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RESEARCH ARTICLE

Blood β-synuclein is related to amyloid PET positivity in memory clinic patients

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Abstract

Introduction: β-synuclein is an emerging blood biomarker to study synaptic degeneration in Alzheimert's disease (AD), but its relation to amyloid-β (Aβ) pathology is unclear.

Methods: We investigated the association of plasma β-synuclein levels with [18F]flutemetamol positron emission tomography (PET) in patients with AD dementia

($n = 51$), mild cognitive impairment (MCI–Aβ+ $n = 18$, MCI– Aβ– $n = 30$), non-AD dementias ($n = 22$), and non-demented controls ($n = 5$).

Results: Plasma β-synuclein levels were higher in Aβ+ (AD dementia, MCI–Aβ+) than in Aβ– subjects (non-AD dementias, MCI–Aβ–) with good discrimination of Aβ+ from Aβ– subjects and prediction of Aβ status in MCI individuals. A positive correlation between plasma β-synuclein and Aβ PET was observed in multiple cortical regions across all lobes.

Discussion: Plasma β-synuclein demonstrated discriminative properties for Aβ PET positive and negative subjects. Our data underline that β-synuclein is not a direct marker of Aβ pathology and suggest different longitudinal dynamics of synaptic degeneration versus amyloid deposition across the AD continuum.

KEYWORDS
Alzheimert's disease, amyloid beta PET, blood biomarker, synaptic degeneration, β-synuclein

Highlights
- Blood and CSF β-synuclein levels are higher in Aβ+ than in Aβ– subjects.
- Blood β-synuclein level correlates with amyloid PET positivity in multiple regions.
- Blood β-synuclein predicts Aβ status in MCI individuals.

1 | INTRODUCTION

β-synuclein is a presynaptic protein and a novel biomarker candidate for synaptic degeneration that can be measured in blood. Synaptic degeneration is one of the major hallmarks of Alzheimert's disease (AD) and the pathological correlate of memory impairment.[1] The measurement of a surrogate biomarker for synaptic degeneration in blood should be of great value for early detection, diagnosis, and evaluation of the outcome and follow-up of clinical trials in patients due to its minimally invasive collection. We recently observed a consistent increase of β-synuclein in cerebrospinal fluid (CSF) and blood of sporadic AD patients[2–5] as an expected result of the release of synaptic proteins during synaptic degeneration into the extracellular space. Correlation with magnetic resonance imaging (MRI) showed that β-synuclein blood levels are mainly related to temporal brain atrophy, a region strongly affected in AD.[5] Higher β-synuclein levels in patients with mild cognitive impairment (MCI) indicate that β-synuclein in blood rises already in the early disease phase.[3,4] This is supported by data from subjects with Down Syndrome, who are known to overproduce amyloid-β (Aβ) due to triplication of the *APP* gene in trisomy of chromosome 21[6] and in whom β-synuclein levels are already increased in the presymptomatic stage.[7] The finding demonstrates that synaptic degeneration belongs to the earliest events in the pathogenesis of AD. However, it is unclear how levels of β-synuclein in blood relate to other key pathological hallmarks of AD, such as accumulation of Aβ plaques in the brain. Amyloid positron emission tomography (PET) is a well-established method for quantification of Aβ plaque deposition in brain both in experimental and clinical settings.[8–10] It has been used in several studies to investigate the relationship of Aβ pathology with blood and CSF biomarkers such as Aβ42/40 ratio or pTau181.[11,12]

The aim of this study was to investigate the relationship of plasma β-synuclein levels with brain Aβ plaque load using quantitative [18F]flutemetamol PET in a cohort of memory clinic patients with uncertain diagnosis after extensive memory assessment and following amyloid PET investigation received the diagnosis of AD dementia (ADD), Aβ positive (Aβ+, prodromal AD [pAD]) and negative (Aβ–) patients with MCI, non-AD dementias, and cognitively unimpaired (CU) individuals. We performed group comparisons and correlation analyses to investigate the association of plasma β-synuclein with Aβ load in the whole brain and receiver operating characteristic (ROC) curve analysis to determine the diagnostic performance.

2 | METHODS

2.1 | Study participants

This study consists of a clinical cohort of 126 patients (mean age = 65.6 ± 8.3, 65F/53 M), who had undergone extensive memory assessments at the Clinic for Cognitive Disorders, Theme Inflammation and Aging, Karolinska University Hospital, Stockholm, Sweden, and owing to a still uncertain diagnosis had been referred for [18F]flutemetamol amyloid PET. The patients had been referred due to cognitive problems from primary care physicians (GPs) and, in a

few cases, from other specialist clinics and secondary memory clinics seeking a second opinion. The patients underwent extensive memory assessment, including physical, neurological, and psychiatric assessment, medical history, neuropsychological testing, computed tomography imaging (CT)/MRI, cerebrospinal fluid (CSF) biomarker analysis, apolipoprotein E (*APOE*) genotyping, [18F]flutemetamol amyloid PET, and, in some cases, [18F]fluorodeoxyglucose PET.

Final diagnoses were achieved by a consensus of dementia expert team composed of specialists in cognitive disorders, clinical neuropsychologists, and specialist nurses. Main diagnostic categories included MCI,[13,14] AD,[15] and non-AD, including dementia of unclear etiology (not otherwise specified) (WHO, 1992), dementia with Lewy bodies,[16] frontotemporal dementia,[17] vascular dementia, including of subcortical type,[18] primary age-related tauopathy,[19–21] and alcohol-related dementia.[22] In a few subjects, after this extensive clinical assessment the diagnosis of neurodegenerative disorder was ruled out (grouped together and referred to as CU individuals).

The Regional Human Ethics Committee of Stockholm, Sweden, and the Isotope Committee of Karolinska University Hospital Huddinge approved this study. All patients gave their written informed consent.

2.2 | Neuropsychological assessments

The neuropsychological assessment included a multidomain battery of tests,[23] including the Mini-Mental State Examination (MMSE), components of the Wechsler Adult Intelligence Scale, Revised (information and similarities, logical memory, block design, and digit symbol), figure classification, a subtest of the Synonyms Reasoning Block Test, the Rey Auditory Verbal Learning Test, the copying and memory subtests of the Rey-Osterrieth Complex Figure Test, parts A and B of the Trail Making Test, and the Verbal Fluency Test.

2.3 | CSF AD biomarkers

Samples of CSF were collected between the L3/L4 or L4/L5 intervertebral space with a 25-gauge needle under non-fasting condition. Samples were collected in polypropylene tubes and centrifuged (3000 rpm, 10 min) within 2 h. Measurements of AD CSF biomarker levels were performed at the Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital in Mölndal, Sweden, where levels of Aβ42, total tau (tTau), and phosphorylated tau-181 (pTau) were determined using commercially available ELISAs (Enzyme-linked Immunosorbent Assay) (Fujirebio, Ghent, Belgium). AD biomarker concentrations were measured in clinical laboratory practice during the diagnostic work-up of patients by board-certified laboratory technicians who were blinded to clinical data. Patient samples were measured as singlicates with one round of freeze-thawing. Analytical variation was monitored using internal quality control (QC) samples (high and low within the clinically relevant concentration ranges). Interassay coefficients of variation were below 10% for all analytes. The laboratory is the coordinating laboratory for the Alzheimer's Association External QC Program for AD biomarkers.

RESEARCH IN CONTEXT

1. **Systematic Review**: Recent data support β-synuclein as an easily accessible blood biomarker to study synaptic degeneration in Alzheimert's disease (AD). We searched the PubMed database, but there is no information how β-synuclein relates to amyloid deposition in the brain, a key pathological hallmark of AD.

2. **Interpretation**: Plasma β-synuclein demonstrates discriminative properties between PET Aβ positivity and PET Aβ negativity, but our data underline that β-synuclein is not a direct marker of Aβ pathology, reflecting the different longitudinal dynamics of synaptic degeneration versus amyloid deposition across the AD continuum

3. **Future Directions**: The investigation of β-synuclein in blood in longitudinal studies can help to elucidate the longitudinal appearance of synaptic degeneration in the AD continuum and its association to amyloid deposition and other pathophysiological processes. It might also be a valuable read-out in clinical trials to assess protective effects on synapse degeneration.

2.4 | PET imaging

[18F]Flutemetamol PET scans were acquired using a Biograph mCT PET/CT scanner (Siemens/CTI, Knoxville, Tennessee, USA) at the Department of Medical Radiation Physics and Nuclear Medicine Imaging, Karolinska University Hospital, Huddinge, Sweden, as detailed elsewhere.[9] Reconstructions of the [18F]flutemetamol PET images were obtained using point-spread-function (PSF) modeling and a time-of-flight (TOF) algorithm (three iterations, 21 subsets, 3.0-mm Gaussian filter), resulting in the resolution of 128 × 128 × 1 (pixels) and a voxel size (mm) of 2.12 × 2.12 × 1. A nuclear medicine physician (I.S.) visually assessed [18F]flutemetamol summation images as positive or negative. Additionally, a quantitative analysis of [18F]flutemetamol uptake was performed based on an automated region of interest (ROI)-based approach implemented in Hermes Medical Solutions Brass software.[24] This analysis is template based and, therefore, probability based and not on an individual's MRI scans. The standardized uptake value ratios (SUVRs) were calculated by dividing average uptake in composite cortical ROI (including frontal, lateral temporal, occipital, parietal, and cingulate cortices) by the average uptake in the reference region (pons). Operational cut-off value for amyloid-positivity was defined based on separation from cognitively normal controls and was equal to 0.60.[25–27]

For ROI analysis, PET images were preprocessed with rPOP pipeline[28] for PET-only datasets in MATLAB (MathWorks, version R2022_a) and SPM 12, and extraction of regional values was carried out according to a simplified Harvard-Oxford atlas[29–32] (modification: subdivisions of gyri were pooled together, resulting in 31 ROIs per brain hemisphere).

2.5 | β-synuclein determination in plasma and CSF

Plasma was collected in sodium-heparin tubes (Vacutainer®, BD Diagnostics) and centrifuged (1,500 × g, +4°C) for 10 min. Following centrifugation, samples were aliquoted in polypropylene tubes and stored at −80°C within 30–60 min of collection. β-synuclein was measured in plasma (500 μL) using immunoprecipitation-mass spectrometry, as previously described.[3] Measurements were performed in a single run with single measurements, and QC samples showed an intra-assay coefficient of variation (CV) of 3.7%–7.3%.

CSF levels of β-synuclein were determined in single measurements using a validated in-house ELISA described by Halbgebauer et al.[4] in one experiment with two runs. CSF QC samples showed intra- and interassay CVs of 5.6%–8.0% and 9.0%.

Samples were thawed once for β-synuclein measurements in CSF and plasma, and all measurements were performed blinded to patient diagnosis.

2.6 | Plasma pTau181 determination

Plasma pTau181 was analyzed using an in-house Simoa method, as described previously in detail.[12] The repeatability was 5.5% and the intermediate precision was 7.5%. All clinical samples were analyzed in single measures.

2.7 | Statistical analysis

Statistical analysis was performed in R (version 1.4.1717, https://www.r-project.org), whereas data visualizations were created using the ggplot2 package (version 3.3.5). To detect whether outliers were present in the data, we applied a Hampel filter and univariate outlier detection (extremevalues package, version 2.3.3). Sex difference was tested by Pearson's chi-squared test and nominal variables by Kruskal-Wallis one-way analysis of variance (stats package version 4.1.1) and Dunn's post hoc test with false discovery rate (FDR) correction (rstatix package, version 0.7.0). The CU group was excluded from biomarker comparisons due to its small sample size ($N = 5$). Due to the variables' distribution, the relationships between β-synuclein levels in plasma and in CSF, plasma pTau181, and amyloid PET burden were tested with Spearman's rank correlation coefficient (correlation package, version 0.8.0).

To identify the best combination of biomarkers able to predict Aβ status as defined by amyloid PET, we implemented a Cox regression model with least absolute shrinkage and selection operator (LASSO) (glmnet package, version 4.1.4), which was validated with 10-fold cross-validation to determine the optimal LASSO penalty. We chose this analysis as it allows for maximizing the discrimination accuracy of the model while reducing its dimension by avoiding overfitting and dropping redundant variables. A total of five variables were included in the LASSO Cox regression: CSF Aβ42, CSF pTau, CSF tTau, plasma β-synuclein, and CSF β-synuclein. The performance of the best LASSO model and of single biomarkers was compared by ROC curve analysis (pROC package, version 1.18.0). The extreme data points that were identified by both methods as the most influential outliers were excluded from further analysis. For ROI analysis of PET images, a linear model was fitted at every region, adjusting for age and sex. The resulting p-values were adjusted with a Bonferroni correction for multiple comparisons. The results were visualized with the ggseg package (version 1.6.5). A p-value < 0.05 was regarded significant.

3 | RESULTS

3.1 | Study participants

Demographic and biomarker data for all participants are shown in Table 1. In total, the study population consisted of 126 individuals with plasma/CSF β-synuclein measures and amyloid PET. Note that in a subgroup of individuals, either CSF or plasma β-synuclein could not be measured due to analytical failure, leading to different numbers of subjects in the plasma ($n = 118$) and CSF ($n = 112$) subgroups. Based on the outlier detection procedure implemented in the study, one outlier was excluded from further analysis. Therefore, the final sample composed of 117 individuals with plasma β-synuclein available, of which 24 were MCI−Aβ−, 16 pAD, 50 with ADD, 22 patients with non-AD (frontotemporal dementia, Lewy body dementia, subcortical vascular dementia), and five CU individuals. For CSF β-synuclein, this distribution was as follows: 26 MCI−Aβ−, 16 pAD, 44 ADD, 21 non-AD, and five CU. Study diagnostic groups were not significantly different in terms of age ($p = 0.76$) and sex ($p = 0.18$) distribution. MMSE scores were significantly different between the groups ($p < 0.001$), and the following pairwise comparisons produced significant results in the post hoc analysis upon FDR correction: MCI−Aβ− > non-AD; MCI Aβ− < CU, pAD > ADD and non-AD, ADD > non-AD.

3.2 | Plasma and CSF β-synuclein is higher in AD

Plasma β-synuclein was significantly higher in ADD (11.85 ± 3.53pg/mL, $p < 0.001$) and pAD (10.85 ± 2.75 pg/mL, $p < 0.01$) participants compared with the other groups (Figure 1A). No significant difference in plasma β-synuclein values was observed between ADD and pAD patients. CSF β-synuclein showed similar changes (pAD (513 ± 142.81pg/mL, $p < 0.001$ vs. MCI−Aβ−, and $p < 0.05$ vs. non-AD), ADD (509.99 ± 236.34pg/mL, $p < 0.001$ vs. MCI-Aβ-, and $p < 0.05$ vs. non-AD)) (Figure 1B). Levels of brain amyloid plaque load quantified with [18F]flutemetamol PET across the diagnostic groups is shown in Figure 1C. In contrast to β-synuclein, CSF Aβ42 was not different between pAD and MCI−Aβ− groups (Figure 1D), whereas differences in levels of pTau and tTau (Figure 1E,F) were observed between MCI-Aβ- and pAD, MCI-Aβ- and ADD, pAD and non-AD, and ADD and non-AD groups. Grouping all patients according

TABLE 1 Characteristics of study population and diagnostic subgroups.

	MCI Aβ− (N = 24)	pAD (N = 16)	ADD (N = 50)	Non-AD (N = 22)	CU (N = 5)	Total (N = 117)	*p*-value
Age							0.450 (1)
Mean (SD)	67.21 (10.80)	67.19 (8.70)	64.12 (7.34)	66.77 (7.22)	61.40 (6.11)	65.56 (8.32)	
Sex							0.295 (2)
F	14 (58.3%)	12 (75.0%)	28 (56.0%)	9 (40.9%)	2 (40.0%)	65 (55.6%)	
M	10 (41.7%)	4 (25.0%)	22 (44.0%)	13 (59.1%)	3 (60.0%)	52 (44.4%)	
MMSE[a]							<0.001 (1)
Mean (SD)	25.79 (3.32)	27.75 (1.81)	25.29 (3.35)	23.10 (4.00)	29.40 (0.55)	25.51 (3.56)	
[18F]Flutemetamol PET SUVRs							<0.001 (1)
Mean (SD)	0.46 (0.05)	0.73 (0.10)	0.77 (0.11)	0.46 (0.08)	0.44 (0.01)	0.63 (0.18)	
Plasma β-synuclein (pg/ml) [b]							<0.001 (1)
Mean (SD)	7.89 (2.67)	10.85 (2.75)	11.85 (3.53)	8.65 (3.45)	6.49 (0.50)	10.07 (3.63)	
CSF β-synuclein (pg/ml)[c]							<0.001 (1)
Mean (SD)	284.12 (142.15)	527.18 (137.58)	509.99 (236.34)	357.37 (206.29)	345.67 (98.30)	430.15 (218.28)	
CSF Aβ42 (pg/ml)							<0.001 (1)
Mean (SD)	665.58 (181.20)	600.75 (139.14)	517.10 (145.60)	755.95 (332.53)	3418.60 (5214.30)	727.91 (1144.52)	
CSF tTau (pg/ml)							<0.001 (1)
Mean (SD)	260.71 (200.54)	546.00 (197.33)	520.14 (231.68)	326.32 (241.72)	209.20 (45.90)	419.87 (248.31)	
CSF pTau (pg/ml)[d]							<0.001 (1)
Mean (SD)	35.62 (13.11)	74.44 (30.10)	76.32 (40.00)	44.14 (22.62)	32.80 (10.62)	59.80 (35.63)	

Note: (1) Kruskal-Wallis rank sum test (2). Pearson's chi-squared test.

Abbreviations: Aβ+, amyloid positive; Aβ−, amyloid negative; CU, cognitively unimpaired; AD, Alzheimer's disease; ADD, AD dementia; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; pAD, prodromal Alzheimert's disease; pTau, phosphorylated tau-181; SUVR, standardized uptake value ratio; tTau, total tau.

[a]MMSE scores were missing for one ADD and one non-AD.
[b]Plasma β-synuclein values were missing for five MCI Aβ−, two pAD, and one ADD.
[c]CSF β-synuclein values were missing for four MCI Aβ-, two pAD, seven ADD, one non-AD.
[d]CSF tTau values were missing for one ADD.

to Aβ status, plasma and CSF β-synuclein were higher in Aβ+ compared with Aβ− individuals (Figure 2A,B, *p* < 0.001 for both comparisons).

3.3 | Plasma and CSF β-synuclein were associated with level of Aβ deposition

To assess whether β-synuclein concentration measured in blood mirrored the concentration of β-synuclein in CSF, we estimated a Spearman's rank correlation coefficient and observed a significant positive correlation between plasma and CSF β-synuclein in the whole group (r_s = 0.38, *p* < 0.001, Figure 3A). Aβ burden positively correlated with plasma β-synuclein in the whole cohort (r_s = 0.46, *p* < 0.0001, Figure 3B) and in MCI individuals (Aβ+ and Aβ−, r_s = 0.38, *p* < 0.05, Figure 3C) but not when Aβ+ or Aβ− subjects were examined separately (r_s = 0.02, *p* = 0.88 in Aβ+ group and r_s = −0.04, *p* = 0.77 in Aβ− group). In addition, plasma β-synuclein showed a significant positive correlation with plasma pTau181 (r_s = 0.32, *p* < 0.001,

Figure 3D), an established blood marker associated with amyloid pathology.

Likewise, association of CSF β-synuclein with Aβ burden was estimated, and a positive association in the whole sample (r_s = 0.44, *p* < 0.001), as well as in MCI individuals (r_s = 0.59, *p* < 0.001), was found. Regional comparison by ROI analysis of the PET images showed that plasma β-synuclein was associated with higher regional [18F]flutemetamol binding in frontal regions, precuneous, posterior cingulate, superior parietal lobule, and other cortical regions (Figure 4A) (see Table 2 for details).

3.4 | Plasma and CSF β-synuclein showed good performance in discriminating between Aβ-positive and -negative cases

To assess the ability of plasma β-synuclein to detect Aβ status determined with [18F]flutemetamol PET in relation to CSF AD biomarkers,

TABLE 2 Association of plasma ß-synuclein with regional [18F]flutemetamol bindings.

| ROIs	p-value	t-value	Beta	F-value
Left frontal pole	0.003	4.227	4.227	8.685
Right frontal pole	0.001	4.566	4.566	10.010
Right insular cortex	0.013	3.830	3.830	7.446
Left superior frontal gyrus	0.009	3.942	3.942	7.821
Right superior frontal gyrus	0.004	4.153	4.153	8.641
Left middle frontal gyrus	0.003	4.251	4.251	8.300
Right middle frontal gyrus	0.001	4.529	4.529	9.663
Left inferior frontal gyrus pars triangularis	0.008	3.983	3.983	7.445
Right inferior frontal gyrus pars triangularis	0.004	4.171	4.171	8.578
Left inferior frontal gyrus pars opercularis	0.019	3.724	3.724	7.572
Right inferior frontal gyrus pars opercularis	0.002	4.314	4.314	8.846
Left superior temporal gyrus	0.044	3.483	3.483	6.089
Right superior temporal gyrus	0.014	3.808	3.808	6.779
Left middle temporal gyrus	0.020	3.718	3.718	6.045
Right middle temporal gyrus	0.017	3.765	3.765	6.050
Left inferior temporal gyrus	0.014	3.810	3.810	6.233
Right inferior temporal gyrus	0.021	3.695	3.695	5.781
Right postcentral gyrus	0.028	3.617	3.617	6.191
Left superior parietal lobule	0.000	4.941	4.941	10.063
Right superior parietal lobule	0.000	4.791	4.791	9.890
Left supramarginal gyrus	0.003	4.203	4.203	7.637
Right supramarginal gyrus	0.002	4.370	4.370	8.566
Left angular gyrus	0.016	3.786	3.786	5.994
Right angular gyrus	0.004	4.128	4.128	7.739
Left lateral occipital cortex	0.015	3.795	3.795	5.916
Right lateral occipital cortex	0.005	4.076	4.076	6.816
Left frontal medial cortex	0.003	4.263	4.263	8.010
Right frontal medial cortex	0.001	4.495	4.495	8.879
Left subcallosal cortex	0.007	4.019	4.019	7.965
Right subcallosal cortex	0.015	3.798	3.798	7.052
Left anterior cingulate gyrus paracingulate	0.011	3.876	3.876	7.460
Right anterior cingulate gyrus paracingulate	0.005	4.093	4.093	8.439
Left cingulate gyrus posterior division	0.002	4.317	4.317	8.123
Right cingulate gyrus posterior division	0.002	4.300	4.300	8.014
Left precuneous cortex	0.004	4.152	4.152	7.128
Right precuneous cortex	0.001	4.490	4.490	7.950
Left frontal orbital cortex	0.011	3.884	3.884	7.109
Right frontal orbital cortex	0.004	4.185	4.185	7.876
Left operculum cortex	0.032	3.583	3.583	6.195
Right operculum cortex	0.006	4.043	4.043	7.317
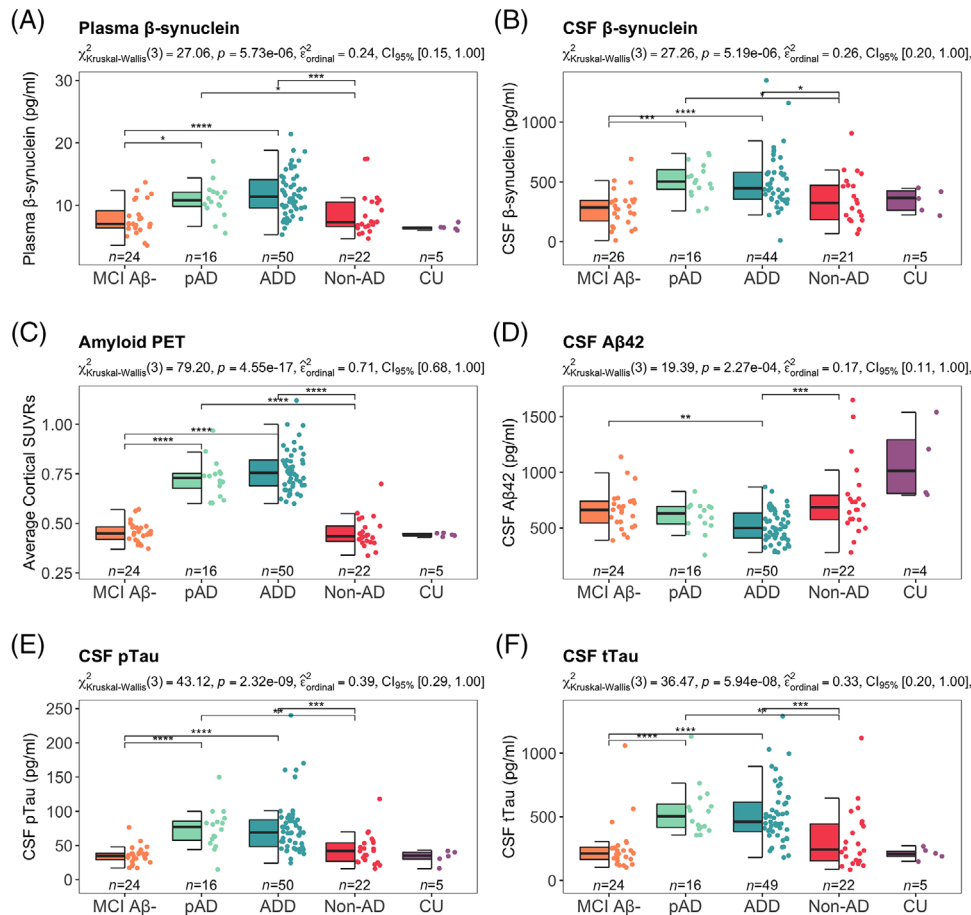
FIGURE 1 Comparison of plasma and CSF β-synuclein, CSF Alzheimer's biomarkers, and amyloid PET SUVRs in different diagnostic groups. (A) Plasma and (B) CSF β-synuclein concentrations were significantly higher in amyloid-positive groups (ADD and pAD) in comparison to amyloid-negative groups (Aβ− mild cognitive impairment, MCI−Aβ− and non-AD dementias). (C) Distribution of amyloid PET average cortical SUVRs across groups. (D) Significantly different concentrations of Aβ42 in CSF between ADD and MCI Aβ− and non-AD. (E–F) CSF phosphorylated (pTau) and total tau (tTau) were significantly different between MCI Aβ− and pAD, MCI Aβ− and ADD, pAD and non-AD, as well as between ADD and non-AD groups. *p*-values in subtitle indicate the result of the analysis of variance with Kruskal-Wallis test, between groups – of post hoc analysis with Dunn's test, and multiple comparisons correction with FDR. Aβ+, amyloid positive; Aβ−, amyloid negative; CU, cognitively unimpaired; AD, Alzheimer's disease; ADD, AD dementia; MCI, mild cognitive impairment; pAD, prodromal AD; pTau, phosphorylated tau-181; SUVR, standardized uptake value ratio; tTau, total tau. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

we used LASSO Cox regression model alongside the ROC curve analysis in the whole sample (Figure 4B). After LASSO Cox regression, two out of five variables had non-zero coefficients and remained significant predictors of Aβ status: CSF pTau and plasma β-synuclein. ROC curve analysis showed that this model had the best discrimination performance with an area under the curve (AUC) of 91.4% and a sensitivity of 97% alongside a specificity of 72% (Figure 4B, Table S1). With respect to individual biomarkers, this result was followed by CSF pTau and tTau separately, which showed AUCs of 88.5% and 86.1%, respectively. Note that, despite the good performance of tTau, this biomarker was dropped by the Cox LASSO model, possibly due to redundancy. Similarly, CSF β-synuclein had a zero coefficient. Plasma β-synuclein could distinguish Aβ+ from Aβ− individuals with an AUC of 80.9% and a sensitivity and specificity of 74% and 82.1% (Table S1). CSF β-synuclein

showed an AUC of 80.4% and a sensitivity and specificity of 84.8% and 68.9%, respectively. Interestingly, the CSF biomarker of Aβ status (Aβ42) had an AUC of 74.9% and specificity of 50%, meaning that at the present memory clinic patient population, the probability of CSF Aβ42 being negative for Aβ+ individuals was at the chance level, which is why patients had been referred for amyloid PET scans due to inconclusive CSF biomarker findings. We observed a similar performance of biomarkers in the prediction of Aβ status in MCI subjects prior to PET scans (Figure 4C, Table S1). In the LASSO Cox regression model, the inclusion of CSF pTau, tTau, Aβ42, and plasma β-synuclein showed the best discrimination with an AUC of 95%. Of the individual biomarkers, CSF pTau (AUC of 92%) and tTau (AUC of 91%) performed best, and CSF β-synuclein (AUC of 89%) performed a bit better here than plasma β-synuclein (AUC of 82%).
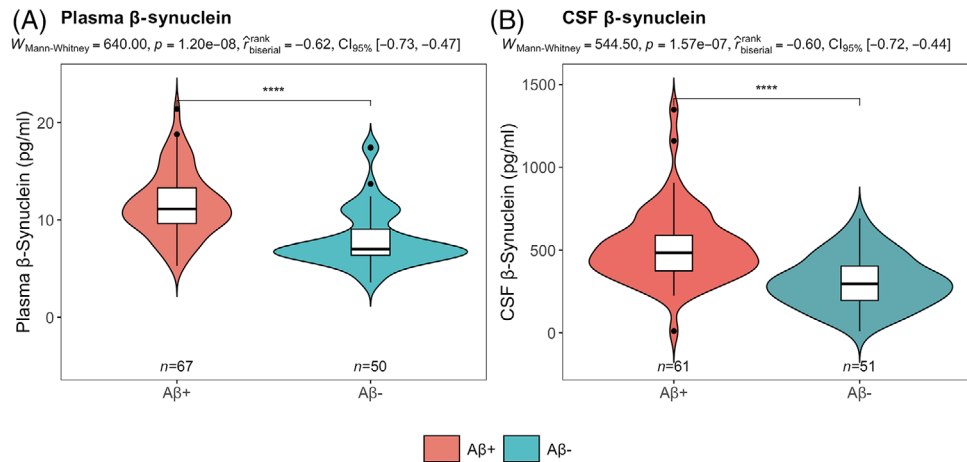
FIGURE 2 Higher β-synuclein levels in plasma and CSF of Aβ+ individuals. (A and B) β-synuclein levels were increased in CSF and plasma of Aβ+ individuals. Violin boxplots with error bars show distribution of individual values and summary statistics such as median and interquartile ranges in Aβ− and Aβ+ groups. ****Indicates levels of significance as $p < 0.0001$. Aβ+, amyloid positive; Aβ−, amyloid negative.
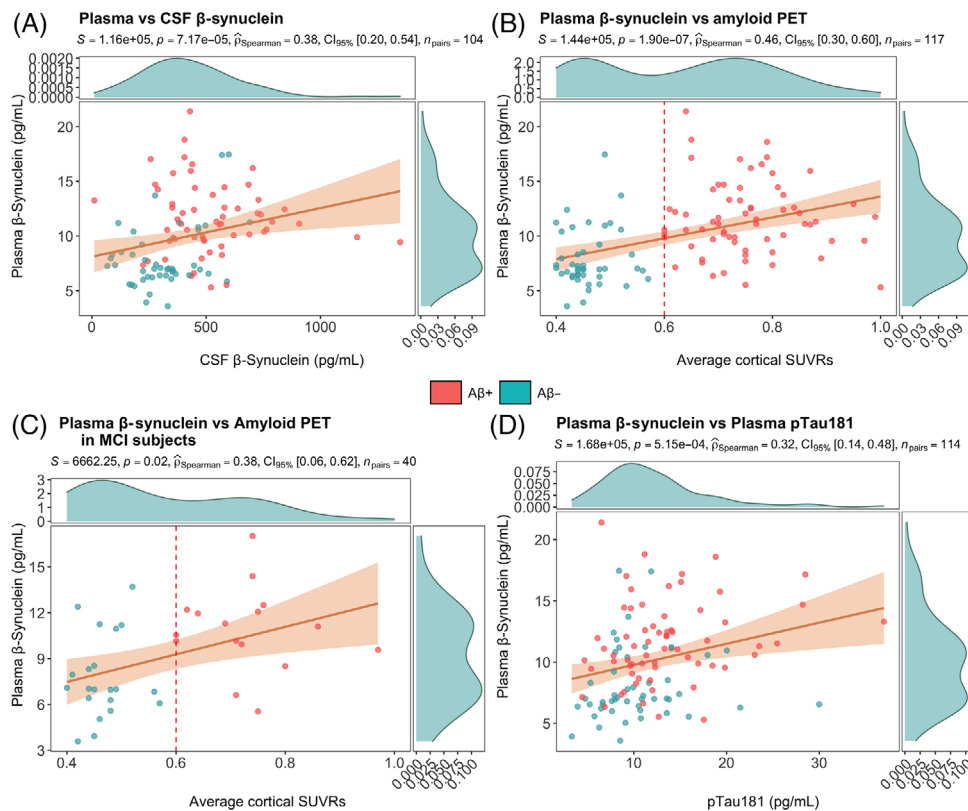
FIGURE 3 Correlation analyses and performance of plasma β-synuclein in discriminating between Aβ− and Aβ+ individuals. (A) Spearman's rank correlation test shows significant relationship between plasma and CSF β-synuclein concentrations. (B) Significant positive correlation between plasma β-synuclein and Aβ plaque load in brains of memory clinic patients. (C) Results of Spearman's rank correlation test in subgroups of MCI individuals showing a significant relationship between Aβ burden on amyloid PET and plasma β-synuclein. (D) Spearman's rank correlation test shows significant relationship between plasma β-synuclein and pTau181 concentrations. Each plot has side panels displaying the distribution density of data points along the corresponding axis (created using ggside version 0.2.0). Red dashed line represents the cut-off for amyloid positivity (SUVRs of 0.6). Dots are individual values colored according to Aβ status. Solid lines are from linear regression and 95% confidence interval band. Aβ+, amyloid positive; Aβ−, amyloid negative.
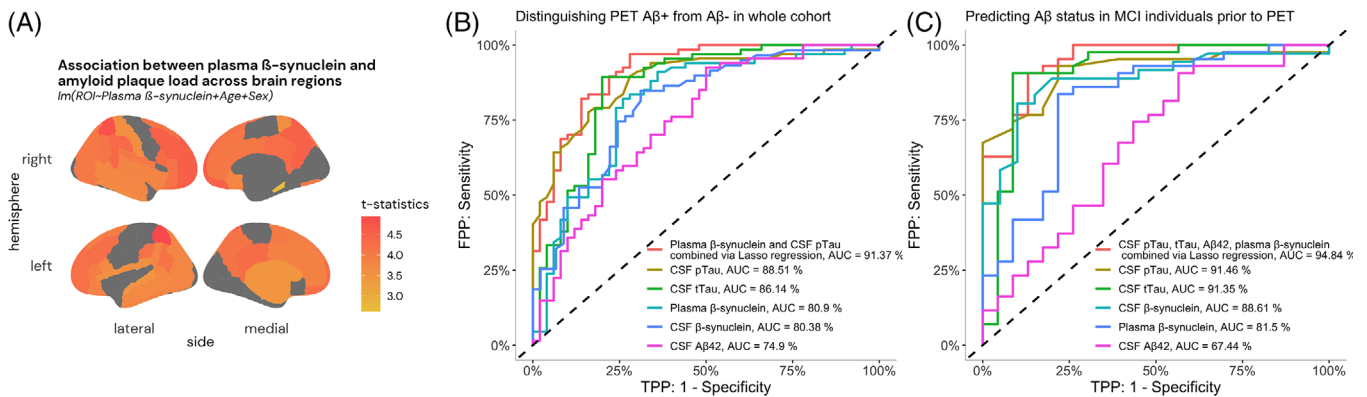
FIGURE 4 Regional associations between β-synuclein and Aβ PET and results of ROC curve analyses. (A) Results of ROI analysis—visualizations of regions showing significant effect of β-synuclein after adjusting for age and sex and correcting for multiple comparisons with Bonferroni correction ($p < 0.05$). (B) Discrimination between Aβ+ and Aβ− status defined by PET scan (whole sample, $N = 117$). (C) Prediction of Aβ status in patients with MCI ($N = 66$) whose diagnosis was clarified after PET. Aβ+, amyloid positive; Aβ−, amyloid negative; FPP, false positive proportion; MCI, mild cognitive impairment; ROC, receiver operating characteristic; TPP, true positive proportion.

4 | DISCUSSION

In this study, we demonstrated higher β-synuclein levels in plasma and CSF of Aβ+ versus Aβ− subjects and showed a significant but moderate correlation with ^{18F}flutemetamol PET, suggesting that β-synuclein is not a direct marker of Aβ pathology.

The main aim of our study was to investigate the relationship between plasma β-synuclein levels and brain Aβ load, which had not been previously investigated. Here, we showed that plasma β-synuclein levels were significantly higher in Aβ+ than in Aβ− subjects and could thus, with β-synuclein measurement, discriminate between patients with AD and other dementias, including frontotemporal dementia, sub-cortical vascular dementia, and Lewy body dementia. In addition, we observed a significant positive correlation between β-synuclein levels and global Aβ deposition in the whole patient cohort suggesting an association of β-synuclein with Aβ pathology. An association with the amount of Aβ load has also been shown for the synaptic marker neurogranin in CSF and is consistent with our finding.[33] However, the correlation of β-synuclein was moderate, and there was no correlation with the Aβ+ subjects alone. It is well known that the Aβ load measured by PET reaches a plateau at the MCI-early AD dementia stage,[34] and our finding thus supports the assumption that β-synuclein is partly associated with brain Aβ load since synaptic degeneration in ADD is ongoing during the progression of the disease. The relation might be stronger in the very early (presymptomatic) stages of AD when β-synuclein levels already rise[7] and Aβ deposition is more dynamic,[34] but this needs to be confirmed.

We also analyzed the association of ROI-based regional Aβ deposition with plasma β-synuclein levels. In the MCI and dementia stages of AD, both synaptic loss[35] and Aβ accumulation[36] are present in nearly all cortical regions. In agreement with this, we observed a significant correlation of plasma β-synuclein with Aβ PET in many cortical regions. No significant correlation was observed in the pre- and postcentral gyri. In these regions, Aβ accumulation seems to occur in the latest stages of AD,[36] which might not yet have been reached by the patients

in our cohort or might be reflected in blood at a later time point with some delay. On the other hand, the regional association of blood β-synuclein levels is hampered by the fact that it reflects global synaptic degeneration, reducing sensitivity for specific brain regions. However, Ot'Dell and colleagues also observed no consistent regional pattern for the ROI-based correlation of synaptic loss and Aβ deposition using PET imaging.[35] Studies on the longitudinal association of synaptic degeneration with Aβ deposition will provide a broader picture of the relation of both pathologies.

Tau pathology has also been related to synaptic degeneration, and it could be a confounding factor in our analyses because the degree of tau pathology is unknown in the patients from our cohort. Therefore, further studies are needed to characterize the association of blood β-synuclein levels with tau pathology. A correlation of β-synuclein with CSF tTau levels has been described,[3] but CSF tau is a general neurodegeneration marker and does not reflect tau pathology as initially thought. The recent developments of tau PET tracers[37] suggest a correlation with neurodegeneration and cognition, and Tau PET appears to be a better predictor of cognitive decline than amyloid PET and CSF biomarkers.[38,39] It will be a promising opportunity to study this association with β-synuclein further. In addition, the comparison of β-synuclein levels with SV2A PET data[40] will provided further information to support it as a marker of synaptic degeneration.

Prodromal AD patients represent the early symptomatic disease phase of AD, and higher β-synuclein in plasma and CSF in this group support the assumption that synaptic degeneration is an early event in AD. A recent study in individuals with Down Syndrome, representing a genetic form of AD due to the triplication of the *APP* gene on chromosome 21, showed that β-synuclein was already increased in the presymptomatic phase of AD and seemed to rise from an age of 27 years.[5] Although this needs to be confirmed in sporadic and monogenetic AD, it is robust evidence that synaptic degeneration belongs to the earliest events in AD pathophysiology and that β-synuclein levels in blood might be used as an early disease biomarker.

We previously showed increased β-synuclein levels in the serum of AD patients[3] and could confirm this observation in the present study also in plasma samples. In addition, absolute β-synuclein concentrations and group differences in serum[3] and plasma are comparable, showing the robustness of β-synuclein as a biomarker and of the assay. Both plasma and CSF β-synuclein levels showed consistent changes in the diagnostic groups, but correlation of quantitative levels was only moderate. This agrees with our previous study[3] and might originate in the use of different techniques to measure plasma and CSF levels (mass spectrometry vs. ELISA). In addition, β-synuclein can enter blood not only from CSF drainage from bulk flow but also via the blood-brain barrier or glymphatic system and thus is affected by additional mechanisms besides CSF levels. An only moderate correlation of blood and CSF levels has also been described for other central nervous system-derived biomarkers such as glial fibrillary acidic protein,[41] supporting this hypothesis. Plasma and CSF β-synuclein levels showed good discriminatory power to separate Aβ+ from Aβ− subjects but lower than the AD core biomarkers in CSF (pTau and tTau). In the combined model, plasma β-synuclein added useful information to the panel of CSF biomarkers and together with CSF pTau showed the best discriminatory power, although the increase of performance in the detection of Aβ positivity was only subtle. Similar results were observed for the prediction of Aβ status in MCI subjects prior to Aβ PET.

We compared plasma β-synuclein levels with a more established AD blood marker, plasma pTau181, and observed a significant but moderate correlation. This is consistent with our previous findings[5] and attributed to the different mechanisms reflected by these two biomarkers. Plasma pTau181 has been shown to mainly reflect AD-related amyloid pathology[42] and seems to be more specific for AD than β-synuclein as a general synaptic marker.[5]

A limitation of our study might be that the clinical patients cohort we studied had undergone [18F]flutemetamol PET since the clinical diagnosis had found to be uncertain despite extensive clinical assessment. Thus, out of 126 patients, 75 had received, prior to amyloid PET investigation, a diagnosis of MCI, and amyloid PET allowed more specific diagnosis such as pAD, MCI-Aβ-, ADD, and non-AD. Since the clinical presentation, including CSF biomarker findings, was in some cases not typical of AD or non-AD (e.g., for CSF Aβ42), the cohort might not entirely reflect a typical cohort of patients from a clinical study. However, it better reflects a real-world situation at a tertiary memory clinic that often receives the referral of more atypical or early memory patients. Also, the non-AD group included several disease entities with a limited number of subjects in each disease group, which should be considered for data interpretation.

In conclusion, our data demonstrate elevated plasma and CSF β-synuclein levels in brain PET amyloid-positive ADD and pAD subjects compared to amyloid-negative patients. The correlation of plasma β-synuclein with quantitative Aβ PET shows an association of synaptic degeneration with Aβ pathology in the brain but also indicates that both pathophysiological mechanisms might have different longitudinal dynamics. Blood β-synuclein is an easily accessible biomarker that can be helpful in further studies to investigate synaptic degeneration in the AD continuum and its relationship with other pathological changes.

CONFLICT OF INTEREST STATEMENT

Markus Otto, Patrick Oeckl, and Steffen Halbgebauer declare that they are co-applicants of a filed patent application for β-synuclein measurement in blood. Henrik Zetterberg has served on scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZPath, Annexon, Apellis, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Optoceutics,

CONSENT STATEMENT

All patients gave their written informed consent.

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SUPPORTING INFORMATION

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