Received: 8 October 2024 | Revised: 28 November 2024 | Accepted: 19 December 2024

DOI: 10.1002/dad2.70073

Alzheimer's & Dementia
Diagnosis, Assessment & Disease Monitoring

RESEARCH ARTICLE

Plasma biomarkers of amyloid, tau, astrogliosis, and axonal injury in a mixed memory clinic cohort

Helena Sophia Gleerup[1] | **Anja Hviid Simonsen**[1] | **Lana Grötschel**[2] | **Mathias Holsey Gramkow**[1] | **Peter Høgh**[3,4] | **Kaj Blennow**[2,5,6,7] | **Henrik Zetterberg**[2,5,8,9,10,11] | **Nicholas Ashton**[2,12,13,14] | **Steen Gregers Hasselbalch**[1,4]

[1]Department of Neurology, Danish Dementia Research Centre, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark

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

[3]Regional Dementia Research Centre, Department of Neurology, Zealand University Hospital, Roskilde, Denmark

[4]Department of Clinical Medicine, Faculty of Health and Medical Science, University of Copenhagen, Copenhagen, Denmark

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

[6]Paris Brain Institute, ICM, Pitié-Salpêtrière Hospital, Sorbonne University, Hôpital Pitié, Paris, France

[7]Neurodegenerative Disorder Research Center, Division of Life Sciences and Medicine, and Department of Neurology, Institute on Aging and Brain Disorders, University of Science and Technology of China and First Affiliated Hospital of USTC, Hefei, P.R. China

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

[9]Institute of Neurology, UK Dementia Research Institute at UCL, London, UK

[10]Hong Kong Center for Neurodegenerative Diseases, Clear Water Bay, Hong Kong, China

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

[12]King's College London, Institute of Psychiatry, Psychology and Neuroscience, Maurice Wohl Clinical Neuroscience Institute, London, UK

[13]NIHR Biomedical Research Centre for Mental Health and Biomedical Research Unit for Dementia at South London and Maudsley NHS Foundation, London, UK

[14]Centre for Age-Related Medicine, Stavanger University Hospital, Stavanger, Norway

Correspondence
Helena Sophia Gleerup, Department of Neurology, Danish Dementia Research Centre, Rigshospitalet, University of Copenhagen, Section 8007, Inge Lehmanns Vej 8, Copenhagen DK-2100, Denmark.
Email: helena.sophia.coley.gleerup@regionh.dk

Web: www.videnscenterfordemens.dk/english

Funding information
Absalon Foundation of 1st May 1978; Alzheimer Drug Discovery Foundation, Grant/Award Number: #201809-2016862; Lundbeck Foundation; Grosserer L. F. Foghts Foundation; Augustinus Foundation; Frimodt-Heineke,; Foundation for Neurological Research; Simons Spies

Abstract

INTRODUCTION: Studies have shown that blood biomarkers can differentiate dementia disorders. However, the diagnosis of dementia still relies primarily on cerebrospinal fluid and imaging modalities. The new disease-modifying treatments call for more widely applicable biomarkers.

METHODS: Plasma samples ($n = 250$) from two mixed memory clinic were included. Participants were divided into amyloid beta positives (Aβ+) and Aβ negatives (Aβ–). Plasma phosphorylated tau (p-tau) 181, p-tau231, Aβ1-42 (Aβ42), Aβ40, Aβ42/Aβ40, glial fibrillary acidic protein (GFAP), and neurofilament light chain (NfL) were measured by single molecule array.

RESULTS: Significant differences were found among cognitively unimpaired, mild cognitive impairment, Alzheimer's disease (AD), and non-AD, and nearly all of the

Foundation; Swedish Research Council, Grant/Award Numbers: #2023-00356, #2022-01018, #2019-02397, #2017-00915, #2022-00732; European Union's Horizon Europe research and innovation programme, Grant/Award Number: 101053962; Swedish State Support for Clinical Research, Grant/Award Number: #ALFGBG-71320; Strategic Fund and the Alzheimer's Association, Grant/Award Numbers: #ADSF-21-831376-C, #ADSF-21-831381-C, #ADSF-21-831377-C, #ADSF-24-1284328-C; Bluefield Project; Cure Alzheimer's Fund; Olav Thon Foundation; Familjen Rönströms Stiftelse; Stiftelsen för Gamla Tjänarinnor; Erling-Persson Family Foundation; Hjärnfonden, Sweden, Grant/Award Numbers: #FO2022-0270, #FO2017-0242, #ALZ2022-0006; European Union's Horizon 2020 research and innovation programme, Grant/Award Number: 860197; European Union Joint Programme – Neurodegenerative Disease Research, Grant/Award Number: JPND2021-00694; National Institute for Health and Care Research University College London Hospitals Biomedical Research Centre; UK Dementia Research Institute, Grant/Award Number: UKDRI-1003; European Union Joint Program for Neurodegenerative Disorders, Grant/Award Number: JPND2019-466-236; Alzheimer's Association 2021 Zenith Award, Grant/Award Number: SG-23-1038904 QC; Kirsten and Freddy Johansen Foundation, Copenhagen; Swedish Alzheimer Foundation, Grant/Award Numbers: #AF-930351, #AF-939721, #AF-968270; Swedish state under the agreement between the Swedish government and the County Councils; ALF-agreement, Grant/Award Numbers: #ALFGBG-715986, #ALFGBG-965240

biomarkers were able to predict amyloid status. When combining p-tau181 and p-tau231 they predicted Aβ positivity with an area under the curve (AUC) of 0.75, and when combining all biomarkers an AUC of 0.86 was found.

DISCUSSION: This study supports previous findings on plasma biomarkers, even when investigated in a typical clinical setting in a consecutive, heterogeneous, mixed memory clinic.

KEYWORDS

Alzheimer's disease, biomarker, dementia, neurodegeneration, plasma

Highlights

- This study investigated seven plasma biomarkers in a mixed memory clinic, regardless of amyloid co-pathology or atypical phenotypes.
- These findings support previous promising results on plasma biomarkers, even when investigated in a heterogeneous population.
- The combination of phosphorylated tau (p-tau)181 and p-231 performed only slightly worse than a panel of multiple biomarkers, aligning with previous studies.
- Plasma biomarkers show potential for future applications in primary care, treatment monitoring, and trial selection.

1 | INTRODUCTION

Precise and timely diagnosis of many dementia disorders is still a challenge. Currently the diagnosis relies on neurological and neuropsychological assessments, imaging modalities (magnetic resonance imaging [MRI], computed tomography [CT], or [^{18}F]fluorodeoxyglucose positron emission tomography PET [FDG-PET]), and cerebrospinal fluid (CSF) tests. These methods are expensive, involve radiation, may have side effects, and often require special training and hospital resources. Furthermore, some of them are specific only to certain dementia disorders, for example Alzheimer's disease (AD).[1] Many of the pathological changes of various dementia disorders begin decades before symptoms evolve and the clinicians see the patients.[2] The latest diagnostic research criteria for AD require biomarkers for diagnosis and staging of the disease,[3] and purely biomarker-driven diagnosis of clinical AD has recently been advocated.[4] Moving from a clinical to a biological diagnosis of AD is partially driven by the recent approval of new disease-modifying treatments.[5] Therefore, new, inexpensive, easily implemented and accessible, non-invasive, and specific biomarkers are required.

Blood (plasma or serum) biomarkers offer one of these widely accessible methods. Several studies have investigated plasma biomarkers in research cohorts and in the most common neurodegenerative disorders, including AD.[6–8] It is well established that many plasma biomarkers are altered in the preclinical and clinical stages of the diseases and correlate with both atrophy and decline in cognition.[9,10] Phosphorylated tau (p-tau) and glial fibrillary acidic protein (GFAP) in plasma have demonstrated amyloid-dependent changes. Studies have shown that p-tau is associated with the density of amyloid beta (Aβ) plaques, and might be better at predicting amyloid status than tau tangles.[11] GFAP has the strongest expression in brain astrocytes and is thought to be linked to a neuroinflammatory response to Aβ pathology, as GFAP has been shown to correlate strongly with Aβ status but not with the number of neurofibrillary tangles in the brain.[6,12] Furthermore, studies have shown that plasma GFAP has a greater association with Aβ pathology than CSF GFAP, which is likely due to instability of the protein in the CSF matrix.[13] Neurofilament light chain (NfL) is a well-established marker of neuroaxonal injury in both CSF and plasma, and depending on the degree of neurodegeneration various amounts of NfL are released into the CSF and plasma.[14]

However, plasma biomarkers are still not used in a broader clinical context. Specialized memory clinics get referrals from primary care or other hospitals to perform diagnostic evaluations of all etiologies of suspected dementia or cognitive dysfunction. Therefore, it is essential for new methods and biomarkers to be tested in a typical mixed-memory clinic population, including patients with presumably mixed pathologies.

In this study, the accuracy of plasma biomarkers to predict Aβ status was challenged and investigated in a typical clinical setting in a consecutive, mixed memory clinic cohort.

RESEARCH IN CONTEXT

1. **Systematic review**: This study investigated the accuracy of seven plasma biomarkers in predicting amyloid status within a typical clinical setting, using a consecutive, mixed memory clinic cohort, regardless of amyloid co-pathology.
2. **Interpretation**: A plethora of studies have shown that blood biomarkers look promising. However, many of these studies are conducted in research cohorts that exclude co-pathology and atypical phenotypes. This study supports previous finding on plasma biomarkers, even when investigated in a real clinical setting in a mixed memory clinic.
3. **Future directions**: With the approval of disease-modifying treatments, it is essential to investigate these biomarkers in real clinical settings. Plasma biomarkers could help in trial selection, design and monitoring, or aid with the identification on who will progress from mild cognitive impairment to dementia. In addition, they could play an important role in primary care as a screening tool.

2 | METHODS

All included patients gave informed consent for their plasma samples and data to be used for research. The study was approved by the Danish Data Protection Agency (VD-2019-105) and the ethical committee of the Capital Region of Denmark (H-19000651). The plasma samples were collected from patients referred for diagnostic evaluation in 2019 at the Copenhagen Memory Clinic, Copenhagen University Hospital, Rigshospitalet, or at the Regional Dementia Research Center, Zealand University Hospital, Roskilde, and later analyzed at the Department of Psychiatry and Neurochemistry, Sahlgrenska Academy, University of Gothenburg.

2.1 | Subjects

A total of 250 subjects were included in the study. They underwent a standard diagnostic evaluation, including medical history, informant-based history, standard blood tests, neurological examinations, cognitive testing including Mini-Mental State Examination (MMSE)[15] and Addenbrooke's Cognitive Examination (ACE),[16] as well as a structural scan (MRI or CT). Based on clinical evaluations, supplementary investigations were administered such as lumbar puncture for AD CSF biomarkers, FDG PET, or full neuropsychological examination. Diagnosis was given at a multidisciplinary consensus conference following international criteria.

All included patients were diagnosed according to the overall clinical evaluation, regardless of, but not blinded to, amyloid co-pathology. All included patients with suspected AD pathology fulfilled the National Institute on Aging–Alzheimer's Association (NIA-AA) criteria for dementia due to AD,[17] or MCI due to AD.[17] Patients with MCI due to other etiologies fulfilled the broad criteria of MCI suggested by the International Working Group on Mild Cognitive Impairment.[18] Patients with dementia with Lewy bodies (DLB) fulfilled the fourth report of the Consortium for Dementia with Lewy Bodies criteria,[19] patients with vascular dementia (VaD) fulfilled the International Society of Vascular Behavioural and Cognitive Disorders (VASCOG) criteria.[20] Patients with mixed dementia (n = 12; AD patients with significant vascular co-pathology determined by symptoms and imaging) fulfilled both the NIA-AA and VASCOG criteria.[17,20] Patients with frontotemporal dementia (FTD) fulfilled the criteria for the behavioral variant,[21] non-fluent aphasia, or the semantic variant.[22] The patients were diagnosed with FTD according to their clinical phenotype and the clinical and supplementary evaluations. Patients with normal pressure hydrocephalus (NPH) fulfilled the international guideline criteria for idiopathic NPH.[23] Patients with alcohol dementia fulfilled the International Classification of Diseases 10th revision (ICD-10) criteria.[24] The cognitively unimpaired (CU) did not fulfill any criteria for dementia or MCI.

The participants were divided into Aβ positives (Aβ+) and Aβ negatives (Aβ−) with a CSF cut-off for p-tau181/Aβ42 locally established at 0.077. The cohort consisted of 46 CU Aβ−, 57 patients with MCI (n = 13 Aβ+, n = 44 Aβ−), 57 patients with AD (all Aβ+), and 90 patients with a non-AD dementia diagnosis (n = 24 Aβ+, n = 66 Aβ−). The non-AD group consisted of patients with alcohol dementia (n = 6; all Aβ−), patients with other non-neurodegenerative causes of dementia (n = 7: n = 1 Aβ+, n = 6 Aβ−), patients with DLB (n = 7: n = 4 Aβ+, n = 3 Aβ−), 12 patients with FTD (n = 2 Aβ+, n = 10 Aβ−), four patients with other specified neurodegenerative disorder (all Aβ−), 10 patients with unknown causes of dementia (n = 1 Aβ+, n = 9 Aβ−), 26 patients with VaD or mixed etiology (n = 14 Aβ+, n = 12 Aβ−), and 18 patients with NPH (n = 2 Aβ+, n = 16 Aβ−). Non-AD Aβ+ patients were classified as having AD as co-pathology if their clinical phenotype was better explained by a non-AD dementia disorder. The seven patients with other non-neurodegenerative causes of dementia, including psychiatric disorders and radiation-induced brain injury damage, while the four patients with other specified neurodegenerative disorders included progressive supranuclear palsy (PSP), primary progressive multiple sclerosis (PPMS), corticobasal degeneration (CBD), and spinocerebellar ataxia type 6 (SCA6).

2.2 | Plasma collection and processing

The plasma samples were collected in ethylenediaminetetraacetic (EDTA)-treated tubes and centrifuged at 2000 g, 4°C, for 10 minutes. After centrifugation, the samples were redistributed in 250 uL aliquots and stored at −80°C.

2.3 | Biomarker assays

Prior to the biomarker analyses, plasma samples were thawed and centrifuged at 4000 g for 10 minutes at room temperature. The plasma biomarker concentrations were analyzed using single molecule array (Simoa) technology on a HD-X analyzer (Quanterix). NfL, GFAP, and Aβ42/Aβ40 were measured using the commercial Neurology 4-Plex E Kit (Lot 502957, Quanterix). p-tau231 and p-tau181 were measured using the University of Gothenburg in-house method as described previously.[7,25] All samples were analyzed as singlicates in one round of experiments using one batch of reagents. Internal quality control samples were added showing mean inter-assay/intra-assay coefficients of variation of NfL 8%/3%, GFAP 15%/3%, Aβ42 6%/5%, Aβ40 6%/6%, p-tau181 8%/7%, and p-tau231 7%/6%.

CSF Aβ42 concentration was measured using sandwich enzyme-linked immunosorbent assay (ELISA; INNOTEST β-AMYLOID$_{(1-42)}$ Fujirebio), and CSF p-tau181 levels was determined using ELISA INNOTEST PHOSPHO-TAU$_{(181P)}$ as part of the routine diagnostic evaluation of the patients.

2.4 | Statistical analyses

The statistics for this study were performed using GraphPad Prism (version 8.4.3 for Mac, GraphPad Software), while the receiver operating characteristic (ROC) analyses were performed in CombiROC.[26] First, Anderson–Darling tests were performed to test for normal distribution. All plasma data followed a non-normal distribution and were log10-transformed. After logarithmic transformation, the plasma data for p-tau181, p-tau231, and Aβ40 followed a normal distribution, and an analysis of variance (ANOVA) or an unpaired *t* test was performed. The plasma data for Aβ42, Aβ42/Aβ40, GFAP, and NfL still did not follow a normal distribution after logarithmic transformation, and therefore the data were analyzed using a Kruskal–Wallis test or a Mann–Whitney test. Patients were divided into Aβ+ and Aβ− by CSF p-tau181/Aβ42. A cut-point for this ratio was calculated from routine CSF data from patients at the memory clinic from February 2018 to May 2022. A finite Gaussian mixture modeling procedure was applied to the dataset and the analysis and resulting plots show the highest Bayesian information criterion value for the two-component unequal variance model. The parameters from the model were entered into a function creating the two Gaussians and calculating the intersection to 0.077. This cut-point is close to a previously published value[27] and was used in the present study. The accuracy of plasma biomarkers in predicting Aβ status was evaluated using CombiROC, a validated web tool that operates in two phases. In the first phase, the user uploads data from two sample categories (in this case, Aβ status), allowing the tool to generate box plots and marker profiles to assess data quality and establish thresholds for signal detection. In the second phase, the tool calculates sensitivity and specificity for the biomarkers based on the optimal cutoff, which is determined using the Youden index. The results are then presented in a bubble plot that visualizes the combinations. Finally, ROC curves are generated using multivariable logistic regression models, enabling visualization of the performance of the selected biomarkers.[26] To compare the area under the curve (AUC) of the different models, a DeLong test was applied. Statistical significance for all analyses was set at $p < 0.05$, two sided.

3 | RESULTS

3.1 | Demographics

A total of 250 subjects were included in the study. Table 1 gives an overview of the overall demographic characteristics and biomarker values of the cohort. Significant differences were found for age, sex, and MMSE among groups.

3.2 | Plasma biomarker results

Plasma biomarker levels can be found in Table 1 and Figure S1 in supporting information. All plasma biomarkers, aside from p-tau181, were significantly different among the groups (CU Aβ−, MCI: Aβ+, MCI: Aβ−, AD, non-AD Aβ+, and non-AD Aβ−). Comparing diagnostic groups, p-tau181 was significantly different between CU Aβ− and MCI: Aβ+ ($p < 0.05$), and between CU Aβ− and AD ($p < 0.001$). For p-tau231, significant differences were observed between CU Aβ− and MCI: Aβ+ ($p < 0.05$), CU Aβ− and AD ($p < 0.001$), CU Aβ− and non-AD Aβ+ ($p < 0.05$), MCI: Aβ− and AD ($p < 0.001$), as well as between AD and both of the non-AD groups ($p < 0.05$). Aβ42 showed no significant differences in pairwise comparisons, while Aβ40 differentiated CU Aβ− from both AD and non-AD Aβ+ ($p < 0.01$). The Aβ42/Aβ40 ratio distinguished CU Aβ− from AD ($p < 0.001$), MCI: Aβ− from AD ($p < 0.05$), and AD from non-AD Aβ− ($p < 0.001$). GFAP was significantly different between CU Aβ− and AD ($p < 0.0001$), CU Aβ− and non-AD Aβ+ ($p < 0.01$), and between AD and non-AD Aβ− ($p < 0.01$), while NfL levels were significantly different between CU Aβ− from AD ($p < 0.01$), CU Aβ− and both non-AD groups ($p < 0.0001$), and between MCI: Aβ+ and non-AD Aβ− ($p < 0.05$).

In Figure 1, violin plots of all plasma biomarkers in Aβ+ and Aβ− subjects can be found. In the supporting information the plasma biomarkers and across the diagnostic groups (Figure S1) can be found.

The accuracy of the plasma biomarkers to predict Aβ status can be seen in Figure 2 and Table 2. p-tau231 predicted Aβ positivity with an AUC of 0.77 (95% confidence interval [CI]: 0.67–0.84), and p-tau181 with an AUC of 0.67 (95% CI: 0.57–0.76). Aβ42 predicted Aβ positivity with an AUC of 0.61 (95% CI: 0.51–0.71), Aβ40 with an AUC of

TABLE 1 Characteristics of the study cohort.

	CU Aβ− (*n* = 46)	MCI: Aβ+ (*n* = 13)	MCI: Aβ− (*n* = 44)	AD Aβ+ (*n* = 57)	non-AD Aβ+ (*n* = 24)	Non-AD Aβ− (*n* = 66)	*p* value
Age, mean (SD)	70.0 (7.5)	65.6 (9.2)	71.7 (8.6)	72.3 (7.7)	74.6 (6.5)	73.7 (9.2)	<0.001
Sex (M/F)	28/18	2/11	29/15	17/40	17/7	39/27	<0.0001
MMSE score, mean (SD)	28.7 (1.6)	26.1 (4.1)	26.8 (3.4)	22.6 (4.6)	21.8 (4.4)	23.4 (4.2)	<0.0001
Plasma p-tau 181 pg/mL, mean (SD)	3.96 (1.93)	7.62 (2.78)	6.91 (4.15)	8.72 (6.54)	7.83 (3.89)	6.73 (4.03)	0.06
Plasma p-tau 231 pg/mL, mean (SD)	15.1 (5.80)	20.0 (5.39)	17.1 (7.42)	24.7 (9.67)	23.6 (9.40)	17.4 (6.56)	<0.0001
Plasma Aβ42 pg/mL, mean (SD)	6.13 (1.46)	5.36 (1.28)	6.70 (1.67)	6.35 (1.68)	7.01 (1.21)	6.87 (2.00)	<0.01
Plasma Aβ40 pg/mL, mean (SD)	90.9 (14.09)	96.2 (17.69)	101.8 (22.97)	107.2 (21.72)	111.1 (13.08)	107.2 (22.93)	<0.001
Plasma Aβ42/Aβ40, mean (SD)	0.067 (0.013)	0.056 (0.0077)	0.066 (0.0086)	0.059 (0.0076)	0.062 (0.010)	0.064 (0.011)	<0.0001
Plasma GFAP pg/mL, mean (SD)	115.1 (66.3)	167.6 (76.0)	148.1 (63.0)	202.8 (71.8)	207.6 (78.5)	143.4 (72.6)	<0.0001
Plasma NfL pg/mL, mean (SD)	16.7 (8.13)	18.3 (7.23)	26.4 (13.3)	26.8 (16.3)	43.1 (41.6)	36.2 (29.3)	<0.0001

Note: *p* values were calculated by a one-way analysis of variance or a Kruskal–Wallis test, aside from the *p* value for sex, which was calculated by a chi-squared test.

Abbreviations: Aβ, amyloid beta; AD, Alzheimer's disease; CSF, cerebrospinal fluid; CU, cognitively unimpaired; F, female; GFAP, glial fibrillary acidic protein; M, male; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; n, number; NfL, neurofilament light chain; p-tau, phosphorylated tau; SD, standard deviation.

0.54 (95% CI: 0.44–0.64), and Aβ42/Aβ40 with an AUC of 0.71 (95% CI: 0.62–0.80). GFAP predicted the Aβ status with an AUC of 0.70 (95% CI: 0.61–0.79) and NfL with an AUC of 0.55 (95% CI: 0.45–0.65). The accuracy of predicting Aβ positivity in the whole cohort was further examined by combining the different biomarkers to one another. When looking at the combination of p-tau181 and p-tau231 they predicted Aβ positivity with an AUC of 0.75 (95% CI: 0.68–0.84), which slightly improved when adding Aβ42/Aβ40 (AUC 0.80, 95% CI: 0.71–0.87). Assessing the combination of GFAP and NfL the AUC was 0.77 (95% CI: 0.67–0.83), and when all biomarkers were combined a further increase was seen (AUC 0.86, 95% CI: 0.78–0.92).

The potential improvement in diagnostic accuracy from combining biomarkers was assessed by comparing the AUC values of ROC models. No significant differences were observed between p-tau181 and p-tau231, when used in combination versus individually. However, adding Aβ42, Aβ40, and the Aβ42/Aβ40 ratio to the combination significantly improved accuracy compared to p-tau181 ($p < 0.05$), Aβ42 ($p < 0.01$), and Aβ40 ($p < 0.0001$) alone. In the assessment of GFAP and NfL, combining the two resulted in a statistically significant improvement compared to NfL alone ($p < 0.01$), but not GFAP. When all plasma biomarkers were combined, the performance was better to that of p-tau181 ($p < 0.01$), Aβ42 ($p < 0.0001$), Aβ40 ($p < 0.0001$), Aβ42/Aβ40 ($p < 0.05$), GFAP ($p < 0.01$), and NfL ($p < 0.0001$) individually.

4 | DISCUSSION

The aim of this study was to investigate the accuracy of various plasma biomarkers to predict Aβ status in a typical clinical setting in a mixed memory clinic cohort. Several studies have investigated plasma biomarkers.[6,11] However, many of these studies are performed in research cohorts of the most common neurodegenerative disorders, where amyloid co-pathology and atypical phenotypes are excluded. This is not a true representation of the patients clinicians meet at memory clinics. Our study adds to the existing literature by exploring the performance of plasma biomarkers in a more heterogeneous clinical population, which better reflects the diversity of patients encountered in everyday clinical practice. By demonstrating that plasma biomarkers retain significant predictive value even in this diverse setting, our research shows that these markers could be viable tools for use beyond specialized research contexts.

In memory clinics, clinicians get referrals from the primary care or other hospital departments, with patients with all types of memory complaints. The patients are often diagnosed according to their clinical phenotype and the clinical and supplementary evaluations in conjunction with AD biomarkers that may be affected by very diverse disease mechanisms. Therefore, it is essential to investigate whether plasma biomarkers reflect findings in CSF and can be used in these heterogenous cohorts, because this will probably account for a major part of how the biomarkers are going to be used in the future.

Our findings suggest that a plethora of plasma biomarkers show promising results, also when challenged in a more heterogeneous cohort, thus bridging a crucial gap between research-based findings and real-world clinical application. In this study, statistically significant differences for nearly all plasma biomarkers were found when investigating them in amyloid-positive and amyloid-negative individuals among different etiologies, and when looking at the different diagnostic groups. Further, we found that the combination of all seven plasma biomarkers was able to detect Aβ status with an AUC of 0.86, which is similar to other studies.[28] However, it is still lower than in more homogenous cohorts when looking at the individual biomarkers.[29] In addition, the combination of p-tau181 and p-tau231 performed only slightly worse than the panel of seven markers. Studies have found that p-tau231 performs slightly better than other p-tau epitopes in the early stages of the diseases, making it a promising marker for memory clinics to be used in the first diagnostic evaluation of a patient.[8] Several recent

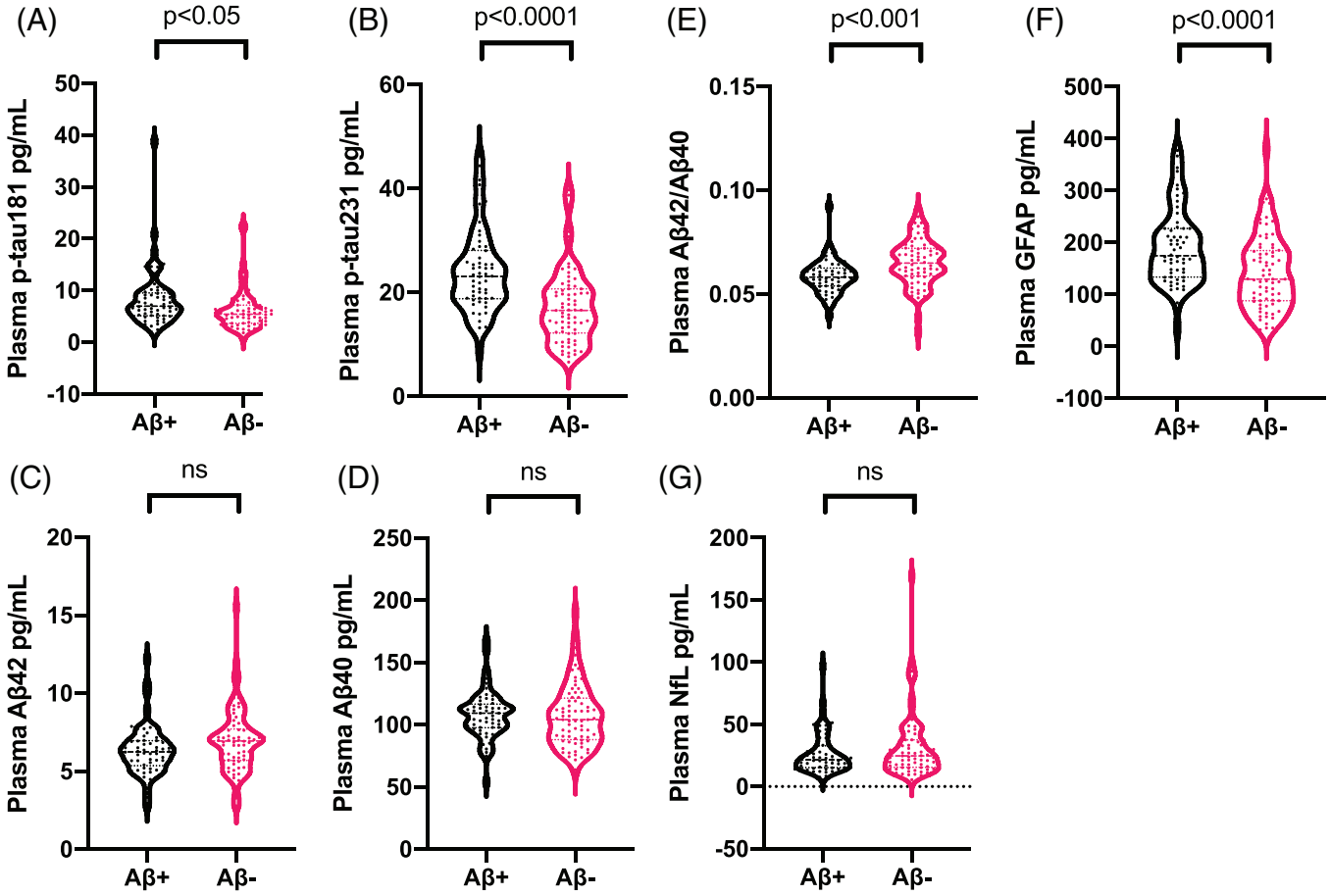

FIGURE 1 Plasma biomarkers in amyloid-positive and amyloid-negative subjects. A, The violin plots show the median, interquartile range, and the extreme values of plasma p-tau181 in amyloid-positive and amyloid-negative subjects across the whole cohort. The minimum and maximum of all data have been included. $p < 0.05$. B, The violin plots show the median, interquartile range, and the extreme values of plasma p-tau231 in amyloid-positive and amyloid-negative subjects across the whole cohort. The minimum and maximum of all data have been included. $p < 0.0001$. C, The violin plots show the median, interquartile range, and the extreme values of plasma Aβ42 in amyloid-positive and amyloid-negative subjects across the whole cohort. The minimum and maximum of all data have been included. $p = 0.051$. D, The violin plots show the median, interquartile range, and the extreme values of plasma Aβ40 in amyloid-positive and amyloid-negative subjects across the whole cohort. The minimum and maximum of all data have been included. $P < 0.84$. E, The violin plots show the median, interquartile range, and the extreme values of plasma Aβ42/Aβ40 in amyloid-positive and amyloid-negative subjects across the whole cohort. The minimum and maximum of all data have been included. $p < 0.001$. F, The violin plots show the median, interquartile range, and the extreme values of plasma GFAP in amyloid-positive and amyloid-negative subjects across the whole cohort. The minimum and maximum of all data have been included. $p < 0.0001$. G, The violin plots show the median, interquartile range, and the extreme values of plasma NfL in amyloid-positive and amyloid-negative subjects across the whole cohort. The minimum and maximum of all data have been included. $p = 0.14$. Plasma data for p-tau181, p-tau231, and Ab40 were analyzed with an unpaired *t* test, while plasma data for Aβ42, Aβ42/Aβ40, GFAP, and NfL were analyzed using a Mann–Whitney test. +, amyloid positives; −, amyloid negatives. (The CSF p-tau181/Aβ42 cut-point was 0.077.) Aβ, amyloid beta; CSF, cerebrospinal fluid; GFAP, glial fibrillary acidic protein; NfL, neurofilament light chain; p-tau, phosphorylated tau.

studies have also pointed to p-tau217 as superior to p-tau181 and p-tau231, which suggests that a diagnostic accuracy > 0.86 is achievable even in heterogenous cohorts.[30]

While the combination of all seven plasma biomarkers achieved an AUC of 0.86, which is comparable to other studies, we acknowledge that the need for multiple biomarkers could pose economic and logistical challenges in clinical practice. Using a panel of six or more biomarkers may not be feasible due to the costs associated with processing and analyzing multiple assays. However, our findings also suggest that certain biomarkers, such as p-tau181 and p-tau231, perform nearly as well in isolation or in smaller combinations. For instance, the combination of just p-tau181 and p-tau231 showed only slightly lower performance than the full panel. This suggests that future research could explore a more cost-effective approach, focusing on a subset of highly predictive markers while maintaining diagnostic accuracy. Additionally, as assay technology improves and becomes more standardized, the costs of multiplex panels may decrease, potentially making this approach more practical.

Plasma biomarkers could help identify pathological changes, which could be used in trial selection and design. Clinicians need methods

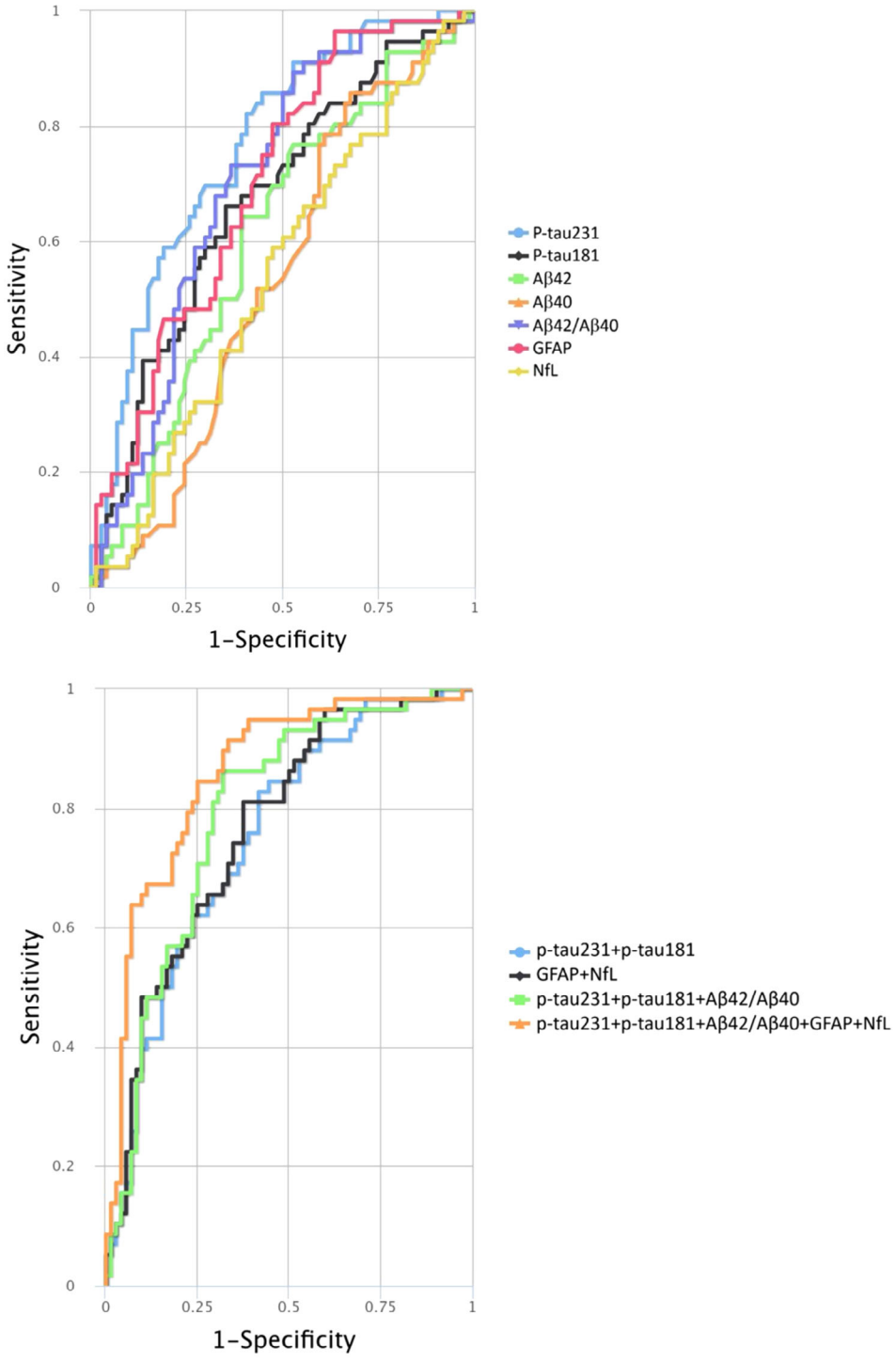

FIGURE 2 ROC curves of plasma biomarkers using CSF p-tau181/Aβ42. The ROC curves and the corresponding AUC show the accuracy of the different plasma biomarkers and various combinations of the plasma biomarkers (demographically uncorrected) to assess amyloid status for the whole cohort. Aβ, amyloid beta; AUC, area under the curve; CSF, cerebrospinal fluid; GFAP, glial fibrillary acidic protein; NfL, neurofilament light chain; p-tau, phosphorylated tau; ROC, receiver operating characteristic.

that can predict who will progress from subjective cognitive decline to MCI: AD, as many of the new upstream therapies might be more effective in the preclinical stages before irreversible damage has occurred. Therefore, it would be of great interest to use plasma biomarkers to identify those patients who will progress clinically and investigate whether these new upstream therapies have an impact on the likelihood of developing a dementia disorder. In addition, plasma biomarkers could be used to monitor the effectiveness of these therapies.[5,31] Plasma biomarkers could help identify amyloid-positive individuals and thereby support the decision on who needs a lumbar puncture or a

TABLE 2 AUC, sensitivity, and specificity of plasma biomarkers using CSF p-tau181/Aβ42.

	AUC	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Opt. cutoff	95% CI
p-tau 231	0.76	81	60	69	70	0.40	0.67–0.84
p-tau 181	0.67	66	65	61	64	0.44	0.57–0.76
Aβ42	0.61	66	61	57	50	0.44	0.51–0.71
Aβ40	0.54	80	40	65	56	0.44	0.44–0.64
Aβ42/Aβ40	0.71	74	65	67	63	0.45	0.62–0.80
GFAP	0.70	97	38	64	61	0.32	0.61–0.79
NfL	0.55	60	54	54	38	0.45	0.45–0.65
Combinations:							
p-tau 231 + p-tau 181	0.75	83	58	69	70	0.40	0.68–0.84
GFAP + NfL	0.77	81	63	68	69	0.40	0.67–0.83
p-tau 231 + p-tau 181 + Aβ42/Aβ40	0.80	86	68	71	65	0.39	0.71–0.87
p-tau 231 + p-tau 181 + Aβ42/Aβ40 + GFAP + NfL	0.86	85	75	77	75	0.41	0.78–0.92

Note: The AUC shows the accuracy of the different plasma biomarkers and various combinations of the plasma biomarkers, to assess amyloid status for the whole cohort. Sensitivity and specificity are expressed in percent (%). The CSF p-tau181/Aβ42 cut-point was 0.077.
Abbreviations: Aβ, amyloid beta; AUC, area under the curve; CI, confidence interval; CSF, cerebrospinal fluid; GFAP, glial fibrillary acidic protein; NfL, neurofilament light chain; NPV (%), negative predictive value in percent; opt. cutoff, optimal cutoff for the receiver operating characteristic curve (the similar thresholds likely reflect the close alignment of the corresponding curves); PPV (%), positive predictive value in percent; p-tau, phosphorylated tau.

PET scan, and thereby lowering the costs for the examinations and the number of unnecessary lumbar punctures and imaging tests.[32] Furthermore, plasma biomarkers could be used as a screening tool to aid the judgment on which patients should get a referral from the primary care to specialized memory clinics.[33] One possible approach involves using plasma biomarkers for risk stratification based on the likelihood of Aβ positivity. By using a two cut-off approach, patients could be categorized into three groups: a high-risk group with a high probability of Aβ-positivity, potentially not requiring additional clinical testing; an intermediate-risk group, which may benefit from advanced testing such as CSF and imaging; and a low-risk group, more likely to be diagnosed with a non-neurodegenerative disorder or another non-AD neurodegenerative condition. Studies have suggested that this strategy could reduce the demand for advanced diagnostics in specialized memory clinics while facilitating more precise diagnostic decisions.[32]

5 | LIMITATIONS

The purpose of the study was to investigate biomarkers in patients with diverse and mixed etiologies. This is also our greatest limitation. In this study, we did not have confirmation of amyloid status by amyloid PET. We therefore determined amyloid status by CSF p-tau181/Aβ42, which may itself be challenged by these diverse etiologies. We could therefore only study to what extent blood-based biomarkers reflected amyloid status as determined by CSF biomarkers. Furthermore, many of the plasma assays used in various studies are preliminary and have different performance, which means they still need to be standardized. Additionally, nearly all plasma studies consist of White cohorts, and therefore the findings might not apply to all races and ethnicities.[33,34] We still need more research on how other co-morbidities might affect the different plasma biomarkers. Another limitation of the study is the lack of plasma p-tau217, which has been shown to outperform other tau biomarkers. Including p-tau217 in future studies could potentially enhance diagnostic accuracy and provide further insights into its performance in heterogeneous clinical populations. In future studies, it could be interesting to compare the best plasma biomarker or the best combination of plasma biomarkers to CSF and imaging, toward the investigation of whether the replacement or addition of plasma biomarkers to the standard diagnostic assessment improves or alters the diagnoses. Furthermore, studies investigating co-morbidities (such as cardiovascular diseases, kidney diseases, body mass index, and psychiatric disorders) and blood work (such as cholesterol and organ biomarkers) are warranted.[35]

6 | CONCLUSIONS

This study supports previous findings on plasma biomarkers and in addition confirms these results, even when investigated in a typical clinical setting in a consecutive, heterogeneous, mixed memory clinic cohort. Plasma biomarkers could potentially soon be used in primary care as an initial test together with a clinical examination, to aid the decision on who should be referred to a specialized memory clinic. Furthermore, with the development of new disease-modifying treatments, plasma biomarkers might be highly useful for treatment monitoring and trial enrollment of Aβ-positive participants. Despite that further validation is required, this study illustrates the usefulness of plasma biomarkers in predicting Aβ status.

ACKNOWLEDGMENTS

All the authors are grateful to the clinical personnel, and the participating patients and controls at the Copenhagen Memory clinic, Copenhagen University Hospital, Rigshospitalet, Denmark. This study was funded by the Lundbeck Foundation, Grosserer L. F. Foghts Foundation, Augustinus Foundation, Frimodt-Heineke, and the Foundation for Neurological Research. The Danish Dementia Biobank (DDBB) was supported by the Absalon Foundation of 1st May 1978 and Simons Spies Foundation. HZ is a Wallenberg Scholar and a Distinguished Professor at the Swedish Research Council supported by grants from the Swedish Research Council (#2023-00356; #2022-01018 and #2019-02397), the European Union's Horizon Europe research and innovation programme under grant agreement No 101053962, Swedish State Support for Clinical Research (#ALFGBG-71320), the Alzheimer Drug Discovery Foundation (ADDF), USA (#201809-2016862), the AD Strategic Fund and the Alzheimer's Association (#ADSF-21-831376-C, #ADSF-21-831381-C, #ADSF-21-831377-C, and #ADSF-24-1284328-C), the Bluefield Project, Cure Alzheimer's Fund, the Olav Thon Foundation, the Erling-Persson Family Foundation, Familjen Rönströms Stiftelse, Stiftelsen för Gamla Tjänarinnor, Hjärnfonden, Sweden (#FO2022-0270, #FO2017-0242, and #ALZ2022-0006), the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 860197 (MIRIADE), the European Union Joint Programme – Neurodegenerative Disease Research (JPND2021-00694), the National Institute for Health and Care Research University College London Hospitals Biomedical Research Centre, and the UK Dementia Research Institute at UCL (UKDRI-1003). KB is supported by the Swedish Research Council (#2017-00915 and #2022-00732), the Swedish Alzheimer Foundation (#AF-930351, #AF-939721, and #AF-968270), Hjärnfonden, Sweden (#FO2017-0243 and #ALZ2022-0006), the Swedish state under the agreement between the Swedish government and the County Councils, the ALF-agreement (#ALFGBG-715986 and #ALFGBG-965240), the European Union Joint Program for Neurodegenerative Disorders (JPND2019-466-236), the Alzheimer's Association 2021 Zenith Award (ZEN-21-848495), the Alzheimer's Association 2022-2025 Grant (SG-23-1038904 QC), and the Kirsten and Freddy Johansen Foundation, Copenhagen.

CONFLICT OF INTEREST STATEMENT

H.Z. has served on scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZPath, Amylyx, Annexon, Apellis, Artery Therapeutics, AZTherapies, Cognito Therapeutics, CogRx, Denali, Eisai, LabCorp, Merry Life, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave; has given lectures in symposia sponsored by Alzecure, Biogen, Cellectricon, Fujirebio, Lilly, Novo Nordisk, and Roche; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). KB has served as a consultant and on advisory boards for Acumen, ALZPath, AriBio, BioArctic, Biogen, Eisai, Lilly, Moleac Pte. Ltd, Novartis, Ono Pharma, Prothena, Roche Diagnostics, and Siemens Healthineers; has served on data monitoring committees for Julius Clinical and Novartis; has given lectures, produced educational materials, and participated in educational programs for AC Immune, Biogen, Celdara Medical, Eisai, and Roche Diagnostics; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program, outside the work presented in this paper. H.S.G., A.H.S., L.G., N.A., S.G.H., M.G., and P.H. report no conflicts of interest. Author disclosures are available in the supporting information.

CONSENT STATEMENT

All included patients gave informed consent for their plasma samples and data to be used for research. The study was approved by the Danish Data Protection Agency (VD-2019-105) and the Ethical Committee of the Capital Region of Denmark (H-19000651).

ORCID

Helena Sophia Gleerup https://orcid.org/0000-0001-7470-4818

REFERENCES

1. Costerus JM, Brouwer MC, van de Beek D. Technological advances and changing indications for lumbar puncture in neurological disorders. *Lancet Neurol* 2018;17:268-278. doi:10.1016/S1474-4422(18)30033-4
2. Serrano-Pozo A, Frosch MP, Masliah E, Hyman BT. Neuropathological Alterations in Alzheimer Disease n.d. CSH Perspectives. doi:10.1101/cshperspect.a006189
3. 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. doi:10.1016/J.JALZ.2018.02.018
4. Working group members to be added. Revised Criteria for Diagnosis and Staging of Alzheimer's Disease: Alzheimer's Association Workgroup (draft 2). AlzOrg; 2023:1-38.
5. Cummings J, Zhou Y, Lee G, Zhong K, Fonseca J, Cheng F. Alzheimer's disease drug development pipeline: 2023. *Alzheimers Dement Transl Res Clin Interv* 2023;9:e12385. doi:10.1002/TRC2.12385
6. Hansson O, Edelmayer RM, Boxer AL, et al. The Alzheimer's Association appropriate use recommendations for blood biomarkers in Alzheimer's disease. *Alzheime's Dement* 2022;18:2669. doi:10.1002/ALZ.12756
7. Thijssen EH, La Joie R, Strom A, et al. Association of Plasma P-tau217 and P-tau181 with clinical phenotype, neuropathology, and imaging markers in Alzheimer's disease and frontotemporal lobar degeneration: a retrospective diagnostic performance study. *Lancet Neurol* 2021;20:739. doi:10.1016/S1474-4422(21)00214-3
8. Ashton NJ, Pascoal TA, Karikari TK, et al. Plasma p-tau231: a new biomarker for incipient Alzheimer's disease pathology. *Acta Neuropathol* 2021;141(5):709-724. doi:10.1007/s00401-021-02275-6
9. Hall S, Janelidze S, Londos E, et al. Plasma phospho-tau identifies Alzheimer's co-pathology in patients with Lewy Body disease. *Mov Disord* 2021;36:767-771. doi:10.1002/mds.28370
10. Deters KD, Risacher SL, Kim S, et al. Plasma Tau Association with brain atrophy in mild cognitive impairment and Alzheimer's disease. *J Alzheimer's Dis* 2017;58:1245-1254. doi:10.3233/JAD-161114
11. Milà-Alomà M, Ashton NJ, Shekari M, et al. Plasma p-tau231 and p-tau217 as state markers of amyloid-β pathology in preclinical Alzheimer's disease 1925. doi:10.1038/s41591-022-01925-w
12. Pereira JB, Janelidze S, Smith R, et al. Plasma GFAP is an early marker of amyloid-β but not tau pathology in Alzheimer's disease. *Brain* 2021;144:3505. doi:10.1093/BRAIN/AWAB223

13. Simrén J, Weninger H, Brum WS, et al. Differences between blood and cerebrospinal fluid glial fibrillary Acidic protein levels: The effect of sample stability. *Alzheimers Dement* 2022;18:1988. doi:10.1002/ALZ.12806
14. Mattsson N, Cullen NC, Andreasson U, Zetterberg H, Blennow K. Association between longitudinal plasma neurofilament light and neurodegeneration in patients with Alzheimer disease. *JAMA Neurol* 2019;76:791-799. doi:10.1001/jamaneurol.2019.0765
15. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state": A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975;12:189-198. doi:10.1016/0022-3956(75)90026-6
16. Larner AJ. Addenbrooke's Cognitive Examination-Revised (ACE-R) in day-to-day clinical practice. *Age Ageing* 2007;36:685-686. doi:10.1093/AGEING/AFM112
17. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's disease. *Neurology* 1984;34:939-944. doi:10.1212/WNL.34.7.939
18. Winblad B, Palmer K, Kivipelto M, et al. Mild cognitive impairment - beyond controversies, towards a consensus: report of the International Working Group on mild cognitive impairment. *J Intern Med*. 2004;256:240-246. doi:10.1111/j.1365-2796.2004.01380.x
19. McKeith IG, Dickson DW, Lowe J, et al. Diagnosis and management of dementia with Lewy bodies: third report of the DLB consortium. *Neurology* 2005;65:1863-1872. doi:10.1212/01.WNL.0000187889.17253.B1
20. Sachdev P, Kalaria R, O'Brien J, et al. Diagnostic criteria for vascular cognitive disorders: A VASCOG statement. *Alzheimer Dis Assoc Disord* 2014;28:206-218. doi:10.1097/WAD.0000000000000034
21. Rascovsky K, Hodges JR, Knopman D, et al. Sensitivity of revised diagnostic criteria for the behavioural variant of frontotemporal dementia. *Brain* 2011;134:2456-2477. doi:10.1093/BRAIN/AWR179
22. Gorno-Tempini ML, Hillis AE, Weintraub S, et al. Classification of primary progressive aphasia and its variants. *Neurology* 2011;76:1006-1014. doi:10.1212/WNL.0b013e31821103e6
23. Relkin N, Marmarou A, Klinge P, Bergsneider M, Black PM. Diagnosing Idiopathic Normal-pressure Hydrocephalus. *Neurosurgery* 2005;57:S2-S4-S2-16. doi:10.1227/01.NEU.0000168185.29659.C5
24. ICD-10 Version:2010 n.d. Accessed October 23, 2020. https://icd.who.int/browse10/2010/en#
25. Karikari TK, Pascoal TA, Ashton NJ, et al. Blood phosphorylated tau 181 as a biomarker for Alzheimer's disease: a diagnostic performance and prediction modelling study using data from four prospective cohorts. *Lancet Neurol* 2020;19:422-433. doi:10.1016/S1474-4422(20)30071-5
26. Mazzara S, Rossi RL, Grifantini R, Donizetti S, Abrignani S, BombaCI: M. CombiROC: an interactive web tool for selecting accurate marker combinations of omics data. *Nat Publ Gr* 2017. doi:10.1038/srep45477
27. Keshavan A, Wellington H, Chen Z, et al. Concordance of csf measures of Alzheimer's pathology with amyloid pet status in a preclinical cohort: A comparison of lumipulse and established immunoassays. *Alzheimer's Dement Diagnosis, Assess Dis Monit* 2021;13:e12131. doi:10.1002/DAD2.12131
28. Palmqvist S, Janelidze S, Stomrud E, et al. Performance of fully automated plasma assays as screening tests for Alzheimer disease-related β-amyloid Status. *JAMA Neurol* 2019;76:1060-1069. doi:10.1001/jamaneurol.2019.1632
29. Janelidze S, Mattsson N, Palmqvist S, et al. Plasma P-tau181 in Alzheimer's disease: relationship to other biomarkers, differential diagnosis, neuropathology and longitudinal progression to Alzheimer's dementia. *Nat Med* 2020;26(3):379-386. doi:10.1038/S41591-020-0755-1
30. Barthélemy NR, Salvadó G, Schindler SE, et al. Highly accurate blood test for Alzheimer's disease is similar or superior to clinical cerebrospinal fluid tests. *Nat Med*. 2024;30:1085–1095. doi:10.1038/s41591-024-02869-z
31. Husain M. Screening for Alzheimer's disease: plasma biomarkers and what else?. *Brain*. 2023;146(2):407-408. doi:10.1093/brain/awac490
32. Brum WS, Cullen NC, Janelidze S, et al. A two-step workflow based on plasma p-tau217 to screen for amyloid β positivity with further confirmatory testing only in uncertain cases. *Nat Aging*. 2023;3:1079-1090. doi:10.1038/s43587-023-00471-5
33. Angioni D, Delrieu J, Hansson O, et al. Blood biomarkers from research use to clinical practice: what must be done? A report from the EU/US CTAD Task Force. *J Prev Alzheimers Dis*. 2022;9(4):569-579. doi:10.14283/jpad.2022.85
34. Schindler SE, Karikari TK, Ashton NJ, et al. Effect of race on prediction of brain amyloidosis by plasma aβ42/aβ40, phosphorylated tau, and neurofilament light. *Neurology*. 2022;99(3):e245-e257. doi:10.1212/WNL.0000000000200358
35. Pichet Binette A, Janelidze S, Cullen N, et al. Confounding factors of Alzheimer's disease plasma biomarkers and their impact on clinical performance. *Alzheimer's Dement* 2023;19:1403-1414. doi:10.1002/ALZ.12787

SUPPORTING INFORMATION

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

How to cite this article: Gleerup HS, Simonsen AH, Grötschel L, et al. Plasma biomarkers of amyloid, tau, astrogliosis, and axonal injury in a mixed memory clinic cohort. *Alzheimer's Dement*. 2025;17:e70073. https://doi.org/10.1002/dad2.70073