from subsequent regression analyses with clinical diagnosis and cognition.

Correlation patterns for CSF analytes related to neurodegeneration and glial activation were consistent across ADB status for all NTK analytes. All neurodegeneration markers (Figure 1B) correlated highly with tTau (range r = .62–.87). Neurogranin was highly correlated with α-synuclein (r = .81), while NfL was only moderately correlated with α-synuclein (r = .50) and neurogranin (r = .38). Glial activation biomarkers (Figure 2C) were all modestly inter-correlated (r = .22–.62) with S100B showing the lowest correlation with other glial activation markers. IL6 values were unrelated to the remaining analytes (Figure S1).

3.4 | CSF analyte observations by clinical diagnosis and ADB status

Descriptive statistics for all CSF analytes and derived ratios stratified by clinical diagnosis and ADB status are shown in Table 2. Distributions of analytes are shown in Figure 2A-D. $A\beta_{40}$, $A\beta_{42}$, and $pTau_{181}$ (Figure 2, panel A) exhibited the expected distributions for combinations of clinical and ADB status. $A\beta_{40}$ did not differ across clinical or ADB groups. $A\beta_{42}$ was lower for all ADB+ and did not differ between clinical groups. Phospho-Tau_{181} was low in all ADB–, was higher in unimpaired ADB+, and was highest in impaired (MCI and dementia) ADB+.

Neurodegeneration analytes (Figure 2, panel B) showed similar patterns between ADB and clinical status groups for neurogranin and α-synuclein. These markers did not differ across clinical groups in ADB– and were higher in ADB+ compared to ADB– both within and across clinical groups (not enough ADB– dementia cases for comparison). NfL indicated stepwise increases in ADB+ with increasing clinical severity.

CSF analytes of glial activation YKL-40, S100B, GFAP, and sTREM2 (Figure 2, panel C) exhibited similar patterns in ADB+ wherein impaired ADB+ had higher values compared to unimpaired ADB+. In general, these analyte distributions had considerable overlap between ADB+ and ADB– in the unimpaired group. YKL-40 and GFAP were higher for ADB+ compared to ADB– in the MCI group. IL6 (Figure 2, panel D) was unrelated to ADB status or clinical group.

3.5 | Relationships between cognitive outcomes and extended NTK analytes

Continuous ADB significantly predicted clinical diagnosis and cognitive performance (Ps < .001). Adding gliosis biomarkers did not improve model fit for either clinical status or PACC3 (clinical status: $\chi^2[4] = 4.0$, P = .41; PACC3: $\chi^2[4] = 6.7$, P = 0.15). For both clinical status (Table 3) and PACC3 (Table 4), adding neurodegeneration biomarkers improved the overall model fit when compared to a model that included continuous ADB and covariates (clinical status: $\chi^2[3] = 17.3$, P = .0006; PACC3: $\chi^2[3] = 23.5$, P = .000032). The regression coefficient for neurogranin was opposite in sign to our expectation. Secondary analyses of individual neurodegeneration biomarkers suggested that this was an artifact of statistical suppression.[25] As individual biomarkers, NfL best predicted clinical status and PACC3 over and above continuous ADB. To visualize the latter findings, we plotted a loess curve of PACC3 against NfL grouping by ADB status (Figure S2 in supporting information).

4 | DISCUSSION

The development of CSF assays for Aβ and tau proteins launched a rapid expansion of biomarker research.[4] Nevertheless, questions surrounding heterogeneity in the clinical manifestation of AD,[26,27] and the contribution of co-occurring pathology to clinical symptoms,[28,29]

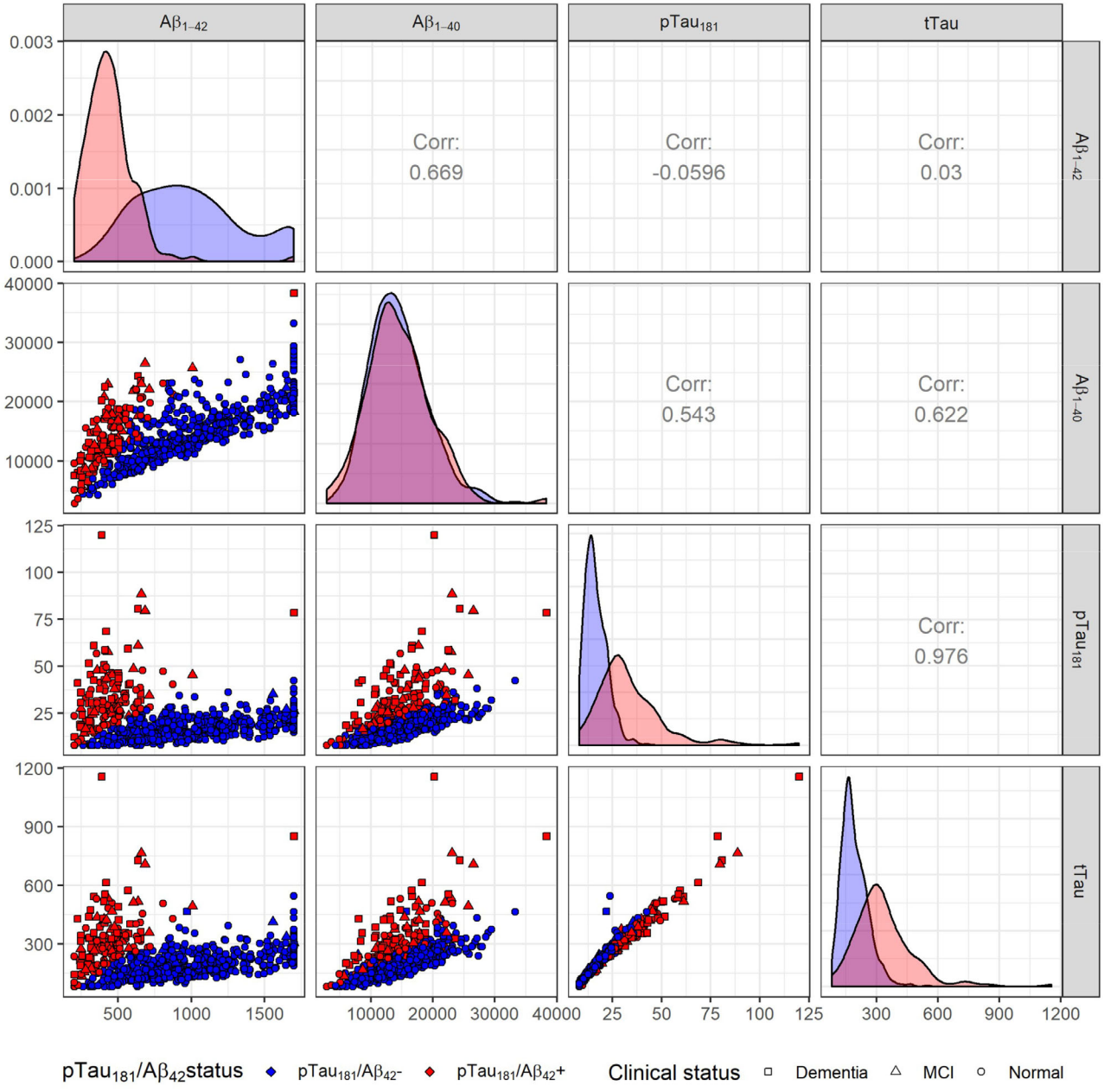

FIGURE 1 Scatterplot histograms and correlation coefficients within related cerebrospinal fluid analytes. Note: Scatterplot histograms and correlation coefficients within core Alzheimer's disease (AD) biomarkers (A), neurodegeneration biomarkers (B), and glial activation biomarkers (C). Scatterplots are shown by biomarker status (ADB− shown in blue, ADB+ shown in red) and clinical diagnosis (square = dementia, triangle = mild cognitive impairment, circle = cognitively unimpaired), in the lower diagonal. Histograms by biomarker status are shown in the diagonal (A–C). Correlation coefficients for the pooled sample (black, A–C) and disaggregated by ADB ($pTau_{181}/A\beta_{42}$) status shown in upper diagonal (B–C). Correlation coefficients are not disaggregated by ADB status in panel (A). Such disaggregation would produce artifactual correlations between analytes used to define biomarker status. For more on this phenomenon, see eg, Cole et al (2009).

onset,[30] and progression[30,31] require an expanded set of biomarkers reflecting neurodegeneration and neuroinflammatory processes. Recent studies have investigated the NTK core AD biomarkers,[10,24] and exploratory NTK biomarkers in cognitively unimpaired adults.[12] We examined established and novel biomarkers in the NTK in subjects that span clinical severity to explore their characteristics in the context of AD biomarker status and clinical diagnosis and their added value in predicting cognition.

4.1 | Biomarker positivity

4.1.1 | Concordance of CSF ratios with amyloid PET

CSF and PET biomarkers of AD provide overlapping, but not completely redundant, information given that their targets differ (eg, the Aβ1-42 protein fragment vs fibrillar amyloid with PET) as do

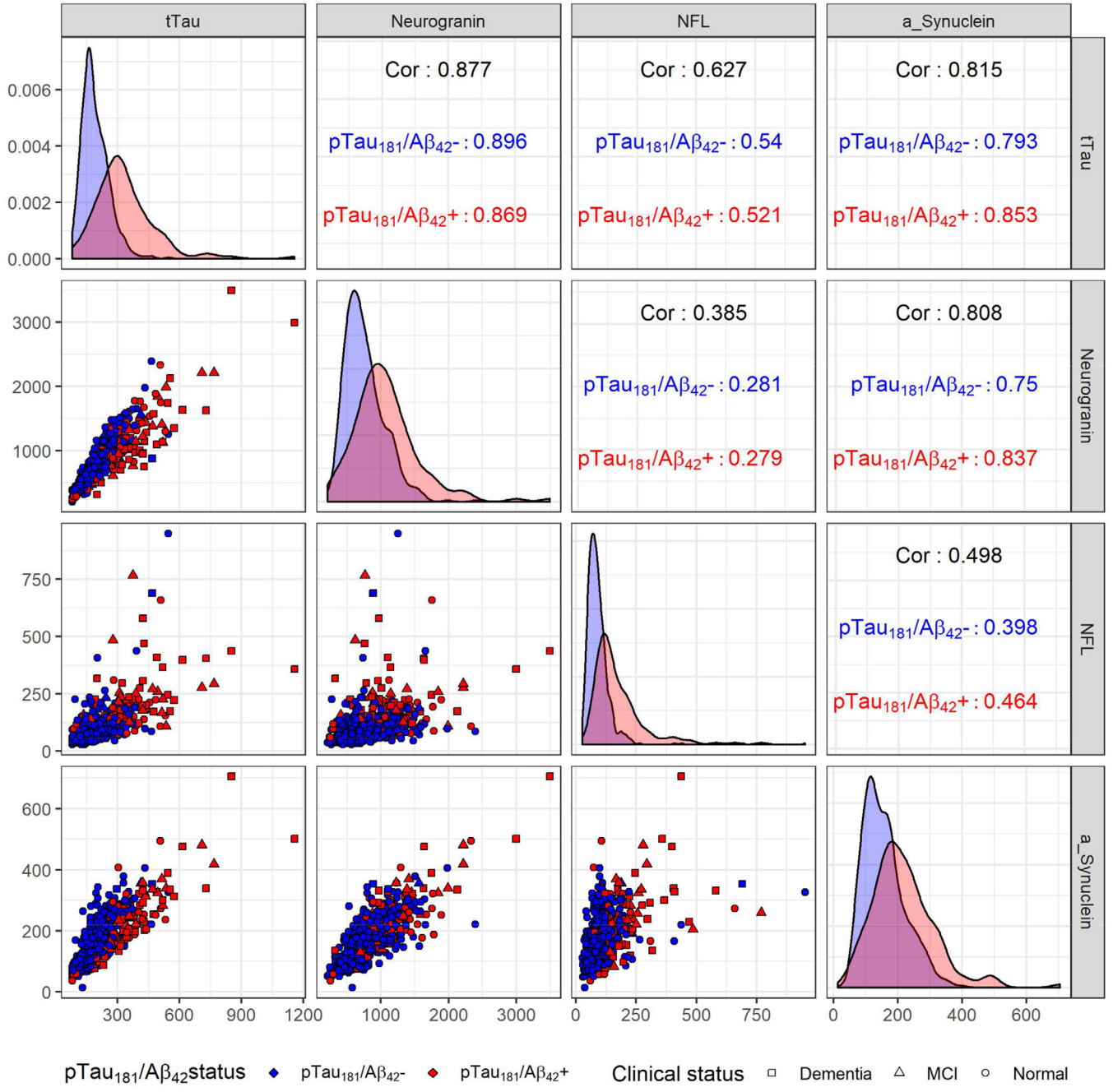

FIGURE 1 Continued

their sensitivity to pathology. Amyloid PET positivity may represent a slightly more mature phase of the disease[32] and has been used as a standard to which AD CSF biomarkers can be compared. For this study, having an empirically derived threshold for CSF Aβ positivity based on maximizing agreement with amyloid PET was an important strength. The derived thresholds for A$\beta_{42/40}$ (0.046) and pTau/Aβ_{42} (0.038) conferred an area under the curve of .97 in classifying participants with known amyloid PET status. While this agreement is excellent, it is important to note the differences in physiologic meaning of the signal—lower CSF levels of Aβ_{42} likely reflect impaired clearance, whereas PET signal likely reflects years of accumulated fibrillar amyloid deposition. CSF is likely to begin reflecting AD pathology earlier than PET imaging, thus some individuals with early amyloid pathology that has not yet shown up on PET imaging may have been misidentified as ADB–. The alternative to using PET amyloid as the standard would be to use autopsy cases (which were not available), distribution-based cutpoints (which we resorted to for other analytes), or published CSF amyloid cut points (assuming site-specific differences in pre-analytic procedures have no effect, which is unlikely). Relying on currently published thresholds[10,24] would have led to overestimating the number of biomarker positive CU participants.

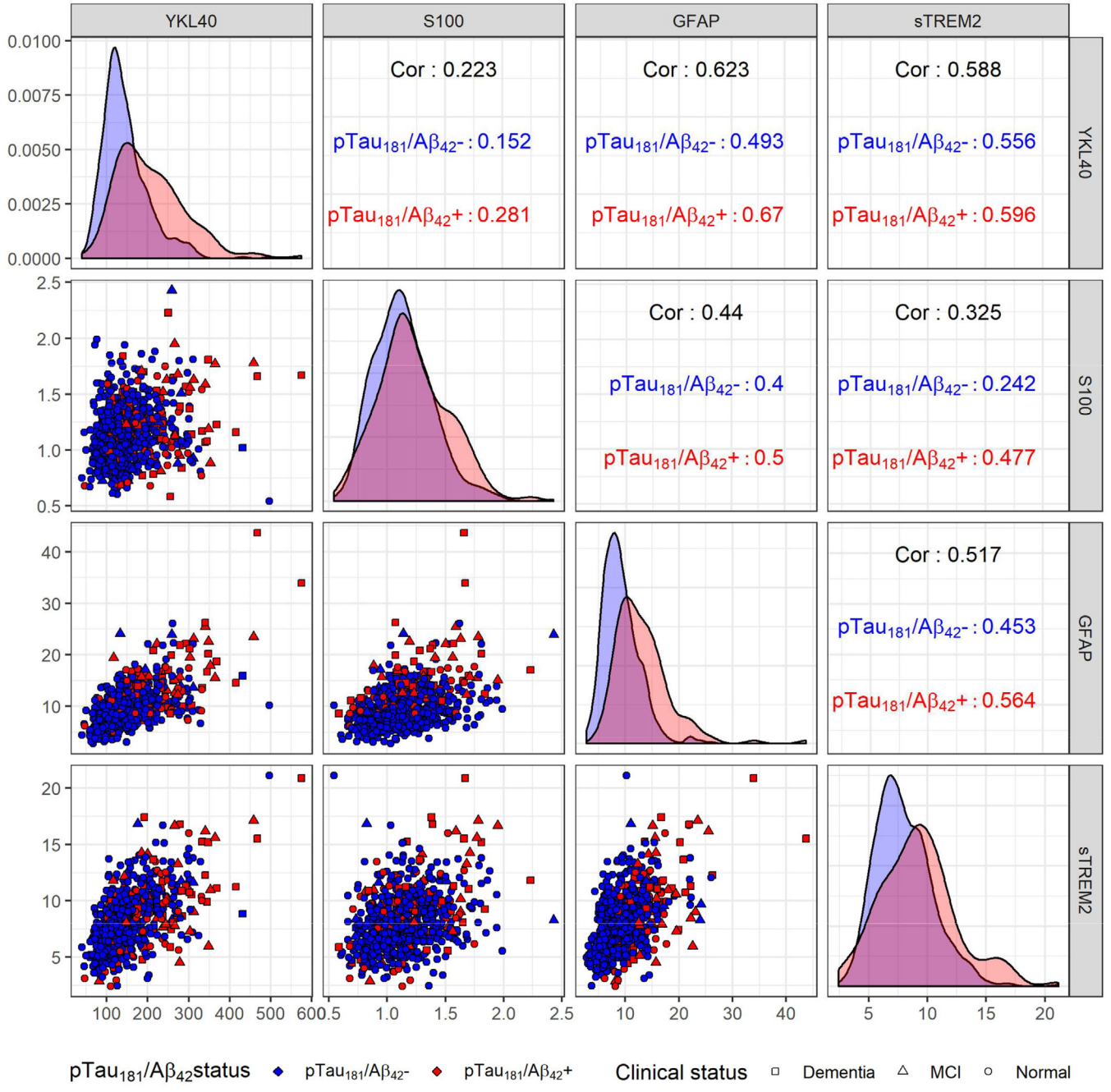

FIGURE 1 Continued

4.1.2 | Concordance of the AD ratios

A$\beta_{42/40}$ and ptau/Aβ_{42} exhibited 95% agreement in this mixed sample of dementia, MCI, and CU participants. This is a high degree of concordance and suggests near equivalence between these ratios for identifying biomarker positive cases defined by PET visual ratings. Because the ptau/Aβ_{42} ratio simultaneously comprises both proteinopathies, concords well with amyloid PET studies, and may be more available to the research and clinical community than A$\beta_{42/40}$, we used ptau/Aβ_{42} as the primary AD biomarker grouping variable. Nevertheless, ptau/Aβ_{42} has the potential to misidentify individuals as ADB– very early in the disease process.

4.2 | Interrelationship between neurodegenerative analytes and clinical diagnosis/ADB status

As noted in the NIA-AA research framework,[6] neurodegeneration is a non-specific feature of several neurodegenerative diseases. Because we[31] and others[33] have observed remarkably high agreement between

TABLE 2 Descriptive statistics and mean differences within clinical diagnosis and Alzheimer's disease biomarker (ADB) status

	Dementia		MCI		CU	
Measure	**ADB+**	**ADB−**	**ADB+**	**ADB−**	**ADB+**	**ADB−**
N	46	4	33	21	70	536
Age, M (SD)	72.3[a] (8.0)	76.5 (14.0)	74.1[e] (7.6)	69.8[b] (9.2)	69.1[e,h] (6.6)	61.6[a,b,c,h] (8.0)
Female, n (%)	18[a] (39%)	0 (0.0%)	13[b] (39%)	10 (48%)	45 (64%)	362[a,b] (68%)
***APOE*4+, n (%)**	32[a] (70%)	1 (25%)	21[a] (63%)	6 (29%)	41 (59%)[c]	176[a,b,c] (33%)
Alzheimer's biomarkers						
$A\beta_{42}$ pg/mL, m (SD)	425[a,f] (229)	1152 (369)	464[b,g] (161)	1061[f,g] (394)	463[c] (152)	991[a,b,c] (366)
$A\beta_{40}$ pg/mL, m (SD)	14002 (5874)	15682 (4296)	15360 (5288)	14896 (4553)	14477 (4439)	14444 (4675)
$A\beta_{42/40}$, m (SD)	0.031[a,f] (0.007)	0.074 (0.008)	0.031[b,g] (0.006)	0.071[f,g] (0.009)	0.034[c] (0.009)	0.069[a,b,c] (0.013)
$pTau_{181}$ pg/mL, m (SD)	39.7[a,f,d] (19.6)	19.1 (5.28)	34.4[g,e,b] (17.6)	17.8[f,g] (6.36)	27.4[d,e,c] (10.27)	16.4[a,b,c] (5.46)
Neurodegeneration biomarkers						
tTau pg/mL, m (SD)	390[a,f,d] (182)	286 (131)	347[b,g] (148)	217[f,g] (77.7)	284[d,c] (99.6)	189[a,b,c] (63.0)a
NfL pg/mL, m (SD)	225[a,f,d] (112)	279 (277)	199[b,g,e] (130)	149[f,g] (123)	129[d,e,c] (80.2)	89.9[a,b,c] (55.6)
Neurogranin pg/mL, m (SD)	1116[a,f] (583)	805 (238)	1067[a,b,g] (481)	795[f,b] (320)	1040[c] (414)	753[a,b,c] (289)
α-Synuclein pg/mL, m (SD)	240[a,d] (118)	246 (116)	231[b,g] (101)	177[a,g] (94.7)	195[d,c] (78.3)	156[b,c] (63.4)
Gliosis biomarkers						
YKL-40 ng/mL, m (SD)	238[a,f,d] (96.7)	239 (132)	226[b,g] (87.2)	176[f,g] (68.1)	179[d] (61.2)	144[a,b] (53.7)
GFAP ng/mL, m (SD)	15.2[a,f,d] (6.72)	10.1 (4.00)	15.2[b,g,e] (5.89)	11.4[f,g] (5.26)	11.2[d,e] (3.14)	9.13[a,b] (3.27)
S100B ng/mL, m (SD)	1.25 (0.331)	1.03 (0.214)	1.31[a,b] (0.303)	1.11[a] (0.381)	1.15[b] (0.248)	1.14 (0.249)
sTREM2 ng/mL, m (SD)	9.95[a](3.57)	8.93 (1.61)	9.75 (3.68)	8.90 (2.56)	8.51[a](2.63)	7.94 (2.43)
Inflammation biomarkers						
IL6 pg/mL, m (SD)	5.47 (5.00)	4.38 (0.724)	3.88 (1.84)	5.25 (5.97)	4.01 (1.99)	4.68 (3.16)

Abbreviations: Aβ, amyloid beta; ADB, Alzheimer's disease biomarker status; *APOE*4+, apolipoprotein E4 carrier; CU, cognitively unimpaired; Dementia, dementia due to suspected AD or other causes; GFAP, glial fibrillary acidic protein; MCI, Mild cognitive impairment due to suspected AD or other causes; Nfl, neurofilament light protein; SD, standard deviation; sTREM2, soluble triggering receptor expressed on myeloid cells 2; YKL-40, chitinase-3-like protein 1.
Notes: Clinical status (MCI/dementia) was determined based on National Institute on Aging-Alzheimer's Association (NIA-AA) criteria without reference to biomarkers. ADB status defined by $pTau_{181}/A\beta_{42}$ threshold .038. Core AD biomarkers that exceeded detectable limits were imputed at the limit threshold: $A\beta_{42}$ lower limit is 200, upper limit is 1700, $pTau_{181}$ lower limit is 8, upper limit is 120, tTau lower limit is 80 upper limit is 1300. In cases in which CSF analyte values exceeded upper or lower detection limits,[9,11] the value of the threshold was imputed; 44 values were imputed for $A\beta_{42}$ ($N_{<LL} = 3$, $N_{>UL} = 41$), and 20 for $pTau_{181}$ ($N_{<LL} = 19$, $N_{>UL} = 1$). Ten CU participants had missing values for core AD biomarker values due to sample abnormalities. Biomarker negative participants with dementia were excluded from statistical comparisons due to low sample size.
[a]ADB+/dementia compared to ADB-/CU, P < .05
[b]ADB+/MCI compared to ADB-/CU, P < .05.
[c]ADB+/CU compared to ADB-/CU. P < .05.
[d]ADB+/dementia compared to ADB+/CU, P < .05.
[e]ADB+/MCI compared to ADB+/CU. P < .05.
[f]ADB+/dementia compared to ADB-/MCI. P < .05.
[g]ADB+/MCI compared to ADB- /MCI. P < .05.
[h]ADB-/MCI compared to ADB-/CU, P < .05.

tTau and $pTau_{181}$, CSF tTau does not appear to be a fully independent measure of neurodegeneration in AD. The other NTK markers may serve an important need in this regard. Indeed, NfL, an indicator of axonal degradation, has been used as a useful neurodegeneration marker in multiple sclerosis, non-AD tauopathies, synucleinopathies,[34] and traumatic brain injury[35] as well as AD.[36] NfL is also in agreement with magnetic resonance imaging metrics in this population[37] and correlates with pre-dementia disease progression.[31,38] In the present analyses, NfL, neurogranin, and α-synuclein exhibited moderate to strong agreement with tTau and at least moderate agreement with each other, suggesting these markers of neurodegeneration are reflecting common aspects of neurodegeneration. Further, they exhibited elevation within diagnostic stage by ADB status or significant elevation differences across diagnoses (as shown in Figure 2). NfL exhibited the most characteristic stepwise increase across clinical diagnosis in AD biomarker positive subjects and this was further evident in Figure S2 in which a steeper relationship between lower cognitive scores and NfL was observed among ADB+ than ADB− participants. In

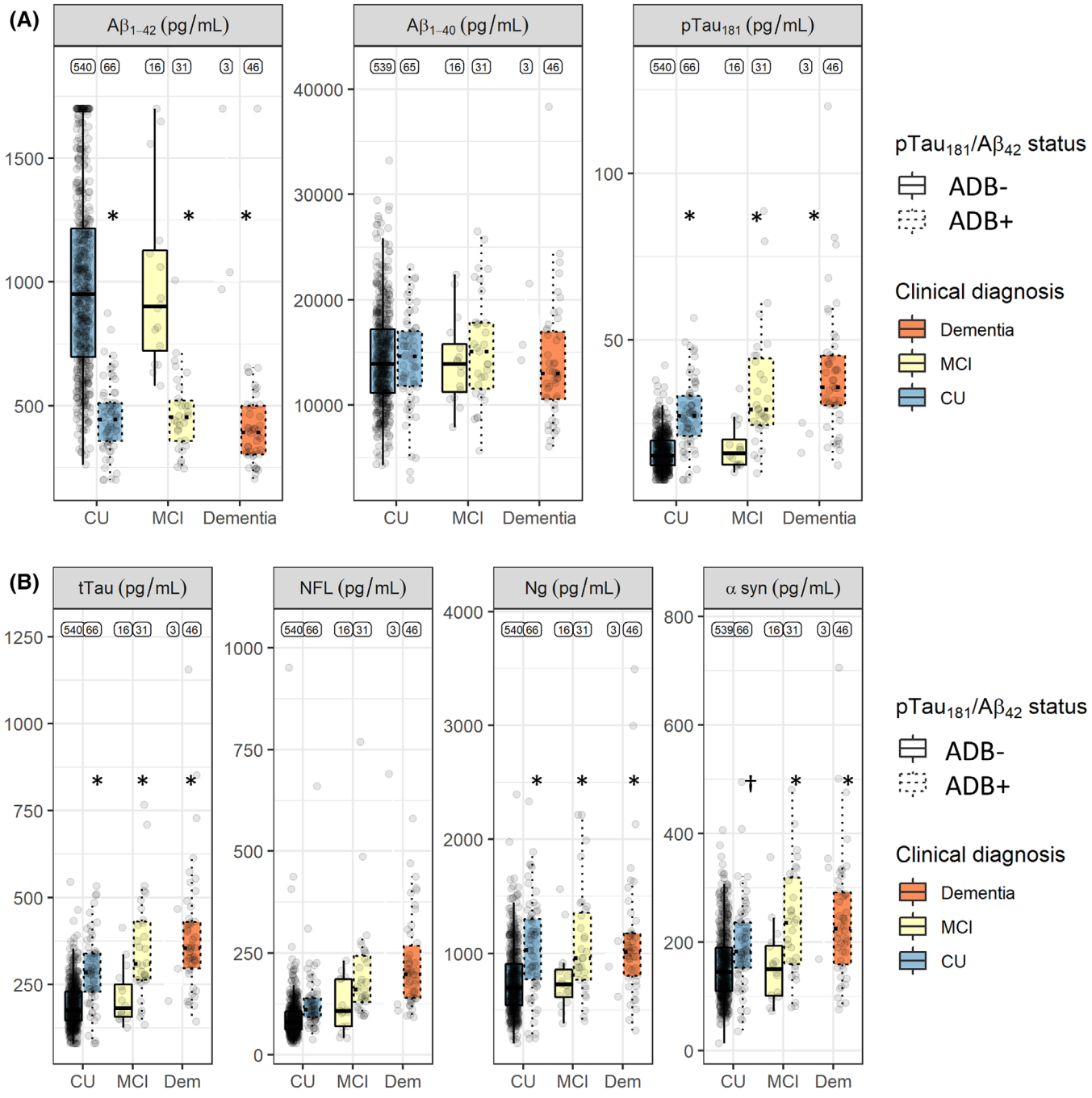

FIGURE 2 Distributions of cerebrospinal fluid analytes by clinical diagnosis and Alzheimer's biomarker (ABD) status. Note: Boxplots are shown for core AD biomarkers (A), non-Tau neurodegeneration biomarkers (B), glial activation biomarkers (C), and IL6 (D). Clinical status was determined based on National Institute on Aging-Alzheimer's Association (NIA-AA) criteria, without reference to biomarkers. Dementia = dementia due to AD clinical syndrome or other related causes, MCI = mild cognitive impairment due to AD clinical syndrome or other causes, CU = cognitively unimpaired. Sample sizes are shown at the top of each plot. Cognitively unimpaired ADB– is the reference group for mean comparisons

contrast, neurogranin, a post-synaptic protein marker, was significantly elevated in the cognitively unimpaired group who were ADB+ and remained elevated across clinical diagnoses consistent with our prior observations.[31,38] Total α-synuclein, a presynaptic marker, exhibited a similar pattern and was also strongly correlated with neurogranin ($r = .80$). CSF α-synuclein was initially found to be slightly decreased in Parkinson's disease and Lewy body dementia, but subsequent studies showed a pronounced increase in CSF α-synuclein in neurodegenerative disorders with marked neurodegeneration, including Creutzfeldt-Jakob disease and AD.[39] Elevation of this protein in our sample likely

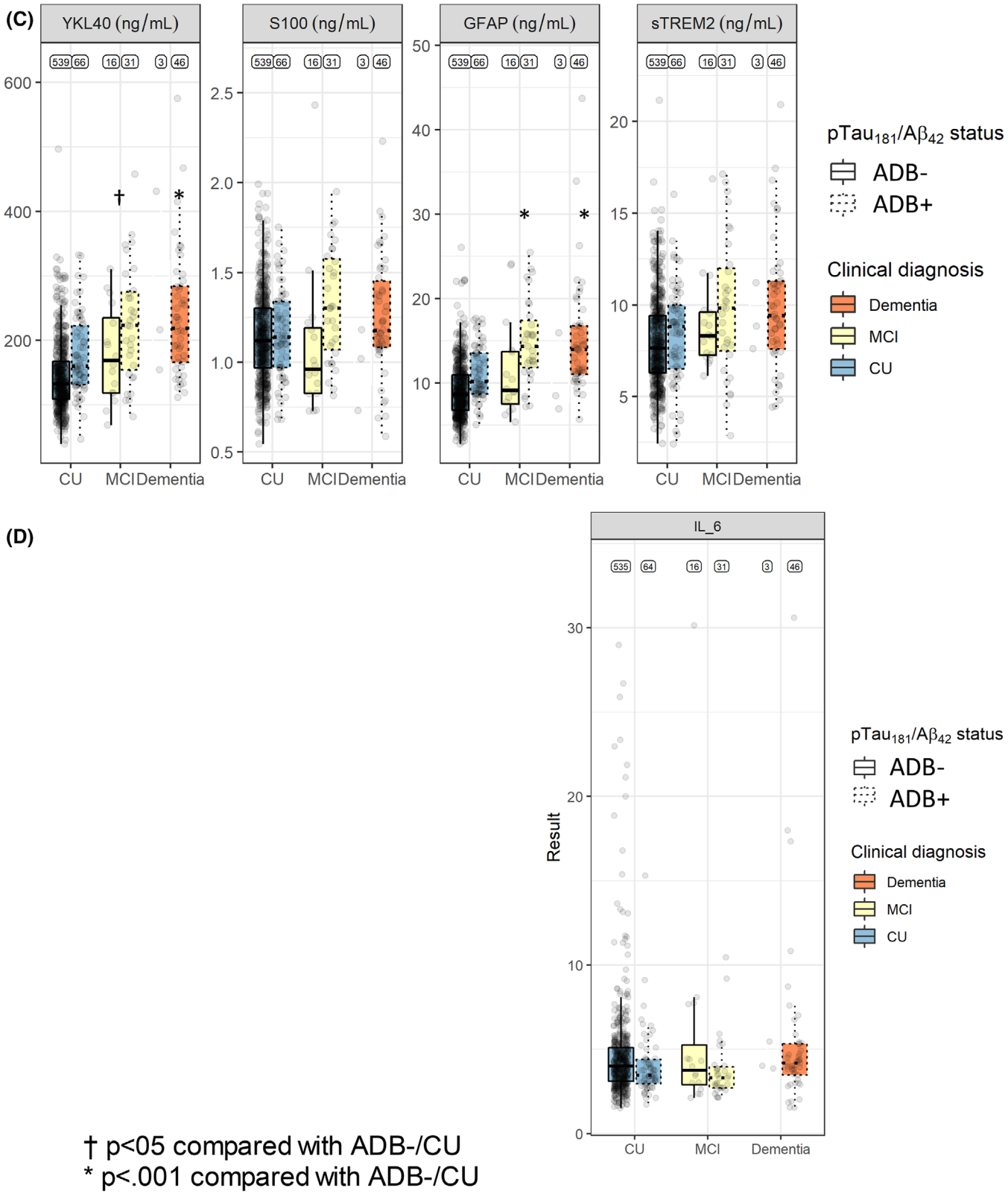

FIGURE 2 Continued

TABLE 3 Results of logistic regression predicting clinical diagnosis (n = 681) from NTK biomarkers

	Neurodegeneration ($\chi^2(3) = 17.3, P = .0006$)				Gliosis ($\chi^2(4) = 4.0, P = .40$)			
Term	Estimate	SE	z value	Pr(> |t|)	Estimate	SE	z value	Pr(> |t|)
(Intercept)	−0.346	1.044	−0.33	.740	−0.634	1.030	−0.62	.537
Sex, male	0.743	0.335	2.22	.026	1.010	0.316	3.18	.002
Parental AD +	−0.471	0.344	−1.37	.171	−0.386	0.333	−1.16	.247
APOE4+	0.446	0.351	1.27	.205	0.507	0.338	1.50	.133
Education, years	−0.170	0.064	−2.66	.008	−0.161	0.062	−2.60	.009
Age at LP	0.096	0.022	4.37	<.001	0.095	0.024	3.91	<.001
$pTau_{181}/A\beta_{42}$	1.365	0.280	7.28	<.001	1.160	0.163	7.10	<.001
NfL	0.325	0.158	2.05	.040				
Neurogranin	−0.891	0.280	−3.18	.002				
α-Synuclein	0.740	0.272	2.72	.007				
YKL-40					0.067	0.194	0.35	.729
S100B					−0.095	0.174	−0.54	.586
GFAP					0.331	0.205	1.61	.106
sTREM2					0.000	0.175	0.00	.990

Abbreviations: *APOE4*+, apolipoprotein E4 carrier; GFAP, glial fibrillary acidic protein; LP, lumbar puncture; Nfl, neurofilament light protein; NTK, NeuroToolKit; sTREM2, soluble triggering receptor expressed on myeloid cells 2;YKL-40, chitinase-3-like protein 1.
Notes: Participants with MCI or dementia were pooled to form the cognitively impaired group. Neurodegeneration and glial activation biomarkers were standardized prior to analysis. Age is mean-centered. $n = 47$ participants were excluded due to missing covariates or missing cerebrospinal fluid values (1 dementia, 5 mild cognitive impairment, 41 cognitively unimpaired). There were no demographic differences between excluded participants and participants in the analyses and no differences in biomarker status.

reflects synaptic degeneration rather than deposition of α-synuclein in Lewy bodies. Its utility as a novel marker of neurodegeneration continues to undergo study.

Although promising as continuous markers of neurodegeneration (N), among AD and CU participants a lower proportion were identified as positive when defined by NFL, neurogranin, or α-synuclein compared to tTau. Agreement across neurodegeneration biomarkers was moderate. The method for choosing thresholds for these analytes (2 SD above the mean of a CU $A\beta_{42/40}$ negative group) is a reasonable approach but assumes a monotonic relationship between age and biomarker concentration. Ongoing work in the field will lead to more precise methods for defining a meaningful threshold for N+/−.

4.3 | Interrelationship between inflammation and gliosis analytes and clinical diagnosis/ADB status

Activation of microglia in response to amyloid plaques is a well-known feature of AD, and inflammatory pathways have been shown to play a role in AD pathogenesis.[40,41] Despite the involvement of inflammatory processes in AD pathophysiology, IL6 was unrelated to either markers of glial activation or clinical diagnosis, and may be more relevant at a more advanced disease state.[42] Markers of glial activation exhibited low to moderate intercorrelation, indicating potentially unique physiologic meaning of each analyte. YKL-40, a glycoprotein expressed by microglia and astrocytes, and GFAP, an indicator of reactive astrocytes,[43] were both elevated in ADB+ cognitively impaired subjects compared to their biomarker negative peers. The YKL-40 finding replicates previous observations.[44] From Figure 2, the effect sizes of glial and microglial markers observed here appear lower than for the neurodegeneration markers. Nevertheless, these results are promising and warrant further study, particularly in the context of co-occurring diseases.

4.4 | Core AD biomarkers and cognition

Before examining the effect of the NTK panel, we first confirmed that AD biomarkers were related to cognition defined by clinical diagnosis and global cognitive performance. $A\beta_{42}$ alone predicted impairment, but did not distinguish between MCI and AD, perhaps due to the well-known observation that levels of this protein plateau by the dementia stage.[45,46] Normalizing against total amyloid production (ie, by the $A\beta_{42/40}$ ratio), led to clear differentiation by clinical diagnosis, as did $pTau_{181}$ and $pTau_{181}/A\beta_{42}$.

4.5 | NTK biomarkers and cognition

Results from hierarchical regression analyses suggest that as a group, neurodegeneration biomarkers add value in predicting both clinical impairment (MCI/dementia vs CU) and global cognitive performance. The information in these markers is overlapping, as can be

TABLE 4 Results of linear mixed model predicting continuous cognitive performance on the preclinical Alzheimer's cognitive composite (PACC3; n = 617) from NTK biomarkers

	Neurodegeneration ($\chi^2(3) = 23.5$, $P = .000032$)		Gliosis ($\chi^2(4) = 6.7$, $P = 0.15$)	
Term	**β(SE)**	***P***	**β (SE)**	***P***
Intercept	−1.88 (0.22)	<.001	−1.82 (0.22)	<.001
Sex, male	−0.5 (0.068)	<.001	−0.58 (0.068)	<.001
Parental AD +	0.012 (0.07)	.86	−0.0013 (0.071)	.99
APOE4+	−0.0054 (0.067)	.94	−0.018 (0.068)	.79
Education, years	0.11 (0.013)	<.001	0.11 (0.013)	<.001
Prior exposure to cognitive tests				
1 exposure	0.18 (0.056)	.001	0.21 (0.056)	<.001
2 exposures	0.28 (0.059)	<.001	0.31 (0.059)	<.001
3 exposures	0.54 (0.065)	<.001	0.56 (0.066)	<.001
4 exposures	0.6 (0.077)	<.001	0.63 (0.077)	<.001
5 exposures	0.54 (0.13)	<.001	0.56 (0.13)	<.001
6 exposures	0.58 (0.25)	.023	0.64 (0.25)	.012
8 exposures	−0.88 (0.53)	.1	−0.98 (0.53)	.066
Age at cognitive testing				
Linear term	−0.041 (0.0044)	<.001	−0.04 (0.0049)	<.001
Quadratic term	−0.0012 (0.00031)	<.001	−0.0012 (0.00031)	<.001
$pTau_{181}/A\beta_{42}$	−0.42 (0.034)	<.001	−0.38 (0.033)	<.001
NfL, sd	−0.1 (0.033)	.002	–	–
Neurogranin, sd	0.17 (0.045)	<.001	–	–
α-Synuclein, sd	−0.059 (0.042)	.16	–	–
YKL-40, sd	–	–	−0.044 (0.044)	.31
S100B, sd	–	–	0.059 (0.028)	.035
GFAP, sd	–	–	−0.058 (0.042)	.17
sTREM2, sd	–	–	0.018 (0.037)	.63

Abbreviations: *APOE4*+, apolipoprotein E4 carrier; GFAP, glial fibrillary acidic protein; Nfl, neurofilament light protein; NTK, NeuroToolKit; PACC, preclinical Alzheimer's cognitive composite; PACC3, Preclinical Alzheimer Cognitive Composite; sTREM2, soluble triggering receptor expressed on myeloid cells 2; YKL-40, chitinase-3-like protein 1.

Notes: NTK biomarkers were standardized prior to analysis, and age is mean-centered on baseline age; PACC3 is comprised of Rey AVLT-total over five trials, Logical Memory IIA (Story Recall Delayed or cross-walked Craft Story), and Trail-Making Test Part B. $n = 111$ participants were excluded due to missing covariates, cerebrospinal fluid values, or cognitive testing (1 dementia, 5 mild cognitive impairment, 105 cognitively unimpaired). Excluded participants were younger ($t[158.5] = 6.9$, $P < .001$) and less likely to be biomarker positive (χ^2 [1] = 16.7, $P < .001$) than participants included in the analyses. No other demographic differences were found.

seen by the suppression effects observed in the full model; however, their relatively moderate concordance with one another indicates that the overlap is only partial. Of the three neurodegeneration markers, NfL appears to have the best concordance with clinical diagnosis. In contrast, adding gliosis markers to the regression did not improve model fit for either clinical status or global cognition, which suggests these markers do not explain additional variance in cognition beyond core AD markers. This conclusion must be tempered by the constraint of the study design. It is possible that glial markers may exhibit effects in certain contexts, such as the presence of vascular disease,[10] or their effects are non-linear across disease state.[47]

4.6 | Limitations

Although the development of immunoassays has the potential to greatly reduce assay variability, raw values, particularly for Aβ, may still be affected by preanalytic fluid collection protocols, which vary across studies.[48] As such, the values and cut points described here may not generalize to studies in which preanalytic protocols differ. Our cohort is typical of dementia research (white, educated, relatively high functioning) but may not represent the typical AD patient, or individuals from higher risk populations like African Americans and Latinx. Results need to be interpreted in light of this limitation. Although the time interval between LP and PET imaging was up to 2 years, given the stability of

amyloid in the brain we do not anticipate a smaller time interval would change our findings.

5 | CONCLUSION

The NTK panel of neurodegeneration and neuroinflammatory markers represents an important array of tools that may play a role in staging AD, provide complementary outcomes for clinical trials, and confer new insights into the pathogenesis of AD and its clinical manifestation. In this sample, which spanned the spectrum of AD clinical stages, we observed informative interrelationships among the analytes and found that the neurodegeneration markers, but not glial activation, improved prediction of cognitive performance.

ACKNOWLEDGMENTS

The authors would like to thank the WRAP, ADRC, SHARP, and ADCP research participants and staff for their generosity and hard work. CSF assays kits were provided by Roche Diagnostics GmbH. Data were collected under the following grants from the NIH National Institute of Aging: R01 AG027161 (Johnson), R01 AG031790 (Carlsson), R01 AG037639 (Bendlin), R01 AG021155 (Johnson), R01AG054059 (Gleason), R01 AG062167 (Okonkwo), UF1AG051216 (Bendlin), P50 AG033514 (Asthana), P30 AG062715 (Asthana). Support was also provided by the NIH National Center for Advancing Translational Sciences (NCATS), grant UL1TR000427 (Clinical and Translational Sciences). Dr. Betthauser was supported by AARF-19-614533. Other support was provided by the Holland and Lange funds. Dr. Blennow is supported by the Swedish Research Council (#2017-00915), the Alzheimer Drug Discovery Foundation (ADDF), USA (#RDAPB-201809-2016615), the Swedish Alzheimer Foundation (#AF-742881), Hjärnfonden, Sweden (#FO2017-0243), the Swedish state under the agreement between the Swedish government and the County Councils, the ALF-agreement (#ALFGBG-715986), and European Union Joint Program for Neurodegenerative Disorders (JPND2019-466-236). These funding sources had no role in the design and conduct of the study or collection, management, and analysis of the data. All authors critically reviewed and edited the article. Sterling Johnson has previously served on an advisory board to Roche Diagnostics. Norbert Wild, and Gwendlyn Kollmorgen are employees of Roche Diagnostics. Richard Batrla was an employee of Roche Diagnostics at the time of the study. The authors would like to thank Katharina Zink and Katharina Buck for critical review of the article.

ELECSYS, COBAS, and COBAS E are registered trademarks of Roche.

CONFLICTS OF INTEREST

Dr. Cynthia Carlsson receives grant support from NIH/Lilly, NIH, Veterans Affairs, and Bader Philanthropies. Dr. Sterling Johnson previously served on an advisory board for Roche Diagnostics, and receives research funding from NIH and from Cerveau Technologies. Dr. Sanjay Asthana serves as a site principal investigator for pharmaceutical trials funded by Merck Pharmaceuticals, Lundbeck, NIH/UCSD, EISAI, and Genetech Inc. Dr. Barbara Bendlin has received precursor and compounds from Avid Radiopharmaceuticals. Dr. Henrik Zetterberg has served at scientific advisory boards for Denali, Roche Diagnostics, Wave, Samumed, 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. Dr. Kaj Blennow has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, Biogen, Julius Clinical, Lilly, MagQu, Novartis, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program.

REFERENCES

1. Blennow K, Shaw LM, Stomrud E, et al. Predicting clinical decline and conversion to Alzheimer's disease or dementia using novel Elecsys Aβ(1-42), pTau and tTau CSF immunoassays. *Sci Rep*. 2019;9:1-11.
2. Price J, Morris JC. Tangles and plaques in nondemented aging and "preclinical" Alzheimer's disease. *Ann Neurol*. 1999;45:358-368.
3. Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol*. 1991;82:239-259.
4. Blennow K, Zetterberg H. The past and the future of Alzheimer's Disease fluid biomarkers. In: Perry G, Avila J, Tabaton M, Zhu X, editors. *J Alzheimers Dis*. 2018;62:1125-1140.
5. Fleisher AS, Chen K, Quiroz YT, et al. Florbetapir PET analysis of amyloid-β deposition in the presenilin 1 E280A autosomal dominant Alzheimer's disease kindred: a cross-sectional study. *Lancet Neurol*. 2012;11:1057-1065.
6. Jack CR, Bennett DA, Blennow K, et al. NIA-AA Research Framework: toward a biological definition of Alzheimer's disease. *Alzheimers Dement*. 2018;14:535-562.
7. Donohue MC, Sperling RA, Petersen R, Sun C-K, Weiner MW, Aisen PS, for the Alzheimer's Disease Neuroimaging Initiativeet al. Association between elevated brain amyloid and subsequent cognitive decline among cognitively normal persons. *JAMA*. 2017;317:2305-2316.
8. Blennow K, Zetterberg H. Biomarkers for Alzheimer's disease: current status and prospects for the future. *J Intern Med*. 2018;284:643-663.
9. Bittner T, Zetterberg H, Teunissen CE, et al. Technical performance of a novel, fully automated electrochemiluminescence immunoassay for the quantitation of β-amyloid (1-42) in human cerebrospinal fluid. *Alzheimers Dement*. 2016;12:517-526.
10. Hansson O, Seibyl J, Stomrud E, et al. CSF biomarkers of Alzheimer's disease concord with amyloid-β PET and predict clinical progression: a study of fully automated immunoassays in BioFINDER and ADNI cohorts. *Alzheimers Dement*. 2018;14:1470-1481.
11. Lifke V, Kollmorgen G, Manuilova E, et al. Elecsys® Total-Tau and Phospho-Tau (181P) CSF assays: analytical performance of the novel, fully automated immunoassays for quantification of tau proteins in human cerebrospinal fluid. *Clin Biochem*. 2019;72:30-38.
12. Milà-Alomà M, Salvadó G, Gispert JD, et al. Amyloid beta, tau, synaptic, neurodegeneration, and glial biomarkers in the preclinical stage of the Alzheimer's *continuum*. *Alzheimers Dement*. 2020. alz.12131.
13. Johnson SC, Koscik RL, Jonaitis EM, et al. The Wisconsin Registry for Alzheimer's Prevention: a review of findings and current directions. *Alzheimers Dement Diagn Assess Dis Monit*. 2018;10:130-142.
14. Albert MS, DeKosky ST, Dickson D, et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement*. 2011;7:270-279.
15. McKhann GM, Knopman DS, Chertkow H, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the

National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement*. 2011;7:263-269.
16. Johnson SC, Christian BT, Okonkwo OC, et al. Amyloid burden and neural function in people at risk for Alzheimer's Disease. *Neurobiol Aging*. 2014;35:576-584.
17. Hildon J. The area under the ROC curve and its competitors. *Med Decis Making*. 1991;11:95-101.
18. Kemppainen NM, Aalto S, Wilson IA, et al. PET amyloid ligand [11C]PIB uptake is increased in mild cognitive impairment. *Neurology*. 2007;68:1603-1606.
19. Jonaitis EM, Koscik RL, Clark LR, et al. Measuring longitudinal cognition: individual tests versus composites. *Alzheimers Dement Diagn Assess Dis Monit*. 2019;11:74-84.
20. Donohue MC, Sperling RA, Salmon DP, et al. Australian Imaging, Biomarkers, and Lifestyle Flagship Study of Ageing, Alzheimer's Disease Neuroimaging Initiative, Alzheimer's Disease Cooperative Study. The preclinical Alzheimer cognitive composite: measuring amyloid-related decline. *JAMA Neurol*. 2014;71:961-970.
21. Monsell SE, Dodge HH, Zhou X-H, et al. Neuropsychology Work Group Advisory to the Clinical Task Force. Results From the NACC Uniform Data Set Neuropsychological Battery Crosswalk Study. *Alzheimer Dis Assoc Disord*. 2016;30:134-139.
22. R Core Team. *R: A Language and Environment for Statistical Computing [Internet]*. Vienna, Austria: R Foundation for Statistical Computing; 2019. Available from: http://www.r-project.org
23. Lenth R, emmeans: Estimated marginal means, aka Least-Squares Means. [Internet]. 2019. Available from: https://CRAN.R-project.org/package=emmeans
24. Schindler SE, Gray JD, Gordon BA, et al. Cerebrospinal fluid biomarkers measured by Elecsys assays compared to amyloid imaging. *Alzheimers Dement*. 2018;14:1460-1469.
25. Cohen J, Cohen P, West SG, Aiken LS. *Applied Multiple Regression/Correlation Analyses for the Behavioral Sciencies*. 3rd ed. Mahwah, NJ US: Lawrence Erlbaum Associates, Inc; 2003.
26. Katzman R, Terry R, DeTeresa R, et al. Clinical, pathological, and neurochemical changes in dementia: a subgroup with preserved mental status and numerous neocortical plaques. *Ann Neurol*. 1988;23:138-144.
27. Perez-Nievas BG, Stein TD, Tai H-C, et al. Dissecting phenotypic traits linked to human resilience to Alzheimer's pathology. *Brain*. 2013;136:2510-2526.
28. Abner EL, Kryscio RJ, Schmitt FA, et al. Outcomes after diagnosis of mild cognitive impairment in a large autopsy series: outcomes of MCI. *Ann Neurol*. 2017;81:549-559.
29. Beach TG, Monsell SE, Phillips LE, Kukull W. Accuracy of the clinical diagnosis of Alzheimer disease at National Institute on Aging Alzheimer Disease Centers, 2005-2010. *J Neuropathol Exp Neurol*. 2012;71:266-273.
30. Betthauser TJ, Koscik RL, Jonaitis EM, et al. Amyloid and tau imaging biomarkers explain cognitive decline from late middle-age. *Brain*. 2020;143:320-335.
31. Merluzzi AP, Vogt NM, Norton D, et al. Differential effects of neurodegeneration biomarkers on subclinical cognitive decline. *Alzheimers Dement Transl Res Clin Interv*. 2019;5:129-138.
32. Blennow K, Mattsson N, Schöll M, Hansson O, Zetterberg H. Amyloid biomarkers in Alzheimer's disease. *Trends Pharmacol Sci*. 2015;36:297-309.
33. Lewczuk P, Riederer P, O'Bryant SE, et al. Cerebrospinal fluid and blood biomarkers for neurodegenerative dementias: an update of the Consensus of the Task Force on Biological Markers in Psychiatry of the World Federation of Societies of Biological Psychiatry. *World J Biol Psychiatry*. 2018;19:244-328.
34. Bacioglu M, Maia LF, Preische O, et al. Neurofilament Light chain in blood and CSF as marker of disease progression in mouse models and in neurodegenerative diseases. *Neuron*. 2016;91:56-66.
35. Shahim P, Zetterberg H, Tegner Y, Blennow K. Serum neurofilament light as a biomarker for mild traumatic brain injury in contact sports. *Neurology*. 2017;88:1788-1794.
36. Zetterberg H, Skillbäck T, Mattsson N, et al. for the Alzheimer's Disease Neuroimaging Initiative. Association of cerebrospinal fluid neurofilament light concentration with Alzheimer disease progression. *JAMA Neurol*. 2016;73:60.
37. Allison SL, Koscik RL, Cary RP, et al. Comparison of different MRI-based morphometric estimates for defining neurodegeneration across the Alzheimer's disease continuum. *Neuroimage Clin*. 2019;23:101895.
38. Merluzzi AP, Carlsson CM, Johnson SC, et al. Neurodegeneration, synaptic dysfunction, and gliosis are phenotypic of Alzheimer dementia. *Neurology*. 2018;91:e436-e443.
39. Parnetti L, Gaetani L, Eusebi P, et al. CSF and blood biomarkers for Parkinson's disease. *Lancet Neurol*. 2019;18:573-586.
40. Heneka MT, Carson MJ, Khoury JE, et al. Neuroinflammation in Alzheimer's disease. *Lancet Neurol*. 2015;14:388-405.
41. Molinuevo JL, Ayton S, Batrla R, et al. Current state of Alzheimer's fluid biomarkers. *Acta Neuropathol*. 2018;136:821-853.
42. Lai KSP, Liu CS, Rau A, et al. Peripheral inflammatory markers in Alzheimer's disease: a systematic review and meta-analysis of 175 studies. *J Neurol Neurosurg Psychiatry*. 2017;88:876-882.
43. Zetterberg H, Bendlin BB. Biomarkers for Alzheimer's disease-preparing for a new era of disease-modifying therapies. *Mol Psychiatry*. 2020.
44. Janelidze S, Mattsson N, Stomrud E, et al. CSF biomarkers of neuroinflammation and cerebrovascular dysfunction in early Alzheimer disease. *Neurology*. 2018;91:e867-77.
45. Palmqvist S, Mattsson N, Hansson O, for the Alzheimer's Disease Neuroimaging Initiative. Cerebrospinal fluid analysis detects cerebral amyloid-β accumulation earlier than positron emission tomography. *Brain*. 2016;139:1226-1236.
46. Buchhave P, Lennart M, Zetterberg H, Wallin A, Blennow K, Hansson O. Cerebrospinal fluid levels of β-Amyloid 1-42, but not of Tau, are fully changed already 5 to 10 years before the onset of Alzheimer dementia. *Arch Gen Psychiatry*. 2012;69:98-106.
47. Suárez-Calvet M, Kleinberger G, Araque Caballero MÁ, et al. sTREM2 cerebrospinal fluid levels are a potential biomarker for microglia activity in early-stage Alzheimer's disease and associate with neuronal injury markers. *EMBO Mol Med*. 2016;8:466-476.
48. Hansson O, Mikulskis A, Fagan AM, et al. The impact of preanalytical variables on measuring cerebrospinal fluid biomarkers for Alzheimer's disease diagnosis: a review. *Alzheimers Dement*. 2018;14:1313-1333.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Van Hulle C, Jonaitis EM, Betthauser TJ, et al. An examination of a novel multipanel of CSF biomarkers in the Alzheimer's disease clinical and pathological continuum. *Alzheimer's Dement*. 2021;17:431–445. https://doi.org/10.1002/alz.12204