RESEARCH ARTICLE

Differential diagnostic performance of a panel of plasma biomarkers for different types of dementia

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Abstract

Introduction: We explored what combination of blood-based biomarkers (amyloid beta [Aβ]1-42/1-40, phosphorylated tau [p-tau]181, neurofilament light [NfL], glial fibrillary acidic protein [GFAP]) differentiates Alzheimer’s disease (AD) dementia, frontotemporal dementia (FTD), and dementia with Lewy bodies (DLB).

Methods: We measured the biomarkers with Simoa in two separate cohorts (n = 160 and n = 152). In one cohort, Aβ1-42/1-40 was also measured with mass spectrometry (MS). We assessed the differential diagnostic value of the markers, by logistic regression with Wald’s backward selection.

Results: MS and Simoa Aβ1-42/1-40 similarly differentiated AD from controls. The Simoa panel that optimally differentiated AD from FTD consisted of NfL and p-tau181 (area under the curve [AUC] = 0.94; cohort 1) or NfL, GFAP, and p-tau181 (AUC = 0.90; cohort 2). For AD from DLB, the panel consisted of NfL, p-tau181, and GFAP (AUC = 0.88; cohort 1), and only p-tau181 (AUC = 0.81; cohort 2).

Discussion: A combination of plasma p-tau181, NfL, and GFAP, but not Aβ1-42/1-40, might be useful to discriminate AD, FTD, and DLB.

KEYWORDS
amyloid beta, blood biomarker, glial fibrillary acidic protein, neurofilament light, phosphorylated tau

1 BACKGROUND

Dementia is one of the major causes of disability and dependency among older people and affects ≈50 million people worldwide.1 Alzheimer’s disease (AD) is the most common form of dementia and represents ≈70% of cases,2 followed by frontotemporal dementia (FTD) and dementia with Lewy bodies (DLB). To support the clinical AD diagnosis, amyloid beta (Aβ) and phosphorylated tau (p-tau) are currently measured in the cerebrospinal fluid (CSF) or visualized on positron emission tomography (PET) scans.2 Efforts are ongoing to translate these markers into blood-based biomarkers, with promising results.3 There are no blood-based biomarkers available for specifically the FTD and DLB pathologies, but AD-specific biomarkers or more general neurodegenerative and astrocyte activation biomarkers might be useful to differentiate patients with AD dementia from patients with other dementias.4 Being able to measure different aspects of neurodegenerative dementias comprehensively in a minimally invasive and low-cost approach, could contribute to accurate differential diagnosis in an early stage of the diagnostic process. This could accelerate drug development and help with timely treatment and patient management.

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Plasma Aβ42/1-40 and p-tau181 can be used to discriminate amyloid PET positive and negative individuals across the clinical AD continuum.5–11 Furthermore, elevated plasma p-tau181 levels could accurately differentiate patients with AD from FTD.12–19 Glial fibrillary acidic protein (GFAP) is a major constituent of reactive astrocytes, and reflects neuronal injury and immune-related processes.20 Combined analysis of plasma GFAP with Aβ42/1-40 improved the prediction of amyloid PET status.6,7 Furthermore, blood-based GFAP was increased in AD, DLB, and some variants of FTD compared to non-demented controls, suggesting use as a cross-dementia biomarker.21,22 Neurofilament light (NfL) is a neurological damage biomarker, with especially high levels in FTD and to a lesser extent in AD, suggesting NfL could contribute to the differential diagnosis between AD and FTD.23

A new Simoa multiplex assay was developed that measures Aβ42, Aβ40, NfL, and GFAP simultaneously with high sensitivity and selectivity.25 It may be interesting and informative to add p-tau181 to this multiplex. How such a panel of combined markers could improve differential diagnoses, however, remains to be established. Furthermore, up to now, it seems that the Aβ42/1-40 ratio, alone or as a composite with amyloid beta precursor protein (APP669-711), reaches a higher accuracy for AD when measured with immunoprecipitation mass spectrometry (IPMS) than immune assay based methods such as Simoa.8–11,26 It remains to be established if a multiplex panel either solely based on Simoa, or in combination with an IPMS Aβ42/1-40 method, would optimally provide a differential diagnosis of AD, DLB, and FTD.

The main goal of this study was to explore the differential diagnostic value of combinations of Aβ42/1-40 ratio, measured by Simoa or IPMS, GFAP, NfL, and p-tau181.

2 | METHODS

2.1 | Clinical samples

Cohort 1 consisted of a selection of 160 participants from the Amsterdam Dementia Cohort27 based on equal diagnostic group size, availability of CSF biomarker data, and availability of ethylenediaminetetraacetic acid (EDTA) plasma samples in the Amsterdam Dementia Biobank. Forty participants were controls (21 with psychiatric symptoms and 19 with subjective cognitive decline), 40 had AD dementia, 40 had FTD (11 with FTD with motor neuron disease [FTD-motor], 29 with FTD with primary progressive aphasia [FTD-PPA]), and 40 had DLB. Cohort 2 consisted of 152 participants, also from the Amsterdam Dementia Cohort, based on equal diagnostic group sizes and availability of EDTA plasma samples in the Amsterdam Dementia Biobank. Forty participants were controls (all with psychiatric symptoms and 38 had AD dementia, 38 had FTD (six with FTD-motor, 32 with FTD-PPA), and 38 had DLB. All visited the Alzheimer Center Amsterdam for extensive dementia screening, consisting of neurological, physical, and neuropsychological evaluation; electroencephalography; brain magnetic resonance imaging; and CSF AD biomarker analysis.27,28 Diagnoses were made upon multi-disciplinary consensus based on applicable clinical criteria.2,29–32 All patients with AD dementia were CSF amyloid positive, and all controls were CSF amyloid negative.

Written informed consent to use medical data and biomaterials for research purposes was in place. The study was approved by the VU University medical center ethical committee, and in accordance with the Declaration of Helsinki.

2.2 | CSF Aβ42 measurements

CSF Aβ42 concentrations were available for all samples of cohort 1 and for n = 148 (97%) of cohort 2. CSF AD biomarkers of 120 and 140 participants were measured with Innotest enzyme-linked immunosorbent assays and of 40 and 8 participants with Elescsys, for cohort 1 and cohort 2, respectively. Innotest Aβ42 concentrations were corrected for the drift in biomarker concentrations that occurred over the years.33 The drift-corrected cut-off for Innotest for CSF amyloid positivity was 813 pg/mL.33 The cut-off for Elescsys was 1000 pg/mL.34 To determine the correlation with the plasma markers, Elescsys results were transformed to the predicted Innotest concentration using the formula Innotest Aβ42 = (Elescsys Aβ42 + 365)/1.87.34

2.3 | Plasma biomarker measurements

K2EDTA-plasma samples were obtained through venipuncture. After 10-minute centrifugation at 1,800 x g within 2 hours, plasma was
aliquoted in 0.5 mL aliquots in polypropylene tubes and stored at −80°C.

2.3.1 Simoa measurements

Samples were thawed at room temperature and centrifuged at 10,000 x g for 10 minutes. For cohort 1, samples were measured using a pre-commercial Neurology 4-plex E kit (Quanterix) that measures Aβ1-42, Aβ1-40, GFAP, and NfL simultaneously. This Neurology 4-plex E was developed in collaboration between Amsterdam University Medical Centers, and biotechnology companies ADx Neurosciences and Quanterix. For cohort 2, the commercial Neurology 4-plex E kit (Quanterix) was used, which gave different absolute values than the pre-commercial assay. In the next freeze–thaw cycle, p-tau181 was measured in 149 of the 160 participant samples of cohort 1 and in all samples of cohort 2, using the p-Tau-181 V2 Advantage kit (Quanterix; same kit batch for both cohorts). All measurements were performed on the Simoa HDx analyzer, according to manufacturer’s instructions.

2.3.2 IPMS

Aβ1-42, Aβ1-40, and APP669-711 were measured in a second freeze–thaw aliquot of the samples of cohort 1 with matrix-assisted laser desorption/ionization–linear time-of-flight mass spectrometer (AXIMA Assurance, Shimadzu) after two consecutive IP steps with Dynabeads M-270 Epoxy used as beads and mouse monoclonal anti-Aβ antibodies 6E10 and 4G8 to coat the beads. The composite marker was based on the average of the Z score of Aβ1-40/1-42 ratio and APP669-711/Aβ1-42 ratio (details in the supporting information). Centre Hospitalier Universitaire (CHU) of Montpellier performed the measurements and modified the normalization from the original method, and followed their own quality control. One APP669-711 measurement failed; therefore, the composite is available for 159 of the 160 participants.

2.4 Statistics

The IPMS Aβ1-42/1-40 ratio, NfL, GFAP, and p-tau181 were right skewed, thus natural log transformed. In cohort 1, one outlier IPMS Aβ1-42/1-40 ratio in the DLB group of 34x the mean was excluded, so was one CSF Aβ1-42 concentration in the FTD group of 4x the mean (note: only excluded in analyses with continuous data). In cohort 1, one outlier NfL concentration in the FTD group of 15x the mean (351 pg/mL) and a GFAP outlier in the AD group of 6x the mean (613 pg/mL) were excluded. We used analysis of variance with Bonferroni correction to assess biomarker differences between the diagnostic groups. Pearson correlations were calculated for the Aβ1-42/1-40 ratios measured with IPMS and 4-plex, and the difference in strength between two correlations was tested using methods described in Diedenhofen and Musch. Receiver operating characteristic (ROC) analyses determined the differentiation accuracy of the plasma markers. Areas under the curve (AUCs) were compared with the DeLong test. Sensitivity and specificity were determined at Youden indices. We selected biomarker combinations using the automated backward elimination logistic regression procedure based on the Wald statistic. As a sensitivity analysis, we ran the same models including the other Aβ1-42/1-40 ratio or IPMS composite score, which did not change the results (data not shown). Statistical analyses were performed using SPSS (version 26) and R (version 3.6.1, packages Cocor, pROC).

3 RESULTS

3.1 Patient characteristics

Characteristics and fluid biomarker concentrations of the cohorts are shown in Table 1. In cohort 1, patients with FTD and DLB were older than the controls and patients with AD. In cohort 2, all four groups were of comparable age, but there were more males in the DLB group than in the other diagnostic groups. For both cohorts, Mini-Mental State Examination scores of patients with FTD and DLB were between those of controls and patients with AD. In both cohorts, NfL (r = 0.35–0.37, both: P < .001) and GFAP (r = 0.27–0.39, both: P < .001) correlated with age, whereas p-tau181 correlated with age only in cohort 2 (r = 0.22, P = .007; cohort 1: r = 0.08, P = .34) and 4-plex Aβ1-42/1-40 correlated with age only in cohort 1 (r = –0.20, P = .01; cohort 2 –0.11, P = .18). The IPMS Aβ1-42/1-40 ratio (r = –0.08, P = .31) and IPMS composite (r = –0.04, P = .63) did not correlate with age. There were no differences in biomarker concentrations between males and females within the diagnostic groups in either of the cohorts.

3.2 Comparison of Aβ1-42/1-40 ratio between the platforms: 4-plex and IPMS

First, we assessed in cohort 1 how the Simoa and IPMS platforms for Aβ1-42/1-40 measurement compare. The Aβ1-42/1-40 ratio measured with 4-plex and with IPMS were only moderately correlated (r = 0.34, P < .001, n = 159; Figure 1A). The 4-plex Aβ1-42/1-40 ratio correlated with similar strength with CSF Aβ1-42 (r = 0.34, P < .001, n = 159; Figure 1B) as the IPMS plasma Aβ1-42/1-40 ratio (r = 0.46, P < .001, n = 158, Δr = 0.12, P = .14; Figure 1C). Assessing differences between CSF amyloid negative controls and CSF amyloid positive patients with AD, to decide which assay should be selected for our further analyses, we observed that with the 4-plex, median Aβ1-42/1-40 ratio was decreased 16% in patients with AD (median 0.12 [interquartile range (IQR): 0.11–0.13] pg/mL) compared to controls (0.14 [0.13–0.16] pg/mL; P < .001; Table 1; Figure 2A), and with the IPMS Aβ1-42/1-40 ratio this decrease was 17% in patients with AD (0.037 [0.035–0.041] pg/mL) compared to controls (0.044 [0.042–0.048] pg/mL, P < .001; Table 1; Figure 2B). The 4-plex Aβ1-42/1-40 ratio differentiated controls and patients with AD with AUC = 0.80 (95% confidence interval [CI]: 0.70–0.90, P < .001; Figure 2C) and a sensitivity of 0.90 and a specificity of 0.65. The IPMS
## Cohort characteristics

|                  | Cohort 1 |               | Cohort 2 |               |
|------------------|----------|---------------|----------|---------------|
|                  | Control  = 40 | ADn  = 40 | FTDn  = 40 | DLBLn  = 40 | Totaln  = 160 | Control  = 38 | ADn  = 38 | FTDn  = 38 | DLBLn  = 38 | Totaln  = 152 |
| **Age**          | 56 (53–59) | 58 (55–59) | 64 (61–70) | 70 (62–74) | 59 (56–66) | 63 (59–66) | 63 (59–67) | 63 (59–67) | 67 (64–69) | 64 (60–68) |
| **Female sex (%)** | 20 (50%) | 20 (50%) | 22 (55%) | 12 (30%) | 74 (46%) | 20 (53%) | 20 (53%) | 20 (53%) | 3 (8%) | 63 (41%) |
| **Education**    | 5 (4–6) | 5 (4–6) | 5 (4–6) | 5 (4–6) | 5 (4–6) | 6 (5–6) | 5 (5–6) | 5 (4–6) | 5 (4–6) | 5 (4–6) |
| **MMSE**         | 28 (25–29) | 18 (13–22) | 25 (20–27) | 23 (17–26) | 23 (18–27) | 29 (28–29) | 20 (18–23) | 24 (18–27) | 23 (21–26) | 24 (20–28) |
| **CSF Aβ42 (pg/mL)** | 1075 (962–1182) | 576 (495–652) | 950 (739–1159) | 724 (615–991) | 818 (616–1094) | 1092 (922–1271) | 602 (512–692) | 942 (761–1270) | 839 (648–1094) | 856 (648–1120) |
| **CSF amyloid positive (%)** | 0 (0%) | 40 (100%) | 10 (25%) | 24 (60%) | 74 (46%) | 0 (0%) | 38 (100%) | 10 (29%) | 18 (47%) | 66 (45%) |
| **Mass spectrometry** |         |               |         |               | N.A.      | N.A.      | N.A.      | N.A.      | N.A.      | N.A.      |
| Aβ42 ratio*      | 0.044 (0.042–0.048) | 0.037 (0.035–0.041) | 0.041 (0.038–0.045) | 0.043 (0.038–0.044) | 0.042 (0.037–0.045) | N.A. | N.A. | N.A. | N.A. | N.A. |
| Aβ42 (pg/mL)*    | 0.49 (0.42–0.53) | 0.41 (0.36–0.45) | 0.46 (0.42–0.52) | 0.48 (0.44–0.56) | 0.46 (0.42–0.52) | N.A. | N.A. | N.A. | N.A. | N.A. |
| Aβ40 (pg/mL)*    | 10.8 (9.8–11.8) | 10.5 (9.5–12.1) | 11.5 (10.3–12.5) | 11.7 (10.4–13.1) | 11.1 (9.9–12.5) | N.A. | N.A. | N.A. | N.A. | N.A. |
| APP 699-711 (pg/mL)* | 0.46 (0.40–0.50) | 0.40 (0.37–0.50) | 0.42 (0.36–0.48) | 0.45 (0.38–0.53) | 0.43 (0.38–0.48) | N.A. | N.A. | N.A. | N.A. | N.A. |
| **Simoa**        |         |               |         |               | N.A.      | N.A.      | N.A.      | N.A.      | N.A.      | N.A.      |
| Aβ42 ratio*      | 0.14 (0.13–0.16) | 0.12 (0.11–0.13) | 0.13 (0.11–0.14) | 0.13 (0.11–0.14) | 0.13 (0.12–0.14) | 0.06 (0.05–0.07) | 0.05 (0.05–0.06) | 0.05 (0.05–0.06) | 0.05 (0.05–0.06) | 0.05 (0.05–0.06) |
| Aβ42 (pg/mL)*    | 12.7 (11.1–14.5) | 10.9 (9.0–11.7) | 11.6 (9.7–13.1) | 11.3 (10.6–13.1) | 11.5 (9.9–13.1) | 7.22 (6.48–7.73) | 6.16 (5.47–6.73) | 7.33 (6.47–8.34) | 6.83 (6.25–8.20) | 6.81 (6.10–7.74) |
| Aβ40 (pg/mL)*    | 88.4 (82.9–97.0) | 90.7 (78.0–97.3) | 93.3 (83.4–103.6) | 90.5 (85.5–102.9) | 90.4 (82.6–100.5) | 115 (105–137) | 119 (102–140) | 136 (117–154) | 125 (114–147) | 124 (109–145) |
| NfL (pg/mL)*     | 17.0 (13.4–23.6) | 31.3 (25.2–40.1) | 45.5 (35.3–86.5) | 30.9 (22.1–53.3) | 30.6 (20.8–44.6) | 110 (8.5–15.3) | 18.8 (14.0–25.7) | 28.7 (21.4–52.0) | 15.5 (11.7–20.2) | 17.6 (12.0–26.8) |
| GFAP (pg/mL)*    | 534 (342–693) | 1580 (1091–1797) | 943 (610–1231) | 1047 (611–1580) | 962 (563–1487) | 666 (471–85.9) | 119 (99.4–178) | 91.0 (54.2–128) | 106 (65.6–142) | 96.4 (59.0–129) |
| P-tau181         | 1.18 (0.92–1.39) | 2.94 (2.04–3.99) | 1.35 (0.94–2.05) | 1.81 (1.09–2.65) | 1.61 (1.03–2.59) | 1.24 (1.09–1.64) | 2.60 (2.01–3.03) | 1.65 (1.09–2.71) | 1.55 (1.07–2.04) | 1.68 (1.20–2.55) |

Notes: Values shown as median (interquartile range). Aβ42, Aβ40, NfL, and GFAP were measured with the 4-plex pre-commercial assay in cohort 1, and with the 4-plex commercial assay in cohort 2. These two assays give different absolute values. CSF Aβ42 was missing for 4 FTD patients of cohort 2. Abbreviations: Aβ, amyloid beta; AD, Alzheimer’s disease; CSF, cerebrospinal fluid; DLBL, dementia with Lewy bodies; FTD, frontotemporal dementia; GFAP, glial fibrillary acidic protein; NfL, neurofilament light; p-tau181, phosphorylated tau 181.

* indicates a statistically significant difference between groups (P < .05) with Control in post hoc pairwise comparisons.

**P < .05 versus AD.**
FIGURE 1  Correlations between amyloid plasma markers and CSF markers. A, Pearson correlation between the 4-plex and IPMS A$_{β}$1-42/1-40 ratio. B, Pearson correlation between the 4-plex A$_{β}$1-42/1-40 ratio and CSF A$_{β}$1-42. C, Pearson correlation between the IPMS A$_{β}$1-42/1-40 ratio and CSF A$_{β}$1-42. Shape indicates clinical diagnosis, color indicates CSF amyloid status. A$_{β}$, amyloid beta; AD, Alzheimer’s disease; CSF, cerebrospinal fluid; DLB, dementia with Lewy bodies; FTD, frontotemporal dementia; IPMS, immunoprecipitation mass spectrometry.

FIGURE 2  Differentiation accuracy of amyloid markers in CSF amyloid negative controls and CSF amyloid positive AD patients. A, The A$_{β}$1-42/1-40 ratio measured with the Simoa 4-plex. B, The A$_{β}$1-42/1-40 ratio measured with IPMS. C, ROC curves of the two plasma amyloid marker for differentiation between control and AD (in blue: Simoa; in green: IPMS). There was no difference in accuracy between the two ratios. A$_{β}$, amyloid beta; AD, Alzheimer’s disease; AUC, area under the curve; CSF, cerebrospinal fluid; IPMS, immunoprecipitation mass spectrometry; ROC, receiver operating characteristic.

A$_{β}$1-42/1-40 ratio had a similar AUC of 0.82 (95% CI: 0.72–0.92, P < .001; Figure 2C), but a sensitivity of 0.78 and a specificity of 0.83. The performance of the 4-plex and IPMS A$_{β}$1-42/1-40 ratio did not differ in terms of AUC (ΔAUC = 0.02, DeLong P = .83) and was similar to the IPMS composite (ΔAUC = 0.01, DeLong P = .83, Figure S1 in supporting information). Given the similarity in AUCs and the value of the Simoa 4-plex as a multiplex assay that measures several independent markers simultaneously and requires less volume than IPMS, we continued the analyses for differential diagnostic value with the 4-plex A$_{β}$1-42/1-40 ratio as amyloid marker.
FIGURE 3 4-Plex plasma (A) Aβ1-42/1-40, (B) NfL, (C) GFAP, and (D) Single-plex p-tau181 in cohort 1. Aβ, amyloid beta; AD, Alzheimer’s disease; CSF, cerebrospinal fluid; DLB, dementia with Lewy bodies; FTD, frontotemporal dementia; GFAP, glial fibrillary acidic protein; NfL, neurofilament light; p-tau, phosphorylated tau. ***P < .001, **P < .01, *P < .05

3.3 | Differential diagnosis

In addition to the Aβ1-42/1-40 ratio, we measured NfL, GFAP, and p-tau181. In cohort 1, the Aβ1-42/1-40 ratio was decreased not only in patients with AD (P < .001), but also in patients with FTD (P = .010) and DLB (P = .002) compared to controls. The Aβ1-42/1-40 ratio did not differ among the AD, FTD, or DLB group in this cohort (P > .48). NfL concentrations were increased in all diagnostic groups compared to controls (all P < .001), and were highest in patients with FTD. GFAP concentrations were increased in all diagnostic groups compared to controls (all P < .001), and were highest in patients with AD. P-tau181 concentrations were increased in patients with AD compared to the other dementia groups and controls (P ≤ .001; Figure 3, Table 1).

When measured in cohort 2, the Aβ1-42/1-40 ratio was decreased only in patients with AD (P = .002) compared to controls. Similar to cohort 1, the Aβ1-42/1-40 ratio did not differ among the AD, FTD, or DLB groups (all: P = 1.000). NfL concentrations were increased in all diagnostic groups compared to controls (all: P < .015). NfL concentrations were again highest in patients with FTD. Contrary to cohort 1, GFAP was increased in patients with AD (P < .001) and in patients with DLB (P = .042) compared to controls, but not in patients with FTD compared to controls (P = .060). GFAP was also increased in patients with AD compared to patients with FTD (P = .011) and patients with DLB (P = .017). In agreement with findings in cohort 1, p-tau181 concentrations were increased in AD compared to the other dementia groups and compared to controls (all: P < .007; Figure S2 in supporting information; Table 1).

3.4 | Classification of patients by the biomarkers

The AUCs of the ROC curve for differentiation between patients with AD and FTD and between patients with AD and DLB are detailed in Table 2. To define the panels, Wald’s backward elimination logistic regression analysis was performed among all biomarkers (4-plex Aβ1-42/1-40, NfL, GFAP, and single-plex p-tau181).

3.5 | AD versus FTD

In cohort 1, Aβ1-42/1-40 had the lowest AUC (AUC = 0.62, 95% CI: 0.49–0.75, P = .065) and p-tau181 had the highest AUC (AUC = 0.85, 95% CI: 0.75–0.94, P < .001, Figure 4A) for differentiating patients with AD from patients FTD. The combination of NfL and p-tau181 was selected as the optimal differential diagnostic panel for AD versus FTD with an AUC of 0.94 (95% CI: 0.87–1.00, P < .001, sens: 0.87, spec: 1.00).
### Table 2: Diagnostic accuracy of the 4-plex $A_\beta_{1-42}/1-40$ ratio, NfL, GFAP, p-tau181, and optimal panel

| Cohort 1 | AD vs. FTD | n   | AUC  | 95% CI    | P-value | Sensitivity | Specificity |
|----------|------------|-----|------|-----------|---------|-------------|-------------|
|          | $A_\beta_{1-42}/1-40$ ratio | 40 vs 40 | 0.62 | 0.49–0.75 | .065    | 0.38        | 0.95        |
|          | NfL        | 40 vs 40 | 0.79 | 0.69–0.89 | <.001   | 0.48        | 0.98        |
|          | GFAP       | 40 vs 40 | 0.81 | 0.71–0.90 | <.001   | 0.75        | 0.73        |
|          | P-tau181   | 36 vs 38 | 0.85 | 0.75–0.94 | <.001   | 0.74        | 0.97        |
|          | Panel (NfL, P-tau181) | 36 vs 38 | 0.94 | 0.87–1.00 | <.001   | 0.87        | 1.00        |

| AD vs. DLB | n   | AUC  | 95% CI    | P-value | Sensitivity | Specificity |
|------------|-----|------|-----------|---------|-------------|-------------|
| $A_\beta_{1-42}/1-40$ ratio | 40 vs 40 | 0.60 | 0.47–0.73 | .124    | 0.38        | 0.95        |
| NfL        | 40 vs 40 | 0.50 | 0.37–0.64 | .946    | 0.28        | 0.98        |
| GFAP       | 40 vs 40 | 0.69 | 0.57–0.81 | <.001   | 0.50        | 0.85        |
| P-tau181   | 36 vs 37 | 0.75 | 0.63–0.87 | <.001   | 0.54        | 0.97        |
| Panel (NfL, GFAP, P-tau181) | 36 vs 37 | 0.88 | 0.80–0.96 | <.001   | 0.84        | 0.81        |

| Cohort 2 | AD vs. FTD | n   | AUC  | 95% CI    | P-value | Sensitivity | Specificity |
|----------|------------|-----|------|-----------|---------|-------------|-------------|
|          | $A_\beta_{1-42}/1-40$ ratio | 38 vs 38 | 0.58 | 0.46–0.71 | .205    | 0.63        | 0.55        |
|          | NfL        | 38 vs 37 | 0.78 | 0.68–0.88 | <.001   | 0.89        | 0.55        |
|          | GFAP       | 37 vs 38 | 0.71 | 0.60–0.83 | .001    | 0.45        | 0.95        |
|          | P-tau181   | 38 vs 38 | 0.71 | 0.59–0.83 | .002    | 0.66        | 0.76        |
|          | Panel (NfL, GFAP, P-tau181) | 37 vs 37 | 0.90 | 0.82–0.98 | <.001   | 0.81        | 0.92        |

| AD vs. DLB | n   | AUC  | 95% CI    | P-value | Sensitivity | Specificity |
|------------|-----|------|-----------|---------|-------------|-------------|
| $A_\beta_{1-42}/1-40$ ratio | 38 vs 38 | 0.56 | 0.43–0.69 | .372    | 0.29        | 0.90        |
| NfL        | 38 vs 38 | 0.63 | 0.51–0.76 | .045    | 0.55        | 0.71        |
| GFAP       | 37 vs 38 | 0.65 | 0.52–0.77 | .027    | 0.40        | 0.87        |
| P-tau181   | 38 vs 38 | 0.81 | 0.71–0.91 | <.001   | 0.76        | 0.76        |
| Panel (p-tau181) | 38 vs 37 | 0.81 | 0.71–0.91 | <.001   | 0.76        | 0.76        |

Notes: The panels were selected with backward logistic regression based on Wald’s statistics, among the plasma markers $A_\beta_{1-42}/1-40$, NfL, GFAP, and p-tau181. Sensitivity and specificity are at Youden’s indices. Abbreviations: $A_\beta$, amyloid beta; AD, Alzheimer’s disease; AUC, area under the curve; CI, confidence interval; DLB, dementia with Lewy bodies; FTD, frontotemporal dementia; GFAP, glial fibrillary acidic protein; NfL, neurofilament light; p-tau181, phosphorylated tau 181.

In cohort 2, $A_\beta_{1-42}/1-40$ had the lowest AUC (AUC = 0.58, 95% CI: 0.46–0.71, P = .205), similar to cohort 1, but NfL had the highest AUC (AUC = 0.78, 95% CI: 0.68–0.88, P < .001, Figure 4C) for differentiating patients with AD from those with FTD. The combination of NfL, GFAP, and p-tau181 was selected as the optimal differential diagnostic panel for AD versus FTD with an AUC of 0.88 (95% CI: 0.80–0.96, P < .001, sens: 0.84, spec: 0.81).

### 3.6 AD versus DLB

In cohort 1, NfL had the lowest AUC (AUC = 0.50, 95% CI: 0.37–0.64, P = .946) and p-tau181 had the highest AUC (AUC = 0.75, 95% CI: 0.63–0.87, P < .001, Figure 4B) for differentiating patients with AD from those with DLB. The combination of NfL, GFAP, and p-tau181 was selected as the optimal differential diagnostic panel for AD versus DLB with an AUC of 0.88 (95% CI: 0.80–0.96, P < .001, sens: 0.84, spec: 0.81).

In cohort 2, $A_\beta_{1-42}/1-40$ had the lowest AUC (AUC = 0.56, 95% CI: 0.43–0.69, P = .372) and similar to cohort 1, p-tau181 had the highest AUC (AUC = 0.81, 95% CI: 0.71–0.91, P < .001, Figure 4D) for differentiating patients with AD from those with DLB. P-tau181 alone was selected as the optimal differential diagnostic panel for AD versus DLB with an AUC of 0.81 (95% CI: 0.71–0.91, P < .001, sens: 0.76, spec: 0.76).

### 4 DISCUSSION

We found that the 4-plex and IPMS $A_\beta_{1-42}/1-40$ ratio and IPMS composite performed similarly in differentiating CSF amyloid positive patients with AD and CSF amyloid negative controls. Furthermore, we showed...
FIGURE 4  ROC AUCs for the different biomarkers. Cohort 1: (A) Differentiation between AD and FTD. The panel consists of NfL and p-tau181. (B) Differentiation between AD and DLB. The panel consists of NfL, GFAP, and P-tau181. Cohort 2: (C) Differentiation between AD and FTD. The panel consists of NfL, GFAP, and P-tau181. (D) Differentiation between AD and DLB. The panel consists of p-tau. The panels were corrected for age and sex. Aβ, amyloid beta; AD, Alzheimer’s disease; AUC, area under the curve; CSF, cerebrospinal fluid; DLB, dementia with Lewy bodies, FTD, frontotemporal dementia; GFAP, glial fibrillary acidic protein; NfL, neurofilament light; p-tau181, phosphorylated tau 181; ROC, receiver operating characteristic.

that the plasma Aβ1-42/1-40 ratio, NfL, GFAP, and p-tau181 differ between diagnostic groups. All markers showed a largely similar pattern of differences between the diagnostic groups in both cohort 1 and cohort 2, though the optimal panel for differential diagnosis differed between the cohorts. Our data suggest that, depending on patient characteristics (e.g., age, sex), a plasma biomarker panel for optimal differentiation between AD and FTD, and between AD and DLB, will include plasma p-tau181 but not plasma Aβ1-42/1-40 ratio, and will likely include plasma GFAP and plasma NfL.

We hypothesized that the IPMS would perform better than the 4-plex, because the differentiation accuracy for amyloid positivity in cohorts was higher in IPMS studies (AUC range 0.84–0.89)8–10,39 than in Simoa studies (AUC range 0.73–0.77).7,40 Also, two recent head-to-head studies showed that IPMS Aβ1-42/1-40 outperformed Simoa Aβ1-42/1-40.11,26 The similarity in performance in our study could be due to our cohort selection, which includes two extremes: CSF amyloid positive symptomatic AD patients compared to CSF amyloid negative controls. In a preclinical cohort, the differences in plasma amyloid might be more subtle and better reflected with IPMS. In addition, the IPMS analyses were performed with the same method but by a different research group than described in this recent comparison study.26 Inter-laboratory variability and modifications in assay set-up could be an explanation for the difference in performance. IPMS has the advantage of high assay specificity and no interference of the sample matrix.41 The 4-plex assay uses antibodies that are highly specific for full-length Aβ1-42 and Aβ1-40, just like IPMS.35

We found that p-tau181 had often the highest accuracy in differentiating AD from FTD, and AD from DLB, and was included as a discriminatory biomarker in both panels for both cohorts. Earlier studies by us and others also convincingly showed that p-tau181 is a highly
specific marker for AD pathology. 12–19 That p-tau181 is useful to differentiate patients with AD from patients with DLB has not been reported extensively yet. In line with earlier publications, 23,42–44 we found that NFL is strongly increased in patients with FTD compared to AD, which explains that it was included in the AD versus FTD panel for both cohort 1 and cohort 2. GFAP showed a similar pattern to p-tau181 for differential diagnoses, but with lower or comparable accuracy than p-tau181. Specifically, GFAP was selected in only cohort 1 in the AD versus DLB panel and only in cohort 2 in the AD versus FTD panel. This suggests that GFAP might be of added value to p-tau181 in a differential diagnostic panel, but it depends on the patient characteristics. FTD and DLB patients were older than AD dementia patients in cohort 1 but not in cohort 2, and over the cohorts, we observed relationships of both NFL and GFAP with age.

The observed high accuracy of GFAP as a standalone marker for AD suggests that this marker reflects the process of Aβ aggregates triggering a neuroinflammatory state and promoting glial cell activation. 45 The primary morphological change in astrogliosis is hypertrophy, which is linked to an increase in expression of intermediate filaments such as GFAP. 46 Astrocytes are the most prevalent cell types in the brain, thus even if only a small amount of GFAP is released per cell, this may still be a major response that may result in high GFAP concentrations in blood. In addition, in AD, GFAP was increased in DBL compared to controls in both cohorts, with concentrations between control and AD, 21 which was driven by the CSF amyloid positive patients with DBL. GFAP levels were also increased in FTD patients compared to controls in cohort 1, to a lesser extent than in AD. Of note, we could not reproduce this in our more age-matched cohort 2. A recent study has shown that GFAP concentrations can be increased in symptomatic progranulin (GRN) mutation carriers. 47 We lack the genetic data to verify this in our cohort. The 4-plex Aβ 1-42/1-40 ratio showed a similar pattern of changes as p-tau181 and GFAP, being deviated in patients with AD, though the differentiation accuracy was lower than for p-tau181 and GFAP (AUC > 0.56, P ≤ .372). Few studies have focused on the Aβ 1-42/1-40 ratio in neurodegenerative diseases outside of the AD spectrum thus far. One study measured Aβ 1-42/1-40 with Simoa in controls, patients with mild cognitive impairment (MCI), AD, and across different FTD syndromes but did not find a difference among all groups. 12 Similarly, our results show no differential diagnostic value of Aβ 1-42/1-40 across patients with AD, FTD, or DBL.

In both cohorts, the differentiation accuracy between AD and FTD strongly improved by using biomarker panels compared to single biomarkers, except that based on our cohort 2, p-tau181 was sufficient as a single marker to differentiate between AD and DBL. This is likely the result of the larger number of CSF amyloid negative patients with DBL in cohort 2, which results in smaller overlap in the p-tau181 concentrations of the AD and DBL groups. Every biomarker tested reflects a different aspect of AD pathology or brain damage in general. In the future, these markers should be tested in a larger and more heterogeneous population, for example, to define cut-off values for clinical use. Our exploratory results, however, suggest that an algorithm based on NFL, GFAP, and p-tau as plasma biomarkers could provide an indication of probability per clinical diagnosis. Combined with clinical assessment this could help provide differential diagnoses of AD and other neurodegenerative diseases and support clinical trial inclusion in a minimally invasive and low-cost manner. 48

This study had several limitations. First, our cohorts were relatively small and were included from our memory clinic only and did not reflect a broader population of people with dementia, that is, with older patients with AD. As a result, our AD patients typically have less copathology, which might have influenced our panel selections. It would also be interesting to assess whether our panels are also successful in differentiating among the MCI stages of DBL, FTD, and AD. Second, we used two different batches of the Neurology 4-plex E kit, due to which absolute values were not comparable. Third, due to the cohort characteristics (large difference in age for cohort 1 and large differences in sex in cohort 2) age and sex were not appropriate covariates. Last, because we did not know the apolipoprotein E (APOE) status of all participants, we did not include this as a variable in the regression analyses, while APOE is an important risk factor for AD and confounded blood biomarker results in some studies. 10,49

One of the strengths of our study was the diversity of our cohort, including different non-AD dementias. Also, the Aβ 1-42/1-40 measured with IPMS and immunoassay in the same cohort allowed us to robustly compare their performance. Last, the Simoa Neurology 4-plex E kit is commercially available for immediate use by other groups, with the advantage that Simoa technology offers scalability for large-scale validation and routine implementation.

In summary, the IPMS and 4-plex immunoassay Aβ 1-42/1-40 ratio showed similar performance in indicating amyloidopathy. Different combinations of NFL, GFAP, and p-tau181 were valuable for differential diagnosis of AD, FTD, and DBL. Further research on cut-off values would help implement these markers in clinical practice.

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AUTHOR CONTRIBUTIONS

Design: ET, IV, CT; experiments: JK, AA; data analysis: ET, IV; manuscript writing: ET, IV with assistance of all authors; study supervision: JV, AB, YP, EL, WF, ES, CH, CT.

CONFLICTS OF INTEREST

ET, IV, JK, AA, and YP report no conflicts of interest. JV is an employee of ADx NeuroSciences. AB was an employee of Quanterix Corporation when the study was performed and holds shares in Quanterix. AL has received funding from stichting Dioraphte, Alzheimer Nederland, and ZonMW Memorabel (project #733050509). AL has performed contract research with Axovant, EIP Pharma, and Combinostics. All funding is paid to her institution. WF has performed contract research for Biogen MA Inc, and Boehringer Ingelheim. All funding is paid to her institution. WF has been an invited speaker at Boehringer Ingelheim, Biogen MA Inc, Danone, Eisai, WebMD Neurology (Medscape), Springer Healthcare. All funding is paid to her institution. WF is a consultant to Oxford Health Policy Forum CIC, Roche, and Biogen MA Inc. All funding is paid to her institution. WF participated in advisory boards of Biogen MA Inc and Roche. All funding is paid to her institution. WF was associate editor of Alzheimer, Research & Therapy in 2020/2021. WF is associate editor at Brain. All funding is paid to her institution. ES is an employee and shareholder of ADx NeuroSciences, Gent, Belgium. CH has a collaboration contract with Shimadzu European Innovation Center including a PhD thesis. CT has a collaboration contract with ADx NeuroSciences and Quanterix, performed contract research for or received grants from AC-Immune, Axon Neurosciences, Biogen, Brainstorm Therapeutics, Celgene, EIP Pharma, Eisai, PeopleBio, Roche, Toyama, Vivoryon. CT serves on editorial boards of Medidact Neurologi/Springer, Alzheimer Research and Therapy, Neurology: Neuroimmunology & Neuroinflammation, and is editor of a Neuromethods book for Springer. All funding is paid to her institution.

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