Alzheimer's Disease Plasma Biomarkers Distinguish Clinical Diagnostic Groups in Memory Clinic Patients

Michelle Gerards[a]   Ann-Katrin Schild[a]   Dix Meiberth[a]   Ayda Rostamzadeh[a]

Jörg Janne Vehreschild[b, c]   Sebastian Wingen-Heimann[b, d]   Wibke Johannis[e]

Pamela Martino Adami[f]   Oezguer A. Onur[g]   Alfredo Ramirez[f, h, i, j, k]

Thomas K. Karikari[l]   Nicholas J. Ashton[l, m, n, o]   Henrik Zetterberg[l, p, q, r, s]

Kaj Blennow[l, p]   Franziska Maier[a]   Frank Jessen[a, j, k]

[a]Department of Psychiatry and Psychotherapy, Faculty of Medicine, University Hospital Cologne, University of Cologne, Cologne, Germany; [b]Department I for Internal Medicine, Faculty of Medicine, University Hospital Cologne, University of Cologne, Cologne, Germany; [c]German Centre for Infection Research (DZIF), Partner Site Bonn-Cologne, Cologne, Germany; [d]FOM University of Applied Sciences, Cologne, Germany; [e]Institute of Clinical Chemistry, Faculty of Medicine, University Hospital Cologne, University of Cologne, Cologne, Germany; [f]Division of Neurogenetics and Molecular Psychiatry, Department of Psychiatry and Psychotherapy, Faculty of Medicine, University Hospital Cologne, University of Cologne, Cologne, Germany; [g]Department of Neurology, Faculty of Medicine, University Hospital Cologne, University of Cologne, Cologne, Germany; [h]Department of Neurodegenerative Diseases and Geriatric Psychiatry, Medical Faculty, University Hospital Bonn, Bonn, Germany; [i]Department of Psychiatry & Glenn Biggs Institute for Alzheimer's and Neurodegenerative Diseases, San Antonio, TX, USA; [j]German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany; [k]Cluster of Excellence Cellular Stress Responses in Aging-associated Diseases (CECAD), University of Cologne, Cologne, Germany; [l]Department of Psychiatry and Neurochemistry, University of Gothenburg, Gothenburg, Sweden; [m]Wallenberg Centre for Molecular and Translational Medicine, University of Gothenburg, Gothenburg, Sweden; [n]Department of Old Age Psychiatry, Maurice Wohl Clinical Neuroscience Institute, King's College London, London, UK; [o]Unit for Dementia at South London & Maudsley, NIHR Biomedical Research Centre for Mental Health & Biomedical Research, London, UK; [p]Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden; [q]UK Dementia Research Institute, University College London, London, UK; [r]Department of Neurodegenerative Disease, UCL Institute of Neurology, London, UK; [s]Hong Kong Center for Neurodegenerative Diseases, Hong Kong, China

Abstract

Introduction: Several recent research studies show high performance of blood biomarkers to identify Alzheimer's disease also in the pre-dementia mild cognitive impairment (MCI) stage, but data from the routine clinical care memory clinic setting are needed. ***Methods:*** We examined plasma samples of 144 memory clinic patients, including dementia of Alzheimer type (DAT, *n* = 54), MCI (*n* = 57), and subjective cognitive decline (SCD, *n* = 33), who either presented as self-referrals or were referred by general practitioners or neurologists or psychiatrists. The plasma biomarkers, amyloid-beta42 (Aß42), amyloid-beta40 (Aß40), phospho-Tau181 (pTau181), total-tau (tTau), and neurofilament light (NFL), as

Correspondence to:
Frank Jessen, frank.jessen@uk-koeln.de

well as different ratios, were measured using the ultrasensitive single molecule array (Simoa) immunoassay technology. Statistical analysis including Kruskal-Wallis test, linear regression, and receiver operating characteristics analyses was performed. ***Results:*** Of the single markers, we observed statistically significant group effects of pTau181 (H(2) = 34.43, *p* < 0.001) and NFL (H(2) = 27.66, *p* < 0.001). All individual group comparisons of pTau181 were significant, while the contrast of SCD versus MCI for NFL was not significant. In addition, the ratios of Aß42/Aß40 (H(2) = 7.50, *p* = 0.02) and pTau181/Aß42 (H(2) = 25.26, *p* < 0.001) showed significant group effects with significant difference between all groups for pTau181/Aß42 and an SCD versus MCI difference for Aß42/Aß40. PTau181 showed the highest area under the curve of 0.85 for the discrimination of SCD and DAT with a sensitivity of 80% and a specificity of 79% at a cut-off of 12.2 pg/mL. Age influenced Aß42, Aß40, and NFL concentrations. ***Conclusion:*** Plasma pTau181 and NFL, as well as the ratios Aß42/Aß40 and pTau181/Aß42, are biomarkers, which can differentiate diagnostic groups in a memory clinic setting outside of research studies.

Introduction

Dementia of Alzheimer's type (DAT) is a major health care challenge of our times [1]. Alzheimer's disease (AD) is neuropathologically characterized by the accumulation of neurofibrillary tangles composed of aggregated tau protein and amyloid deposition [2]. There is evidence that these pathological processes begin more than two decades before the onset of symptoms. Hence, biomarkers of amyloid deposition and tau aggregation can detect the disease in patients already in early stages [3]. Subjective cognitive decline (SCD) occurs at the late preclinical stage of AD, recently labeled as stage 2 [4], and is also a risk factor for the development of mild cognitive impairment (MCI) and DAT. SCD is not specific to AD and can also be caused by normal aging, depression, and other psychiatric and neurologic disorders [5]. In addition, a recent meta-analysis reported substantial variation of the proportion of SCD cases with amyloid pathology among individual samples, depending on the specific recruitment criteria and settings [6]. In those SCD cases, who progress to dementia, DAT is the most common, but other dementia types also occur [7].

The detection of neuropathological changes in patients currently requires amyloid-positron emission tomography (amyloid-PET) or biomarkers obtained from cerebrospinal fluid (CSF) [8–10]. Core biomarkers in CSF include amyloid-beta42 (Aß42), total-tau protein (ttau), and phosphorylated tau protein (pTau) [11]. Both the use of amyloid-PET and CSF biomarkers are limited concerning access and are either costly or invasive, and rare complications can occur [12, 13]. The recent evolution of plasma biomarkers provides potentially novel opportunities in the future regarding improved accessibility as well as lower risk [14, 15]. Since the early diagnosis of AD before the stage of DAT is most likely critical for the success of future therapies, accessible tests for the identification of pre-dementia stages of AD are becoming increasingly important [16, 17].

There was early inconsistent evidence about potential changes of plasma Aß42 throughout the disease course [18], with some studies not being able to differentiate between DAT and controls in a cross-sectional setting [16, 19]. Stable lower effects in DAT are found from 2018 onward using new measurement techniques, with the Aß42/amyloid-beta40 (Aß40) ratio providing promising results [20–26].

Plasma phospho-Tau181 (pTau181) distinguishes between DAT and controls and is elevated to a lesser extent in patients with MCI [14, 27–32]. There is also evidence that plasma pTau181 differentiates between DAT and other neurodegenerative diseases [29, 30]. Other plasma pTau markers, such as pTau217, show similar results [33, 34]. The ratio of pTau181/Aß42 in CSF predicted greater clinical decline in MCI patients and showed a comparable accuracy as PET imaging [35].

Some studies showed increased levels of tTau in DAT compared to MCI and controls and provided evidence for an association of tTau with poor cognition and brain atrophy [20, 36, 37]. Some findings suggest that the overlap between normal aging and AD is large, implying that tTau may not be a suitable biomarker when measured in plasma [36]. Increased tTau values were also detected in other neurodegenerative diseases suggesting that tTau is a nonspecific biomarker of neuronal damage [38, 39].

The biomarker neurofilament light (NFL) is considered an unspecific marker for neurodegeneration which can be measured in CSF, but also in plasma [40]. NFL concentration differentiates between patients with DAT and controls [41]. Changes in plasma NFL were associated with amyloid deposition in amyloid-PET [42]. NFL was associated with cognitive deficits and magnetic resonance imaging (MRI) characteristics of DAT at early stages and throughout the disease course [41]. Higher baseline plasma levels were associated with poorer longitudinal cognition [41, 43]. The goal of this study is to test how

these plasma biomarkers perform with regard to the differentiation of the diagnostic groups of patients with DAT, MCI, and SCD in a routine care memory clinic setting outside of a highly standardized research setting.

Methods

We included samples and data from patients of the memory clinic of the Centre for Memory Disorders (ZfG) at the University Hospital of Cologne who gave written consent to provide blood samples for research purposes. The study was approved by the Ethics Commission of the medical faculty of the University of Cologne. The study complied with the Declaration of Helsinki.

Study Sample
Our study included 144 patients who were clinically diagnosed with SCD ($n = 33$), MCI ($n = 57$), or DAT ($n = 54$) at the memory clinic between 2016 and 2018. Patients either presented as self-referrals or were referred by general practitioners or neurologists or psychiatrists.

Clinical Examination and Diagnosis
All patients underwent a comprehensive clinical examination, including medical history, caregiver report, psychopathological and physical examination, the Mini-Mental State Examination (MMSE), extended neuropsychological testing, standard blood laboratory measures, and MRI. CSF for diagnostic purposes was obtained in 31 DAT and 17 MCI cases. Note that according to the current guidelines, CSF biomarker sampling is not recommended in SCD outside of research due to the yet unclear meaning in determining the individual cause and prognosis of SCD. For this reason, CSF biomarkers were only obtained in 2 SCD cases, who specifically demanded AD biomarker assessment.

The syndromal diagnosis of DAT, MCI, and SCD was established based on all available information by the treating physician. Patients with DAT and MCI met the clinical National Institute on Aging and Alzheimer's Association (NIA-AA) criteria [4]. Both were defined by cognitive impairment as documented by the MMSE and by extended neuropsychological testing. The groups were discriminated by the presence of impairment in activities of daily living, which interfere with independence based on clinical judgment in the case of DAT and the absence thereof in the case of MCI. Thirty-five MCI patients (61%) were multi-domain MCI. Of these, 34 patients were amnestic multi-domain. One was non-amnestic multi-domain (language and executive function domain affected). Twenty-one patients (37%) were single-domain MCI of which 18 were single-domain amnestic MCI, two were single-domain visuo-construction, and one single-domain language. Following proposed criteria [5], SCD was defined by a complaint of cognitive decline and age-, sex-, and education-adjusted unimpaired cognitive performance in extended neuropsychological testing.

The clinical and neuropsychological work-up together with the laboratory and MRI information was used in all groups to exclude non-AD causes of cognitive impairment as good as possible, including psychiatric disorders. Patients fulfilling clinical criteria of a depressive episode, or any other detectable non-AD cause of cognitive impairment, were not included. The DAT group comprised early- and late-onset cases. Note that the definition of groups in this study is based on the clinical diagnoses of DAT, MCI, and SCD only and does not incorporate CSF biomarker information as inclusion or exclusion criterion.

Plasma Biomarkers
All patients agreed to plasma sampling for research purposes when they first presented in the clinic. Research plasma samples were obtained within the diagnostic process of the respective participant and stored directly at −20° and permanently at −80°C. None of the samples was thawed and refrozen before this study. The samples were shipped on dry ice to and analyzed in Gothenburg (Clinical Neurochemistry Laboratory, Institute of Neuroscience and Physiology, Sahlgrenska Academy). Biomarker measurements were performed through ultrasensitive single molecule array (Simoa) immunoassay technology (Quanterix, Billerica, MA, USA) [44]. The laboratory was blinded to patient diagnoses and other clinical data. The following numbers of measurements were obtained: Aß42 – $n = 142$; Aß40 – $n = 144$; pTau181 – $n = 143$; tTau – $n = 144$; NFL – $n = 144$. Calibrators were run in duplicates, and samples were diluted 4-fold and run in singlicates. Results were compensated for the dilution. Two QC levels were run in duplicates at the beginning and the end of each run. Intra-assay coefficients of variation were below 10%.

Statistical Analysis
Differences between diagnostic groups in age, years of education, and plasma biomarkers were assessed using the nonparametric Kruskal-Wallis test, χ^2 test, and post hoc comparison. Linear regression was used to examine the correlation between plasma biomarkers, age, sex, and years of education. In the case of significant associations, the group difference analyses were performed with ANCOVAs adjusted for respective covariates. To determine the diagnostic accuracy, receiver operating characteristic (ROC) analysis was used to calculate areas under the curve (AUCs) for the comparisons of SCD versus (vs.) MCI plus DAT, SCD vs. MCI, SCD vs. DAT, and MCI vs. DAT. Potential cut-off values are proposed.

In addition, the plasma biomarkers across all groups of those cases with pathological CSF biomarkers were plotted against those with normal CSF biomarkers. Note that for this step, the clinical routine CSF and the respective cut-off data obtained from the central laboratory of the University Hospital of Cologne were used. The following local cut-offs were applied to define CSF positivity: Aß42 <629 pg/mL, pTau >61 pg/mL, tTau <290 pg/mL, and Aß42/Aß40 <0.095 pg/mL. In addition to plotting, the plasma measures of CSF-positive and CSF-negative cases across all groups were compared with t tests. All analysis and statistics were performed in SPSS (Version 26.0, IBM, Armonk, NY, USA).

Results

Sample Characteristics
The age ranged from 42 to 90 years with a mean age of 69.7 (standard deviation [SD] = 10.1) years (Table 1) with 31% of the patients being under the age of 65 (55% of SCD, 32% of MCI, and 15% of DAT patients). Statisti-

Table 1. Characteristics of the diagnostic groups and plasma biomarker measures

Sample characteristics	SCD mean (±SD)/	MCI mean (±SD)/	DAT mean (±SD)/	$H(2)/\chi^2$[1]	p value[1]	z^2	p value[2]	z^3	p value[3]	z^4	p value[4]
N	33	57	54								
Age, years	63.1 (9.6)	69.1 (9.7)	74.3 (8.2)	27.2	**<0.001**	−25.9	0.01	−47.8	<0.001	−21.9	0.01
Male, n (%)	12 (36.4)	33 (57.9)	24 (44.4)	4.3	0.12						
Years of education	14 (3)	13 (3)	12 (2)	12.4	**0.02**	18.0	0.03	30.6	<0.001	12.6	0.08
MMSE results	29 (2.0)	27 (2.1)	23 (3.6)	73.5	**<0.001**	31.8	<0.001	75.8	<0.001	44.1	<0.001
Verbal fluency (semantic)	21 (3.4)	17 (4.8)	13 (4.7)	49.3	**<0.001**	36.3	<0.001	63.4	<0.001	27.1	<0.001
Boston naming test	23 (3.9)	16 (4.9)	11 (3.8)	70.9	**<0.001**	37.8	<0.001	75.4	<0.001	37.6	<0.001
Wordlist learning	15 (1.6)	14 (1.6)	12 (2.3)	33.4	**<0.001**	24.6	0.01	49.8	<0.001	25.2	<0.001
Wordlist delayed recall	8 (1.5)	5 (2.6)	2 (1.6)	74.4	**<0.001**	41.5	<0.001	77.4	<0.001	35.9	<0.001
Wordlist recognition	9 (1.0)	8 (3.2)	6 (4.4)	21.9	**<0.001**	21.5	0.02	38.0	<0.001	16.5	0.01
Constructional praxis	11 (0.9)	10 (1.2)	9 (1.9)	30.8	**<0.001**	13.5	0.11	44.1	<0.001	30.6	<0.001
Delayed constructional savings	10 (1.8)	6 (2.9)	3 (2.4)	71.9	**<0.001**	24.3	<0.001	74.7	<0.001	40.4	<0.001
CERAD sum score	87 (8.3)	69 (13.0)	52 (12.0)	77.1	**<0.001**	40.8	<0.001	78.6	<0.001	37.8	<0.001
Aβ42, pg/mL	13.2 (4.1)	12.4 (3.9)	13.0 (3.2)	1.8	0.40						
Aβ40, pg/mL	278.8 (88.3)	297.9 (83.9)	313.9 (60.6)	4.7	0.09						
pTau181, pg/mL	9.5 (6.4)	17.2 (21.2)	18.6 (8.8)	**34.4**	**<0.001**	−28.3	0.01	−53.4	<0.001	−25.0	0.01
tTau, pg/mL	2.4 (1.0)	2.6 (2.1)	2.2 (0.8)	1.2	0.56						
NFL, pg/mL	12.9 (9.3)	14.5 (7.2)	22.1 (14.1)	27.7	**<0.001**	−14.5	0.34	−45.2	<0.001	−30.6	<0.001
Aβ42/Aβ40	0.045 (0.008)	0.042 (0.012)	0.042 (0.007)	7.5	**0.02**	20.3	0.03	24.3	0.01	4.0	0.61
pTau181/Aβ42	0.9 (1.1)	1.7 (2.4)	1.5 (0.7)	**25.3**	**<0.001**	−29.5	<0.001	−45.8	<0.001	−16.3	0.04

SD, standard deviation; n, number; SCD, subjective cognitive decline; MCI, mild cognitive impairment; DAT, dementia of Alzheimer's type; H, Kruskal-Wallis-H; χ^2, chi²-test; z, post hoc test; Aβ42, amyloid-beta42; Aβ40, amyloid-beta40; pTau181, phospho-Tau181; tTau, total-tau; NFL, neurofilament light. [1] p values reported are for comparison between all diagnostic groups, [2] SCD-MCI, [3] SCD-DAT, [4] MCI-DAT using analysis of variance. Individual group comparisons were only made in cases of a significant overall group effect using post hoc tests.

Table 2. ROC performance of plasma biomarkers

Plasma biomarkers	SCD versus MCI AUC (p value)	SCD versus MCI + DAT AUC (p value)	SCD versus DAT AUC (p value)	MCI versus DAT AUC (p value)
Aß42	0.58 (*p* = 0.20)	0.57 (*p* = 0.25)	0.55 (*p* = 0.44)	0.54 (*p* = 0.47)
Aß40	0.55 (*p* = 0.44)	0.59 (*p* = 0.10)	**0.64 (*p* = 0.03)**	0.57 (*p* = 0.18)
pTau181	**0.72 (*p* < 0.001)**	**0.78 (*p* < 0.001)**	**0.85 (*p* < 0.001)**	**0.69 (*p* < 0.001)**
tTau	0.52 (*p* = 0.80)	0.51 (*p* = 0.48)	0.57 (*p* = 0.30)	0.54 (*p* = 0.43)
NFL	0.61 (*p* = 0.10)	**0.70 (*p* < 0.001)**	**0.81 (*p* < 0.001)**	**0.72 (*p* < 0.001)**
Aß42/Aß40	**0.64 (*p* = 0.03)**	**0.66 (*p* = 0.01)**	**0.67 (*p* = 0.01)**	0.53 (*p* = 0.62)
pTau181/Aß42	**0.72 (*p* < 0.001)**	**0.77 (*p* < 0.001)**	**0.81 (*p* < 0.001)**	**0.62 (*p* = 0.03)**

ROC, receiver operating characteristics; SCD, subjective cognitive decline; MCI, mild cognitive impairment; DAT, dementia of Alzheimer's type; AUC, area under the curve; Aß42, amyloid-beta42; Aß40, amyloid-beta40; pTau181, phospho-Tau181; tTau, total-tau; NFL, neurofilament light.

Table 3. Possible cut-off points SCD versus MCI and SCD versus DAT

Plasma biomarker	SCD versus MCI			SCD versus DAT		
	cut-off, pg/mL	sensitivity, %	specificity, %	cut-off, pg/mL	sensitivity, %	specificity, %
Aß40	na	na	na	≥273.6	80	42
pTau181	≥8.4	80	55	≥12.2	80	79
NFL	na	na	na	≥12.7	80	67
Aß42/Aß40	≤0.048	80	47	≤0.048	80	47
pTau181/Aß42	≥0.58	80	50	≥0.77	80	75

Possible cut-off points for plasma biomarkers with significant results in ROC analysis for SCD versus MCI and SCD versus DAT. Shown are exemplary cut-off values with a sensitivity of 80% for comparison of biomarkers. ROC, receiver operating characteristics; SCD, subjective cognitive decline; MCI, mild cognitive impairment; DAT, dementia of Alzheimer's type; Aß40, amyloid-beta40; Aß42, amyloid-beta42; pTau181, phospho-Tau181; NFL, neurofilament light; na, not available due to no significant ROC analysis.

cally significant group differences in age ($p < 0.001$) and years of education ($p < 0.001$) were observed (Table 1). Post hoc tests showed group effects of age for SCD-MCI ($p = 0.01$), SCD-DAT ($p < 0.001$), and MCI-DAT ($p = 0.01$) and of years of education for SCD-MCI ($p = 0.03$) and SCD-DAT ($p < 0.001$), but not for MCI-DAT ($p = 0.08$). There were no significant group differences for sex ($p = 0.12$).

Association of Plasma Biomarkers, Age, Years of Education, and Sex

There was no association of any plasma biomarker with years of education or sex. The age of participants affected the concentration of Aß42 ($p < 0.001$), of Aß40 ($p < 0.001$), and of NFL ($p < 0.001$). Older patients showed increased plasma values. Other biomarkers or their ratios were not correlated with age.

There was an association of Aß42 with age also in all individual diagnostic groups: SCD ($p = 0.01$), MCI ($p = 0.02$), and DAT ($p = 0.02$), and of Aß40 with age in patients with SCD ($p = 0.02$) and DAT ($p = 0.01$), but not in patients with MCI ($p = 0.12$). Furthermore, an increase of NFL with age was observed for SCD ($p < 0.001$), MCI ($p < 0.001$), and DAT ($p = 0.01$).

Group Differences of Plasma Biomarkers

All plasma biomarker measures for all groups are listed in Table 1. The AUCs of all comparisons are displayed in Table 2. In Table 3, cut-offs are proposed for individual markers at a sensitivity level of 80%. Figure 1 shows
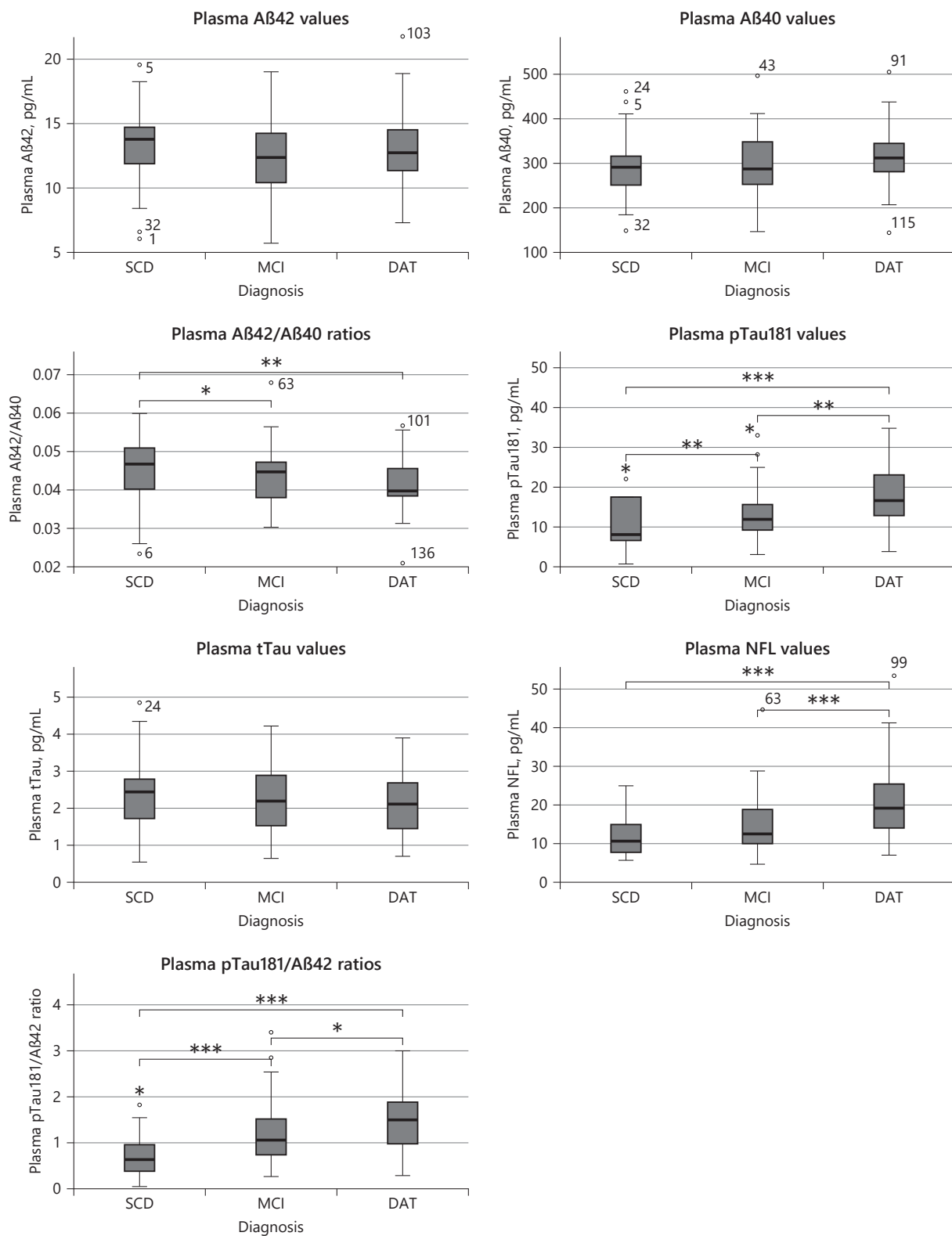
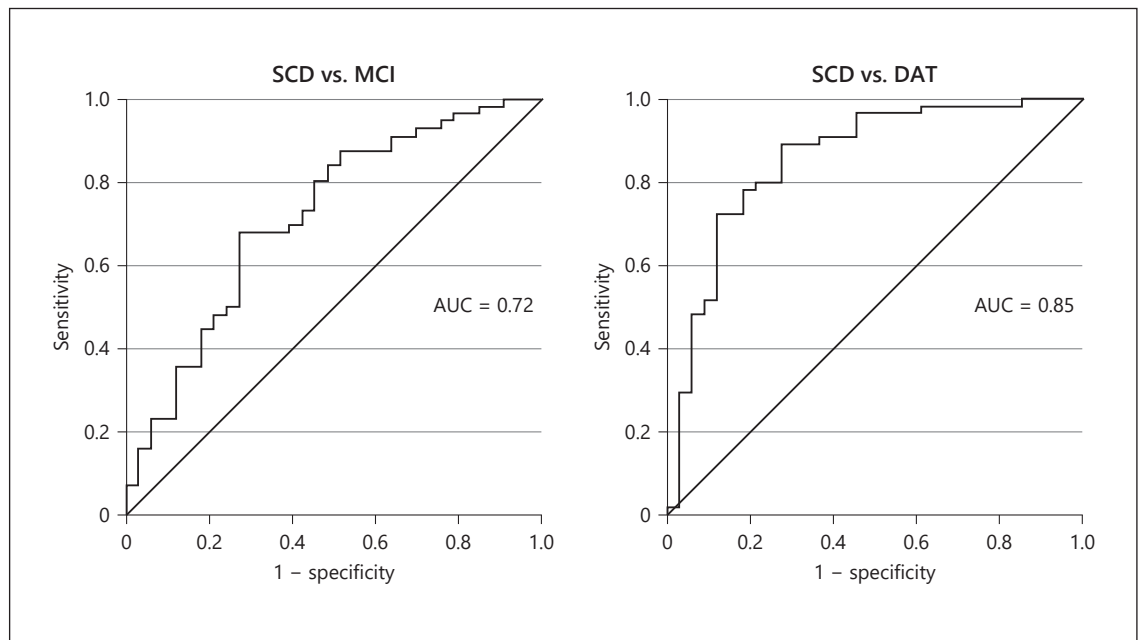**Fig. 1.** Plasma biomarker distribution across the diagnostic groups.

Fig. 2. ROC performance of pTau181 for SCD versus MCI and SCD versus DAT.

the distribution of the biomarker data separated by the diagnostic groups.

Aß42 concentrations were not significantly different between the diagnostic groups ($p = 0.4$). Neither were there any significant differences in Aß42 between groups when adjusted for age ($p = 0.07$). The AUC in ROC analysis was 0.58 for SCD vs. MCI ($p = 0.20$), 0.57 for SCD vs. MCI + DAT ($p = 0.25$), 0.55 for SCD vs. DAT ($p = 0.44$), and 0.54 for MCI vs. DAT ($p = 0.47$) indicating that the performance of Aß42 to discriminate between diagnostic groups is poor.

The ANOVA for Aß40 did not show a significant group effect ($p = 0.09$). Furthermore, there were no significant differences in Aß40 between groups when adjusting for age ($p = 0.94$). ROC analysis showed an AUC of 0.55 for SCD vs. MCI ($p = 0.44$), 0.59 for SCD vs. MCI + DAT ($p = 0.10$), and 0.57 for MCI vs. DAT ($p = 0.18$). Only the ROC for SCD vs. DAT with an AUC of 0.64 ($p = 0.03$) was significant.

Significant group effects for plasma pTau181 were observed ($p < 0.001$). Post hoc test showed differences in all diagnostic groups with pTau181 increasing with the severity of impairment: SCD-MCI ($p = 0.01$), SCD-DAT ($p < 0.001$), and MCI-DAT ($p = 0.01$). The AUC for SCD vs. MCI was 0.72 ($p < 0.001$), for SCD vs. MCI + DAT 0.78 ($p < 0.001$), for SCD vs. DAT 0.85 ($p < 0.001$), and for MCI

vs. DAT 0.69 ($p < 0.001$) (Fig. 2). At a cut-off of 10.2 pg/mL, pTau181 had a sensitivity of 80% and a specificity of 79% to distinguish between SCD and DAT. Furthermore, at a cut-off point with a sensitivity of 80%, a specificity of 55% to distinguish between SCD and MCI was reached.

tTau was not significantly different between diagnostic groups ($p = 0.56$). ROC analysis showed an AUC of 0.52 for SCD vs. MCI ($p = 0.80$), 0.51 for SCD vs. MCI + DAT ($p = 0.48$), 0.57 for SCD vs. DAT ($p = 0.30$), and 0.54 for MCI vs. DAT ($p = 0.43$).

Significant group effects for NFL were observed ($p < 0.001$). Post hoc test showed differences between SCD-DAT ($p < 0.001$) and MCI-DAT ($p > 0.001$) with NFL values increasing with the severity of impairment. The contrast SCD-MCI was not significant ($p = 0.34$). Significant differences in NFL between groups remained when adjusted for age ($p = 0.04$). ROC analysis was significant for SCD vs. MCI + DAT with an AUC of 0.7 ($p < 0.001$), for SCD vs. DAT with an AUC of 0.81 ($p < 0.001$), and for MCI vs. DAT with an AUC of 0.72 ($p < 0.001$), but not for SCD vs. MCI with an AUC of 0.61 ($p = 0.09$). A cut-off of 12.7 pg/mL for the differentiation of SCD and DAT showed a sensitivity of 80% and a specificity of 67%.

The plasma biomarkers in relation to CSF positivity or negativity of the respective marker are shown in Figure 3. Direct comparison of the plasma biomarkers between
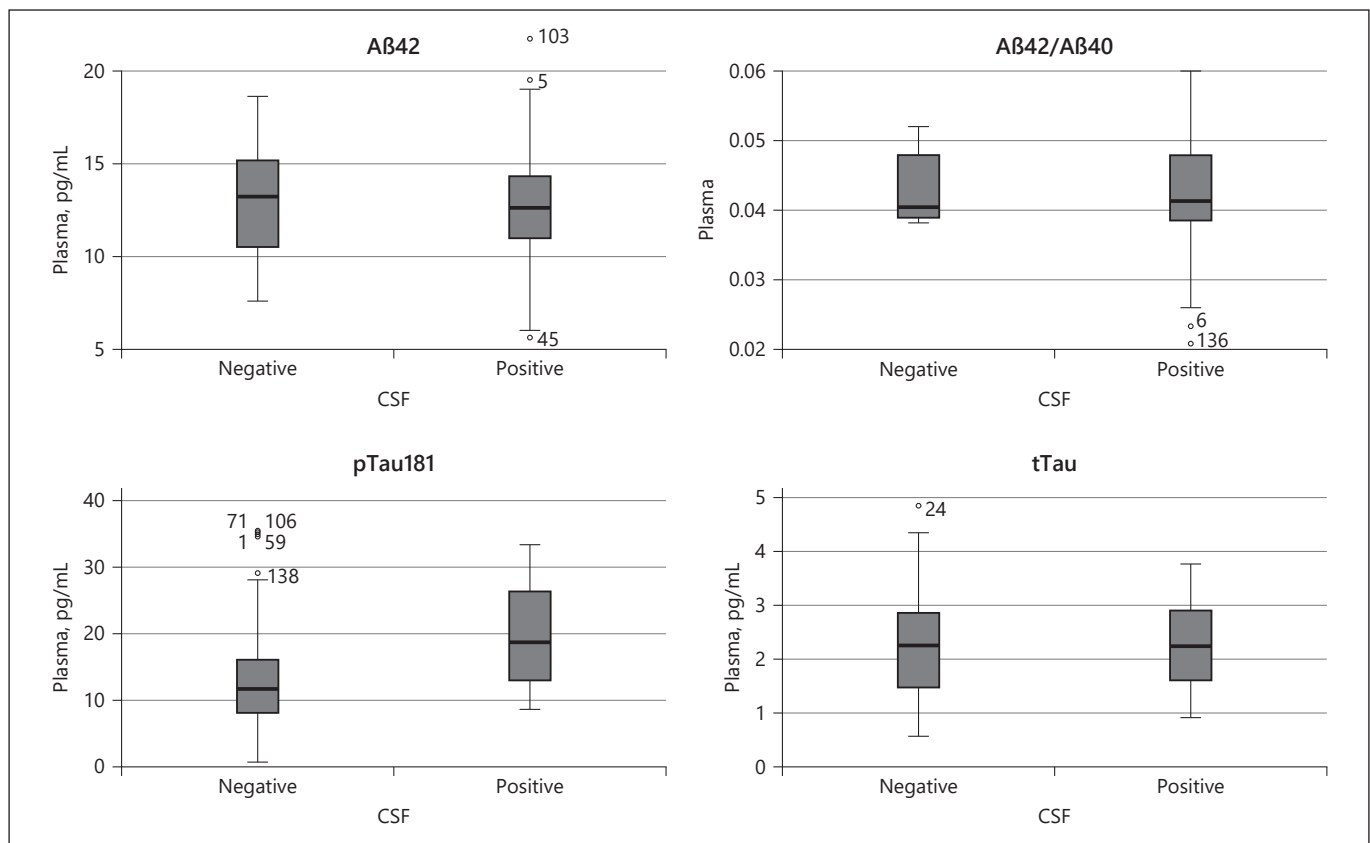
Gerards et al.

Fig. 3. Plasma biomarker values in relation to CSF biomarker classification as positive or negative according to the measurements and cut-off obtained and applied locally in routine care.

CSF-positive and CSF-negative cases revealed a significant group difference for plasma pTau181 when tested one-sided ($p = 0.034$, a priori hypothesis of increased concentration in CSF-positive cases) and a trend-level difference at two-sided testing ($p = 0.069$). None of the other plasma biomarkers showed a significant difference between the respective CSF-positive and CSF-negative cases.

Biomarker Ratios

Significant group effects ($p = 0.02$) were observed for Aß42/Aß40. Post hoc test showed a decrease of the ratio between SCD-DAT ($p = 0.01$) and SCD-MCI ($p = 0.03$), but not between MCI-DAT ($p = 0.61$). The ability to discriminate between SCD vs. MCI with an AUC of 0.64 ($p = 0.03$), between SCD vs. MCI + DAT with an AUC of 0.66 ($p = 0.01$), and between SCD vs. DAT with an AUC of 0.67 ($p = 0.01$) indicated better diagnostic performance than Aß42 or Aß40 alone. The ability to discriminate between MCI vs. DAT with an AUC of 0.53 ($p = 0.62$) was poor. Cut-off points reached a specificity of 47 at a sensitivity of 80% for SCD vs. DAT and SCD vs. MCI.

Significant group effects for pTau181/Aß42 were observed ($p < 0.001$). Post hoc test showed an increase in the ratio between SCD-MCI ($p < 0.001$), SCD-DAT ($p < 0.001$), and MCI-DAT ($p = 0.04$). Furthermore, pTau181/Aß42 showed an AUC of 0.72 ($p < 0.001$) for SCD vs. MCI, of 0.77 ($p < 0.001$) for SCD vs. MCI + DAT, of 0.81 ($p < 0.001$) for SCD vs. DAT, and of 0.62 ($p = 0.03$) for MCI vs. DAT. Cut-off points reached a specificity of 75% at a sensitivity of 80% for SCD vs. DAT and a specificity of 50% at a sensitivity of 80% for SCD vs. MCI.

Discussion/Conclusion

Plasma biomarkers analyzed with the Simoa technology can discriminate between diagnostic groups in a routine memory clinic setting outside of specific research

studies. The ratio of Aß42/Aß40 was higher in SCD compared to MCI and DAT. This agrees with the model of amyloid level plateauing in the early phase of the disease [3]. In agreement with previous studies, we also found that neither plasma Aß42 nor Aß40 alone can distinguish between groups [16, 19]. Aß42/Aß40 achieved AUCs between 0.64 and 0.67 providing the best discrimination between SCD and DAT. With AUCs <0.7, these results are of low clinical relevance but may be meaningful in the future in combination with additional diagnostic markers. PTau181 differed between all groups and showed higher values in more advanced stages of impairment. It provided the best discrimination between SCD and DAT achieving an AUC of 0.85 and a specificity of over 79% at a sensitivity of 80%. With a slightly lower sensitivity and specificity, pTau181 also differentiates between SCD and MCI. Plasma pTau181 concentrations were higher in CSF pTau181-positive cases compared with negative cases. These findings agree with previous studies showing that pTau181 is increased in patients with DAT, but also discriminates between different early stages of the disease [28, 29]. We confirm that the ratio of plasma pTau181/Aß42 differs between the groups as previously shown in CSF [35]. The combination of pTau181/Aß42 was not superior to pTau181 alone, indicating that the combination does not improve the ability to discriminate between diagnostic groups. Our study indicates that tTau discriminates poorly between the diagnostic groups. Some previous studies showed a modest association of tTau with conversion to DAT [37], whereas others suggested that tTau is not a suitable biomarker for discriminating DAT from other groups, which is in agreement with our findings [36]. In contrast to pTau181, NFL did not differentiate between SCD and MCI. NFL differed, however, between SCD and MCI in comparison with DAT. NFL values increased with the severity of impairment and achieved a sensitivity of 80% and specificity of 67% for discriminating SCD and DAT. An association between higher NFL values and DAT diagnosis has been shown previously [41]. NFL was correlated with age; however, the overall statistically significant difference between the diagnostic groups remained with adjustment for age.

Our study has limitations. We did not include CSF biomarkers of the full sample, because the numbers of CSF samples in the SCD group and also in the MCI group were low due to the lack of clinical recommendation for standard use in these groups. Furthermore, CSF was measured locally in the clinical diagnostic context, whereas plasma biomarkers were measured in the research context in the laboratory in Gothenburg. We did not include a healthy control group as we restricted the sampling to memory patients attending the memory clinic only. Furthermore, longitudinal data were not available as these data are derived from routine care, which does not comprise systematic follow-up of patients. Finally, while highly promising for wider application in routine care in the future, at present, the Simoa technology is mainly used in research and has not yet been in clinical practice.

The strength of our study is the real-world design. All patients who presented with SCD, MCI, or DAT in clinical routine and who were willing to participate were included. There were no additional study-specific inclusion or exclusion criteria.

We found that the plasma measures of pTau181 and NFL, as well as the ratios Aß42/Aß40 and pTau181/Aß42, are sufficiently robust to differentiate diagnostic groups with limited sample size in a memory clinic setting. In agreement with recent research studies, pTau181 proved to be the most promising biomarker that distinguishes between all three groups of SCD, MCI, and DAT suggesting potential clinical use also in early symptomatic stages. The implementation of plasma biomarkers in memory clinic procedures and potentially even in nonspecialized and general practice settings will substantially increase accessibility to biomarker-based diagnostics.

Statement of Ethics

Conflict of Interest Statement

Henrik Zetterberg has served at scientific advisory boards and/or as a consultant for AbbVie, Alector, Eisai, Denali, Roche, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics, NervGen, AZTherapies, CogRx, and Red Abbey Labs, has given lectures in symposia sponsored by Cellectricon, Fujirebio, AlzeCure, and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). Kaj Blennow has served as a consultant, at advisory boards or at data monitoring committees for Abcam, Axon, Biogen, JOMDD/Shimadzu, Julius Clinical, Lilly, MagQu, Novartis, Prothena, 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 Incuba-

Author Contributions

Michelle Gerards, Ann-Katrin Schild, Dix Meiberth, Ayda Rostamzadeh, Oezguer A. Onur, Franziska Maier, and Frank Jessen contributed to the overall design and the implementation of the study. Michelle Gerards, Ann-Katrin Schild, Dix Meiberth, Ayda Rostamzadeh, Franziska Maier, and Frank Jessen conducted the study. Jörg Janne Vehreschild, Sebastian Wingen-Heimann, Pamela Martino Adami, Ayda Rostamzadeh, Thomas K. Karikari, Nicholas J. Ashton, Henrik Zetterberg, and Kaj Blennow were responsible for methodological core central data management and data analyses. Michelle Gerards and Ann-Katrin Schild were responsible for the methodological core manuscript. Michelle Gerards, Ann-Katrin Schild, Dix Meiberth, Ayda Rostamzadeh, Jörg Janne Vehreschild, Sebastian Wingen-Heimann, Wibke Johannis, Pamela Martino Adami, Oezguer A. Onur, Alfredo Ramirez, Thomas K. Karikari, Nicholas J. Ashton, Henrik Zetterberg, Kaj Blennow, Franziska Maier, and Frank Jessen contributed to the interpretation of data, drafting of the manuscript, and approval of the final version. They agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Data Availability Statement

The data of this study are not publicly available but can be obtained upon request.

References

1 GBD 2016 Neurology Collaborators. Global, regional, and national burden of neurological disorders, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol. 2019 May;18(5):459–80.

2 Blennow K, de Leon MJ, Zetterberg H. Alzheimer's disease. Lancet. 2006 Jul 29; 368(9533):387–403.

3 Bateman RJ, Xiong C, Benzinger TL, Fagan AM, Goate A, Fox NC, et al. Clinical and biomarker changes in dominantly inherited Alzheimer's disease. N Engl J Med. 2012 Aug 30; 367(9):795–804.

4 Jack CR Jr, Bennett DA, Blennow K, Carrillo MC, Dunn B, Haeberlein SB, et al. NIA-AA research framework: toward a biological definition of Alzheimer's disease. Alzheimers Dement. 2018 Apr;14(4):535–62.

5 Jessen F, Amariglio RE, van Boxtel M, Breteler M, Ceccaldi M, Chetelat G, et al. A conceptual framework for research on subjective cognitive decline in preclinical Alzheimer's disease. Alzheimers Dement. 2014 Nov;10(6): 844–52.

6 Janssen O, Jansen WJ, Vos SJB, Boada M, Parnetti L, Gabryelewicz T, et al. Characteristics of subjective cognitive decline associated with amyloid positivity. Alzheimers Dement. 2021 Dec 8. Epub ahead of print.

7 Slot RER, Sikkes SAM, Berkhof J, Brodaty H, Buckley R, Cavedo E, et al. Subjective cognitive decline and rates of incident Alzheimer's disease and non-Alzheimer's disease dementia. Alzheimers Dement. 2019 Mar;15(3): 465–76.

8 Frisoni GB, Boccardi M, Barkhof F, Blennow K, Cappa S, Chiotis K, et al. Strategic roadmap for an early diagnosis of Alzheimer's disease based on biomarkers. Lancet Neurol. 2017 Aug;16(8):661–76.

9 Blennow K, Zetterberg H. Biomarkers for Alzheimer's disease: current status and prospects for the future. J Intern Med. 2018 Dec; 284(6):643–63.

10 Shea YF, Barker W, Greig-Gusto MT, Loewenstein DA, Duara R, DeKosky ST. Impact of amyloid PET imaging in the memory clinic: a systematic review and meta-analysis. J Alzheimers Dis. 2018; 64(1):323–35.

11 Blennow K, Hampel H. CSF markers for incipient Alzheimer's disease. Lancet Neurol. 2003 Oct;2(10):605–13.

12 Costerus JM, Brouwer MC, van de Beek D. Technological advances and changing indications for lumbar puncture in neurological disorders. Lancet Neurol. 2018 Mar;17(3):268–78.

13 Wittenberg R, Knapp M, Karagiannidou M, Dickson J, Schott J. Economic impacts of introducing diagnostics for mild cognitive impairment Alzheimer's disease patients. Alzheimers Dement. 2019;5:382–7.

14 Zetterberg H, Blennow K. Blood biomarkers: democratizing Alzheimer's diagnostics. Neuron. 2020 Jun 17;106(6):881–3.

15 Blennow K. Phenotyping Alzheimer's disease with blood tests. Science. 2021 Aug 6; 373(6555):626–8.

16 Toledo JB, Vanderstichele H, Figurski M, Aisen PS, Petersen RC, Weiner MW, et al. Factors affecting Aβ plasma levels and their utility as biomarkers in ADNI. Acta Neuropathol. 2011 Oct;122(4):401–13.

17 Blennow K, Dubois B, Fagan AM, Lewczuk P, de Leon MJ, Hampel H. Clinical utility of cerebrospinal fluid biomarkers in the diagnosis of early Alzheimer's disease. Alzheimers Dement. 2015 Jan;11(1):58–69.

18 Schupf N, Tang MX, Fukuyama H, Manly J, Andrews H, Mehta P, et al. Peripheral Aβ subspecies as risk biomarkers of Alzheimer's disease. Proc Natl Acad Sci U S A. 2008 Sep 16; 105(37):14052–7.

19 Feinkohl I, Schipke CG, Kruppa J, Menne F, Winterer G, Pischon T, et al. Plasma amyloid concentration in Alzheimer's disease: performance of a high-throughput amyloid assay in distinguishing Alzheimer's disease cases from controls. J Alzheimers Dis. 2020;74(4):1285–94.

20 Zetterberg H, Wilson D, Andreasson U, Minthon L, Blennow K, Randall J, et al. Plasma tau levels in Alzheimer's disease. Alzheimers Res Ther. 2013;5(2):9.

21 Nakamura A, Kaneko N, Villemagne VL, Kato T, Doecke J, Dore V, et al. High performance plasma amyloid-beta biomarkers for Alzheimer's disease. Nature. 2018 Feb 8; 554(7691):249–54.

22 Shahpasand-Kroner H, Klafki HW, Bauer C, Schuchhardt J, Huttenrauch M, Stazi M, et al. A two-step immunoassay for the simultaneous assessment of Aβ38, Aβ40 and Aβ42 in human blood plasma supports the Aβ42/Aβ40 ratio as a promising biomarker candidate of Alzheimer's disease. Alzheimers Res Ther. 2018 Dec 8;10(1):121.

23 Verberk IMW, Slot RE, Verfaillie SCJ, Heijst H, Prins ND, van Berckel BNM, et al. Plasma amyloid as prescreener for the earliest alzheimer pathological changes. Ann Neurol. 2018 Nov;84(5):648–58.

24 Vogelgsang J, Shahpasand-Kroner H, Vogelgsang R, Streit F, Vukovich R, Wiltfang J. Multiplex immunoassay measurement of amyloid-β42 to amyloid-β40 ratio in plasma discriminates between dementia due to Alzheimer's disease and dementia not due to Alzheimer's disease. Exp Brain Res. 2018 May; 236(5):1241–50.

25 Schindler SE, Bollinger JG, Ovod V, Mawuenyega KG, Li Y, Gordon BA, et al. High-precision plasma beta-amyloid 42/40 predicts current and future brain amyloidosis. Neurology. 2019 Oct 22;93(17):e1647–59.

26 Giudici KV, de Souto Barreto P, Guyonnet S, Li Y, Bateman RJ, Vellas B, et al. Assessment of plasma amyloid-beta42/40 and cognitive decline among community-dwelling older adults. JAMA Netw Open. 2020 Dec 1;3(12): e2028634.

27 Mielke MM, Hagen CE, Xu J, Chai X, Vemuri P, Lowe VJ, et al. Plasma phospho-tau181 increases with Alzheimer's disease clinical severity and is associated with tau- and amyloid-positron emission tomography. Alzheimers Dement. 2018 Aug;14(8):989–97.

28 Yang CC, Chiu MJ, Chen TF, Chang HL, Liu BH, Yang SY. Assay of plasma phosphorylated tau protein (Threonine 181) and total tau protein in early-stage alzheimer's disease. J Alzheimers Dis. 2018;61(4):1323–32.

29 Janelidze S, Mattsson N, Palmqvist S, Smith R, Beach TG, Serrano GE, 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 Mar;26(3): 379–86.

30 Karikari TK, Pascoal TA, Ashton NJ, Janelidze S, Benedet AL, Rodriguez JL, 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 May;19(5):422–33.

31 Hansson O, Cullen N, Zetterberg H; Alzheimer's Disease Neuroimaging Initiative, Blennow K, Mattsson-Carlgren N. Plasma phosphorylated tau181 and neurodegeneration in Alzheimer's disease. Ann Clin Transl Neurol. 2021 Jan;8(1):259–65.

32 Zetterberg H, Blennow K. Moving fluid biomarkers for Alzheimer's disease from research tools to routine clinical diagnostics. Mol Neurodegener. 2021 Feb 19;16(1):10.

33 Barthelemy NR, Horie K, Sato C, Bateman RJ. Blood plasma phosphorylated-tau isoforms track CNS change in Alzheimer's disease. J Exp Med. 2020 Nov 2;217(11):e20200861.

34 Palmqvist S, Janelidze S, Quiroz YT, Zetterberg H, Lopera F, Stomrud E, et al. Discriminative accuracy of plasma phospho-tau217 for Alzheimer disease vs other neurodegenerative disorders. JAMA. 2020 Aug 25;324(8): 772–81.

35 Hansson O, Seibyl J, Stomrud E, Zetterberg H, Trojanowski JQ, Bittner T, et al. CSF biomarkers of Alzheimer's disease concord with amyloid-beta PET and predict clinical progression: a study of fully automated immunoassays in BioFINDER and ADNI cohorts. Alzheimers Dement. 2018 Nov;14(11):1470–81.

36 Mattsson N, Zetterberg H, Janelidze S, Insel PS, Andreasson U, Stomrud E, et al. Plasma tau in Alzheimer disease. Neurology. 2016 Oct 25;87(17):1827–35.

37 Pase MP, Beiser AS, Himali JJ, Satizabal CL, Aparicio HJ, DeCarli C, et al. Assessment of plasma total tau level as a predictive biomarker for dementia and related endophenotypes. JAMA Neurol. 2019 May 1;76(5):598–606.

38 Ost M, Nylen K, Csajbok L, Ohrfelt AO, Tullberg M, Wikkelso C, et al. Initial CSF total tau correlates with 1-year outcome in patients with traumatic brain injury. Neurology. 2006 Nov 14;67(9):1600–4.

39 Skillback T, Rosen C, Asztely F, Mattsson N, Blennow K, Zetterberg H. Diagnostic performance of cerebrospinal fluid total tau and phosphorylated tau in Creutzfeldt-Jakob disease: results from the Swedish Mortality Registry. JAMA Neurol. 2014 Apr;71(4):476–83.

40 Moscoso A, Grothe MJ, Ashton NJ, Karikari TK, Lantero Rodriguez J, Snellman A, et al. Longitudinal associations of blood phosphorylated tau181 and neurofilament light chain with neurodegeneration in Alzheimer disease. JAMA Neurol. 2021 Apr 1;78(4):396–406.

41 Mattsson N, Andreasson U, Zetterberg H, Blennow K; Alzheimer's Disease Neuroimaging Initiative. Association of plasma neurofilament light with neurodegeneration in patients with Alzheimer disease. JAMA Neurol. 2017 May 1;74(5):557–66.

42 Mielke MM, Syrjanen JA, Blennow K, Zetterberg H, Vemuri P, Skoog I, et al. Plasma and CSF neurofilament light: relation to longitudinal neuroimaging and cognitive measures. Neurology. 2019 Jul 16;93(3):e252–60.

43 Preische O, Schultz SA, Apel A, Kuhle J, Kaeser SA, Barro C, et al. Serum neurofilament dynamics predicts neurodegeneration and clinical progression in presymptomatic Alzheimer's disease. Nat Med. 2019 Feb;25(2): 277–83.

44 Rissin DM, Kan CW, Campbell TG, Howes SC, Fournier DR, Song L, et al. Single-molecule enzyme-linked immunosorbent assay detects serum proteins at subfemtomolar concentrations. Nat Biotechnol. 2010 Jun; 28(6):595–9.