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FEATURED ARTICLE

Relationship of serum beta-synuclein with blood biomarkers and brain atrophy

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Abstract

Background: Recent data support beta-synuclein as a blood biomarker to study synaptic degeneration in Alzheimer's disease (AD).

Methods: We provide a detailed comparison of serum beta-synuclein immunoprecipitation – mass spectrometry (IP-MS) with the established blood markers phosphorylated tau 181 (p-tau181) (Simoa) and neurofilament light (NfL) (Ella) in the

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German FTLD consortium cohort ($n = 374$) and its relation to brain atrophy (magnetic resonance imaging) and cognitive scores.

Results: Serum beta-synuclein was increased in AD but not in frontotemporal lobar degeneration (FTLD) syndromes. Beta-synuclein correlated with atrophy in temporal brain structures and was associated with cognitive impairment. Serum p-tau181 showed the most specific changes in AD but the lowest correlation with structural alterations. NfL was elevated in all diseases and correlated with frontal and temporal brain atrophy.

Discussion: Serum beta-synuclein changes differ from those of NfL and p-tau181 and are strongly related to AD, most likely reflecting temporal synaptic degeneration. Beta-synuclein can complement the existing panel of blood markers, thereby providing information on synaptic alterations.

KEYWORDS
Alzheimer's disease, beta-synuclein, blood biomarker, brain atrophy, dementia, frontotemporal lobar degeneration, FTLD, NfL, p-tau181, synaptic degeneration

Highlights

- Blood beta-synuclein is increased in Alzheimer's disease (AD) but not in frontotemporal lobar degeneration (FTLD) syndromes.
- Blood beta-synuclein correlates with temporal brain atrophy in AD.
- Blood beta-synuclein correlates with cognitive impairment in AD.
- The pattern of blood beta-synuclein changes in the investigated diseases is different to phosphorylated tau 181 (p-tau181) and neurofilament light (NfL).

1 | BACKGROUND

Beta-synuclein is a presynaptic protein and a promising synaptic biomarker candidate that can be measured in cerebrospinal fluid (CSF) and blood.[1,2] Synapses are the sites of signal transduction, signal integration, and memory formation in the brain, and impairment of synaptic function or integrity is linked to many neurological diseases.[3] Synaptic degeneration is an early hallmark of Alzheimer's disease (AD),[4] the most common form of dementia. Synaptic alterations have also been observed in other types of dementia including syndromes of the frontotemporal lobar degeneration (FTLD) spectrum of diseases (i.e., behavioral variant frontotemporal dementia [bvFTD], semantic variant of primary progressive aphasia [svPPA], non-fluent variant of PPA [nfvPPA], progressive supranuclear palsy [PSP], and corticobasal syndrome [CBS]).[5] Fluid biomarkers offer the opportunity to characterize synaptic alterations in patients during the lifetime, are capable of uncovering differences between neurological diseases, and can be measured longitudinally to mirror the neuropathology and pathophysiology. They are also in the center of interest to support clinical (differential) diagnosis and to evaluate treatment effects in clinical trials. Especially brain-derived biomarkers in blood have recently become available and are attractive due to their minimally invasive collection and applicability in a broad population.[6]

Since the first description of increased beta-synuclein concentrations in cerebrospinal fluid (CSF) of patients with AD,[7] we[1,2] and others[8,9] have confirmed this observation in different patient cohorts and with different techniques (mass spectrometry and enzyme-linked immunosorbent assay [ELISA]), supporting its value as a reliable synaptic biomarker. A major challenge in synaptic biomarker research so far has been the successful translation of changes observed in the CSF to the measurement in blood. Previous efforts for other synaptic proteins failed, mainly because of low sensitivity or confounding expression of markers in peripheral tissues.[10] We recently developed a sensitive and specific assay to measure beta-synuclein concentration in blood and showed increased levels in patients with AD,[1] similar to the observations in CSF. In patients with Down syndrome, representing a genetic form of AD, beta-synuclein levels are already increased in individuals without clinical signs of AD, indicating that synaptic degeneration belongs to the earliest events in AD.[11] This makes beta-synuclein a promising synaptic biomarker candidate in blood, which might complement the available panel of central nervous system (CNS)–derived blood markers. However, because beta-synuclein is a relatively new synaptic biomarker candidate, a thorough characterization of its changes in different neurological diseases and its relation to structural changes in the brain and to other blood markers, is of fundamental importance to

estimate its potential additive value to the existing panel of blood markers.

The aim of our study was the comparison of serum beta-synuclein changes in AD and FTLD with the established blood biomarkers tau protein phosphorylated tau 181 (p-tau181) and neurofilament light (NfL) and investigate its relation to structural brain changes in a large multicentric cohort. We investigated serum beta-synuclein levels in 374 individuals from the German FTLD Consortium cohort comprising patients with AD ($n = 74$), bvFTD ($n = 81$), svPPA ($n = 41$), nfvPPA ($n = 55$), logopenic variant of PPA (lvPPA, $n = 25$), PSP ($n = 42$), CBS ($n = 25$) and 31 cognitively unimpaired individuals (CUs). The ability to distinguish diagnostic groups by the three biomarkers was assessed with receiver-operating characteristic (ROC) curve analysis. Association with cognitive performance and severity of dementia was examined by correlation analysis with clinical scores. In addition, we investigated the relationship of biomarker levels with atrophy in different brain regions using unbiased quantitative analysis of structural magnetic resonance imaging (MRI).

RESEARCH IN CONTEXT

1. **Systematic review**: Recent data support beta-synuclein as an easily accessible blood biomarker to study synaptic degeneration in Alzheimer's disease (AD). We searched the PubMed database but there is no information about how beta-synuclein relates to the established blood biomarkers phosphorylated tau 181 (p-tau181) and neurofilament light (NfL). Changes in the frontotemporal lobar degeneration (FTLD) spectrum and its association with regional brain atrophy are unknown.
2. **Interpretation**: Beta-synuclein changes in blood are strongly related to AD and mainly reflect synaptic degeneration in the temporal lobe. The pattern of beta-synuclein changes in the investigated diseases is different to the established markers NfL and p-tau181 supporting the added value of beta-synuclein to the existing panel of markers.
3. **Future directions**: A minimally invasive beta-synuclein blood test can fill an important gap in AD research and enable the study of synaptic degeneration in large longitudinal cohorts and also in clinical trials. The application spectrum goes beyond AD and can help to monitor synaptic alterations also in other diseases such as traumatic brain injury or stroke.

2 | METHODS

2.1 | Patients

In this case-control study, we included samples from patients ($n = 374$) recruited between 2011 and 2018 within the German FTLD Consortium (www.ftld.de), a quality-controlled, monitored, multicenter initiative. Participating centers were Bonn, Erlangen, Goettingen, Hamburg, Homburg, Leipzig, Munich (TU and LMU), Rostock, Ulm, and Wuerzburg. All individuals or their legal proxies provided written informed consent for inclusion into this study and it was approved by the local ethics committees of the participating centers (approval number 39/11).

All participants underwent a standardized neurological and neuropsychological examination and MRI of the brain in all centers. Cognitive performance and severity of dementia was assessed using the Mini-Mental State Examination (MMSE), Clinical Dementia Rating sum-of-boxes (CDR-SB), and the FTLD-specific CDR score. Serum was extracted from venous blood and stored within 2 hours at −80°C. All serum samples were stored centrally at the Ulm University Hospital, Department of Neurology.

Clinical diagnoses of AD, bvFTD, svPPA, nfvPPA, lvPPA, PSP, and CBS were established according to internationally accepted criteria.[12–16] CSF amyloid beta 42 (Aβ42) was determined during diagnostic workup at the different centers but not for all patients. Cognitively unimpaired individuals (or CUs) in the same age range and without neurological disease served as controls. In the subgroup analysis, AD patients were divided by their CDR-SB value into patients with mild cognitive impairment due to AD (MCI-AD, CDR-SB <2.5) and AD dementia (CDR-SB ≥2.5) as described previously.[17] The FTLD group included 47 patients with a known FTLD-associated mutation including 9× *C9orf72*, 8× *GRN*, 4× *MAPT*, and other mutations with lower frequency.[18]

2.2 | Brain MRI and atlas-based volumetry

All participants underwent a 3T MRI scan of the brain according to the same standard operating procedures (SOPs) of the FTLD Consortium in all study centers. Data acquisition and postprocessing have been described previously.[19] We used atlas-based volumetry (ABV)[20] based on the LONI Probabilistic Brain Atlas[21] to determine the volumes (white and gray matter combined) of 55 different brain regions. Volumes were normalized to the total intracranial volume to control for inter-individual variability in head size. We used the BrainPainter software[22] to generate drawings of the brain and indicate brain regions with a significant correlation.

2.3 | Measurement of beta-synuclein, p-tau181, and NfL in serum

All serum biomarker measurements were performed at the Ulm University Hospital, Department of Neurology. Beta-synuclein concentration was measured by immunoprecipitation-mass spectrometry as described previously.[1] In brief, 490 μL serum were mixed with 70 μL of an internal standard solution containing ^{15}N-labelled full-length

beta-synuclein (rPeptide, Watkinsville, GA, USA), 0.82 M triethylammonium bicarbonate (TEAB), and 0.37% Tween-20. After pre-clearing with unconjugated magnetic beads, beta-synuclein was immunoprecipitated with a recombinant monoclonal anti-beta-synuclein antibody (EP1537Y from Abcam, Cambridge, UK) covalently coupled to magnetic beads (#14311D, Thermo, Waltham, MA, USA). Beads were washed with 100 mM TEAB and beta-synuclein was eluted. Digestion was performed by addition of trypsin/LysC (Promega, Walldorf, Germany) in 500 mM TEAB. Digestion was stopped by addition of 2% trifluoroacetic acid in 72% acetonitrile, and 20 μL was injected into a QTRAP6500 mass spectrometer coupled to an Eksigent MicroLC200 and Agilent 1260. Separation of peptides was performed using an Acclaim PepMap100, C18 trap column (5 μm, 0.3 × 5 mm, Thermo) and a HALO Fused-Core C18, 100 × 0.5 mm analytical column (Eksigent, Framingham, MA, USA) and a gradient time of 15.5 minutes. Two proteotypic peptides of beta-synuclein were quantified in multiple reaction monitoring mode (aa46-58 and aa61-85). Calibrators were prepared using recombinant human full-length beta-synuclein (without tags) from rPeptide and the exact concentration of the beta-synuclein stock solution was quantified by amino acid analysis (Alphalyse A/S, Odense, Denmark). Calibration range was 2 to 40 pg/mL. Serum quality control (QC) samples were included in all seven runs (intraassay CV 1.7%–8.7%, interassay CV 6.2%).

p-Tau181 was measured with single molecule array (Simoa pTau-181 V2 Advantage Kit from Quanterix, Billerica, MA, USA) and NfL with an automated microfluidic immunoassay (Ella Human NF-L Kit from ProteinSimple, San Jose, CA, USA)[23] according to the manufacturer's instructions. Samples were randomly assigned to the different assay plates and the analysts were blinded to the patient diagnoses. Serum QC samples were included in all runs.

2.4 | Statistics

Statistical analysis was performed with GraphPad Prism 8.3.0, R version 4.1.0 and SPSS version 26.0.0.0. Normal distribution of variables in the diagnostic groups was tested with the Shapiro-Wilk test. Since for all variables the normal distribution was not given in most groups, the Kruskal-Wallis test and Dunn post hoc test were used to compare group differences of age, CDR score, FTLD-CDR score, and MMSE. The values of serum biomarkers were log2-transformed for statistical analysis to enable a normal distribution and groups were compared by multiple linear regression (ANCOVA) with age and sex as covariates. Sex distribution was compared with Fisher's exact test. We performed ROC curve analysis to compare diagnostic performance of biomarkers and the Youden index was used for cut-off selection. Relationship of blood biomarkers with brain atrophy was determined with Spearman's rank correlation coefficient and Benjamini-Hochberg false discovery rate was used to adjust for multiple comparisons. A *P*-value < .05 was regarded as statistically significant. If not other stated, biomarker levels are given as median and interquartile range (IQR).

3 | RESULTS

3.1 | Patients

Patient characteristics are described in Table 1. In total, serum biomarker levels and MRI data were available from 374 individuals including 74 AD, 81 bvFTD, 41 svPPA, 55 nfvPPA, 25 lvPPA, 42 PSP, 25 CBS and 31 CU individuals. Age and sex distribution were not significantly different between groups ($P = .13$ and $P = .22$).

As expected, clinical scores (CDR, FTLD-CDR, MMSE) were significantly altered in all disease groups compared with the CU group (see Table 1). The CDR score was highest in AD (median 4.5, IQR 3.5–7.0) and bvFTD (median 5.0, IQR 3.0–7.0) patients and significantly different to lvPPA (median 2.5, IQR 1.5–4.0, $P < .05$) and nfvPPA (median 2.0, IQR 0.5–3.5, $P < .001$). The bvFTD group showed the highest FTLD-CDR score (median 6.5, IQR 5.0–9.5) which was significantly higher compared with nfvPPA (median 4.0, IQR 2.5–6.5, $P < .001$). Memory impairment indicated by the MMSE score was strongest in AD (median 23, IQR 18–26) and significantly different to bvFTD (median 26, IQR 22–28, $P < .05$).

3.2 | Serum beta-synuclein levels in diagnostic groups

Serum beta-synuclein levels (Figure 1A) were increased significantly in AD patients (median 11.7 pg/mL, IQR 9.3–15.7 pg/mL) compared with CU (median 9.4 pg/mL, IQR 6.9–10.9 pg/mL, $P = .006$), bvFTD (median 8.3 pg/mL, IQR 6.6–10.5 pg/mL, $P < .0001$) and PSP patients (median 8.4 pg/mL, IQR 6.6–9.3 pg/mL, $P < .0001$). lvPPA patients also showed significantly higher serum beta-synuclein levels (median 10.8 pg/mL, IQR 8.8–13.2 pg/mL) than PSP ($P = .046$). ROC curve analysis was performed to test the diagnostic performance of biomarkers. Serum beta-synuclein levels could discriminate AD from CU with an area under the curve (AUC) of 0.74 (sensitivity 49%, specificity 94%) and from bvFTD with an AUC of 0.75 (sensitivity 68%, specificity 77%, Figure 1B). Cutoff values and 95% confidence intervals (CIs) are listed in Table 2.

3.3 | Serum p-tau181 in diagnostic groups

Blood levels of p-tau181 (Figure 1C) were significantly higher in patients with AD (median 2.73 pg/mL, IQR 196–3.66 pg/mL) compared with CU (median 1.40 pg/mL, IQR 1.12–2.38 pg/mL, $P < .0001$), bvFTD (median 1.26 pg/mL, IQR 0.85–1.72 pg/mL, $P < .0001$), svPPA (median 1.36 pg/mL, IQR 0.92–2.55 pg/mL, $P < .0001$), nfvPPA (median 1.30 pg/mL, IQR 1.03–1.82 pg/mL, $P < .0001$), PSP (median 1.36 pg/mL, IQR 1.15–1.88 pg/mL, $P < .0001$), and CBS (median 1.64 pg/mL, IQR 1.12–2.21 pg/mL, $P = .001$). Patients with lvPPA (median 2.04 pg/mL, IQR 1.52–3.10 pg/mL) had higher serum p-tau181 levels than bvFTD patients ($P = .02$). ROC curve analysis of serum p-tau181 levels showed

TABLE 1 Patient characteristics

	CU	AD	bvFTD	svPPA	nfvPPA	lvPPA	PSP	CBS	*P*-value
N	31	74	81	41	55	25	42	25	
No. female (%)	12 (38.7)	42 (56.8)	29 (35.8)	19 (46.3)	25 (45.5)	13 (52)	23 (54.8)	10 (40)	.22
Age (years)[1]	66 (61–72)	67 (59–73)	66 (59–71)	65 (60–70)	69 (63–74)	71 (66–73)	68 (63–74)	69 (65–71)	.13
CDR-SB score[1,2]	0.0 (0.0–0.0)	4.5 (3.5–7.0)[b,c,d]	5.0 (3.0–7.0)[b,c,d]	2.8 (2.0–5.0)[b]	2.0 (0.5–3.5)[a]	2.5 (1.5–4.0)[a]	2.5 (1.5–7.0)[b]	4.0 (2.0–7.0)[b]	<.001
FTLD-CDR score[1,3]	0.0 (0.0–0.0)	5.5 (4.0–8.5)[a]	6.5 (5.0–9.5)[a,d]	4.5 (3.0–8.0)[a]	4.0 (2.5–6.5)[a]	4.5 (3.0–6.5)[a]	3.8 (2.5–7.5)[a]	6.0 (2.5–8.5)[a]	<.001
MMSE[1]	29 (28–30)	23 (18–26)[a]	26 (22–28)[a,e]	24 (15–26)[a]	26 (21–28)[a]	24 (19–27)[a]	27 (24–28)[a,e]	25 (19–28)[a]	<.001
Serum NfL (pg/mL)[1]	22.9 (16.7–30.7)	35.2 (25.4–42.7)	44.3 (26.3–66.7)[f,g]	56.4 (41.4–78.0)[f,h,i]	63.3 (38.5–86.9)[f,i,j]	31.8 (26.3–49.3)	49.1 (29.1–64.4)[k]	41.4 (32.1–66.4)[l]	<.0001
Serum p-tau181 (pg/mL)[1]	1.40 (1.12–2.38)	2.73 (1.96–3.66)[f]	1.26 (0.85–1.72)[j,m]	1.36 (0.92–2.55)[j]	1.30 (1.03–1.82)[j]	2.04 (1.52–3.10)	1.36 (1.15–1.88)[j]	1.64 (1.12–2.21)[g]	<.0001
Serum beta-synuclein (pg/mL)[1]	9.4 (6.9–10.9)	11.7 (9.3–15.7)[l]	8.3 (6.6–10.5)[j]	9.5 (7.6–13.4)	9.9 (7.3–13.1)	10.8 (8.8–13.2)	8.4 (6.6–9.3)[j,m]	10.0 (7.7–11.6)	<.0001

Note: Group comparisons: Fisher exact test for sex distribution. Kruskal-Wallis test and Dunn post hoc test for age, CDR score, FTLD-CDR score, and MMSE: [a]$P < .01$ vs Con; [b]$P < .001$ vs Con; [c]$P < .05$ vs lvPPA; [d]$P < .001$ vs nfvPPA; [e]$P < .05$ vs AD. Multiple linear regression (ANCOVA) with age and sex as covariates for serum NfL p-tau181, and beta-synuclein: [f]$P < .0001$ vs Con; [g]$P < .01$ vs AD; [h]$P < .001$ vs AD; [i]$P < .01$ vs lvPPA; [j]$P < .0001$ vs AD; [k]$P < .001$ vs Con; [l]$P < .01$ vs Con; [m]$P < .05$ vs lvPPA.

Abbreviations: AD, Alzheimer's disease; bvFTD, behavioral variant frontotemporal dementia; CBS, corticobasal syndrome; CDR-SB, Clinical Dementia Rating sum-of-boxes; CU, cognitively unimpaired individual; FTLD, frontotemporal lobar degeneration; lvPPA, logopenic variant primary progressive aphasia; MMSE, Mini-Mental State Examination; NfL, neurofilament light; nfvPPA, non-fluent variant primary progressive aphasia; PSP, progressive supranuclear palsy; p-tau181, phosphorylated tau 181; svPPA, semantic variant primary progressive aphasia.

[1]Values are median and interquartile range.

[2]Missing values CDR score: AD 11, bvFTD 4, CBS 4, Con 8, lvPPA 2, nfvPPA 4, PSP 5, svPPA 3.

[3]Missing values FTLD-CDR score: AD 12, bvFTD 4, CBS 4, Con 8, lvPPA 2, nfvPPA 4, PSP 6, svPPA 3.

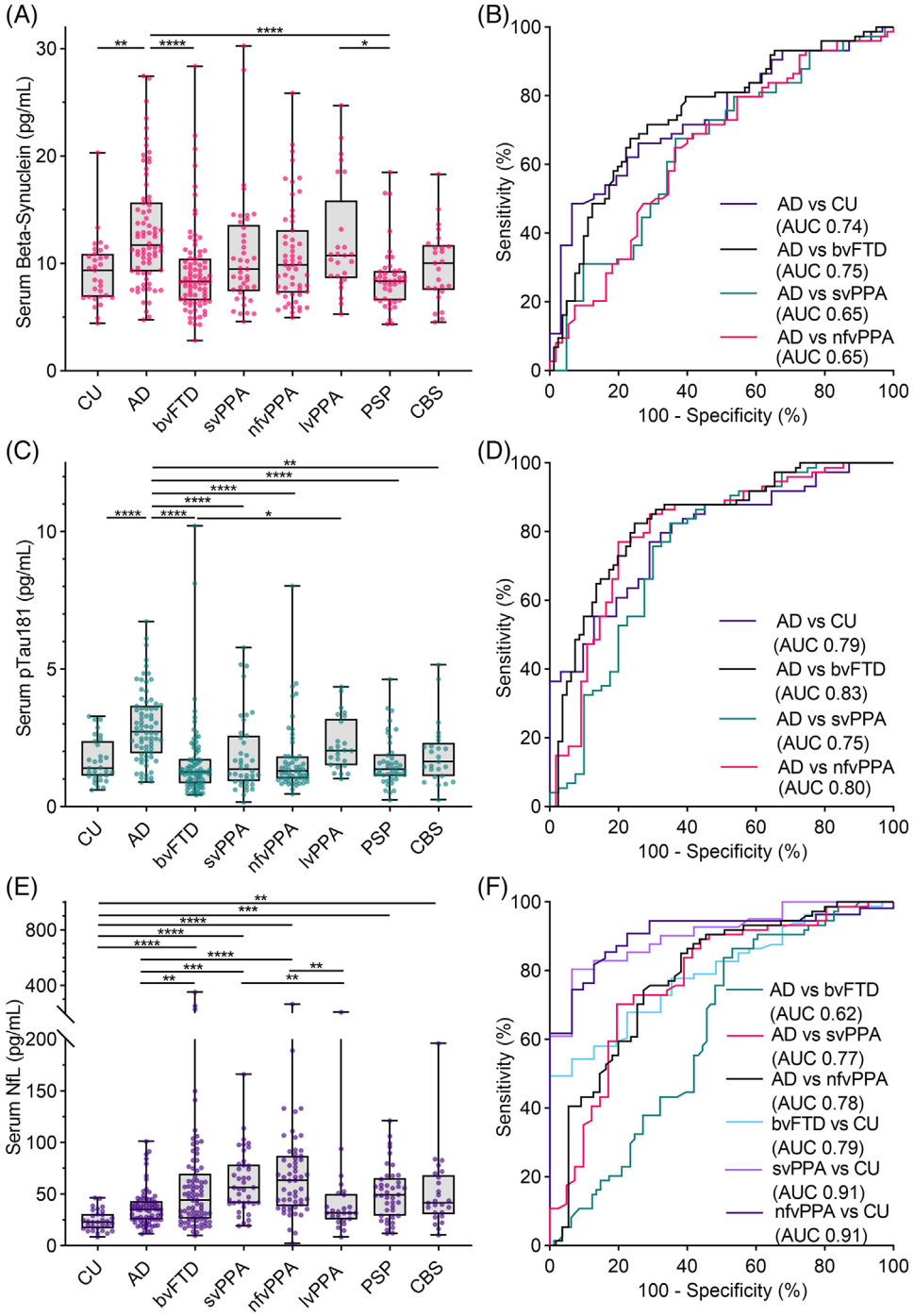

FIGURE 1 Group comparison of blood biomarkers and diagnostic performance. (A,C,E) Serum concentration of beta-synuclein, p-tau181, and NfL was measured in patients with AD ($n = 74$), bvFTD ($n = 81$), svPPA ($n = 41$), nfvPPA ($n = 55$), lvPPA ($n = 25$), PSP ($n = 42$), CBS ($n = 25$), and CU ($n = 31$). Boxes are median and interquartile range, whiskers are min and max. Groups were compared by multiple linear regression of the log2-transformed biomarker values including age and sex as covariates. $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$, $^{****}P < .0001$. (B,D,F) Receiver-operating characteristic (ROC) curve analysis for comparison of diagnostic performance. AD, Alzheimer's disease; AUC, area under the curve; bvFTD, behavioral variant frontotemporal dementia; CBS, corticobasal syndrome; CU, cognitively unimpaired individuals; NfL, neurofilament light chain; nfvPPA, non-fluent variant primary progressive aphasia; lvPPA, logopenic variant primary progressive aphasia; PSP, progressive supranuclear palsy; p-tau181, phosphorylated tau 181; ROC curve, receiver-operating characteristic curve; svPPA, semantic variant primary progressive aphasia; vs, versus.

TABLE 2 Diagnostic performance of serum biomarkers

	AUC (95% CI)	Cutoff (pg/mL)	Sens. (%)	Spec. (%)
Beta-Synuclein				
AD vs CU	0.74 (0.65–0.84)	12.2	49	94
AD vs bvFTD	0.75 (0.67–0.83)	10.5	68	77
AD vs svPPA	0.65 (0.54–0.76)	10.5	68	63
AD vs nfvPPA	0.65 (0.55–0.74)	10.8	65	64
lvPPA vs CU	0.66 (0.51–0.80)	8.5	80	48
lvPPA vs bvFTD	0.69 (0.58–0.81)	10.1	64	72
lvPPA vs svPPA	0.59 (0.45–0.73)	8.8	76	46
lvPPA vs nfvPPA	0.59 (0.46–0.72)	8.1	84	38
p-tau181				
AD vs CU	0.79 (0.70–0.88)	1.87	77	71
AD vs bvFTD	0.83 (0.77–0.90)	1.72	82	75
AD vs svPPA	0.75 (0.65–0.85)	1.71	82	65
AD vs nfvPPA	0.80 (0.72–0.88)	1.87	77	80
lvPPA vs CU	0.70 (0.57–0.84)	1.43	84	55
lvPPA vs bvFTD	0.76 (0.66–0.86)	1.43	84	64
lvPPA vs svPPA	0.67 (0.54–0.80)	1.18	96	43
lvPPA vs nfvPPA	0.74 (0.63–0.85)	1.47	80	64
NfL				
AD vs CU	0.74 (0.64–0.84)	25.1	77	65
AD vs bvFTD	0.62 (0.53–0.71)	49.1	87	47
AD vs svPPA	0.77 (0.68–0.87)	40.1	70	81
AD vs nfvPPA	0.78 (0.70–0.87)	42.5	74	73
lvPPA vs CU	0.74 (0.60–0.87)	31.2	64	77
lvPPA vs bvFTD	0.60 (0.48–0.72)	39.0	72	54
lvPPA vs svPPA	0.75 (0.62–0.87)	39.5	72	81
lvPPA vs nfvPPA	0.76 (0.65–0.88)	38.3	72	76
bvFTD vs CU	0.79 (0.71–0.87)	46.9	49	100
svPPA vs CU	0.91 (0.84–0.97)	39.8	81	94
nfvPPA vs CU	0.91 (0.84–0.97)	35.0	86	84
PSP vs CU	0.84 (0.74–0.93)	40.0	67	94
CBS vs CU	0.82 (0.70–0.94)	36.4	68	87

Abbreviations: AD, Alzheimer's disease; AUC, area under the curve; bvFTD, behavioral variant frontotemporal dementia; CBS, corticobasal syndrome; CI, confidence interval; CU, cognitively unimpaired individual; lvPPA, logopenic variant primary progressive aphasia; NfL, neurofilament light chain; nfvPPA, non-fluent variant primary progressive aphasia; PSP, progressive supranuclear palsy; p-tau181, phosphorylated tau 181; Sens, sensitivity; Spec, specificity; svPPA, semantic variant primary progressive aphasia.

good discriminatory power to differentiate AD from CU (AUC 0.79, sensitivity 77%, specificity 71%), bvFTD (AUC 0.83, sensitivity 82%, specificity 75%), svPPA (AUC 0.75, sensitivity 82%, specificity 65%), and from nfvPPA (AUC 0.80, sensitivity 77%, specificity 80%, Figure 1D and Table 2).

3.4 | Serum NfL levels in diagnostic groups

NfL levels in serum (Figure 1E) were significantly higher in bvFTD (median 44.3 pg/mL, IQR 26.3–66.7 pg/mL, $P < .0001$), svPPA (median 56.4 pg/mL, IQR 41.4–78.0 pg/mL, $P < .0001$), nfvPPA (median 63.3 pg/mL, IQR 38.5–86.9 pg/mL, $P < .0001$), PSP (median 49.1 pg/mL, IQR 29.1–64.4 pg/mL, $P = .0002$), and CBS (median 41.4 pg/mL, IQR 32.1–66.4 pg/mL, $P = .003$) if compared with CU (median 22.9 pg/mL, IQR 16.7–30.7 pg/mL). Patients with bvFTD, svPPA, and nfvPPA also had higher NfL levels compared with AD (35.2 pg/mL, IQR 25.4–42.7 pg/mL, $P = .008$, $P = .0001$, and $P < .0001$) and svPPA and nfvPPA compared with lvPPA (median 31.8 pg/mL, IQR 26.3–49.3 pg/mL, $P = .009$ and $P = .005$).

Serum NfL levels could discriminate CU individuals from AD with an AUC of 0.74 (sensitivity 77%, specificity 65%), bvFTD with AUC 0.79 (sensitivity 49%, specificity 100%), svPPA with AUC 0.91 (sensitivity 81%, specificity 94%), nfvPPA with AUC 0.91 (sensitivity 86%, specificity 84%), PSP with AUC 0.84 (sensitivity 67%, specificity 94%), and from CBS with AUC 0.82 (sensitivity 68%, specificity 87%). In addition, serum NfL levels distinguished patients with AD from bvFTD with AUC 0.62 (sensitivity 87%, specificity 47%), svPPA with AUC 0.77 (sensitivity 70%, specificity 81%), and nfvPPA with AUC 0.78 (sensitivity 74%, specificity 73%, Figure 1F and Table 2).

3.5 | Subgroup analysis of biomarker levels

We compared biomarker levels in diagnostic subgroups for a more detailed characterization of changes. AD patients were divided into patients with MCI-AD, representing the early disease phase, and AD dementia according to their CDR-SB score. We observed a non-significant increase of serum beta-synuclein (10.8 pg/mL, IQR 7.6–13.8 pg/mL) and p-tau181 levels (2.49 pg/mL, IQR 1.67–3.05 pg/mL) in MCI-AD patients compared with CU (Figure 2A–C). In AD dementia, serum levels of all three biomarker were increased significantly (beta-synuclein 11.5 pg/mL, IQR 9.2–15.8 pg/mL, $P < .001$; p-tau181 2.81 pg/mL, IQR 1.92–3.64 pg/mL, $P < .0001$; NfL 35.6 pg/mL, IQR 25.9–22.3 pg/mL, $P < .0001$).

In agreement with the observation in all FTLD patients (Figure 1), there were no significant changes of serum beta-synuclein and p-tau181 in genetic FTLD cases with a *C9orf72* (beta-synuclein 7.8 pg/mL, IQR 4.7–12.0 pg/mL, $P = .90$; p-tau181 1.25 pg/mL, IQR 0.58–1.32 pg/mL, $P = .11$), *GRN* (beta-synuclein 8.9 pg/mL, IQR 6.8-11.8 pg/mL, $P = 1.00$; p-tau181 0.81 pg/mL, IQR 0.55–1.12 pg/mL, $P = .26$), or *MAPT* mutation (beta-synuclein 8.5 pg/mL, IQR 8.2–10.6, $P = .96$; p-tau181 0.86 pg/mL, IQR 0.53–1.36 pg/mL, $P = .68$) compared with CU, although p-tau181 levels tended to be lower (Figure 2A–C). Serum NfL levels were increased significantly in all genetic FTLD subgroups (*C9orf72* 56.4 pg/mL, IQR 19.7—145 pg/mL, $P < .001$; *GRN* 92.5 pg/mL, IQR 76.9—104 pg/mL, $P < .0001$; *MAPT* 62.2 pg/mL, IQR 58.0–89.6 pg/mL, $P < .0001$).

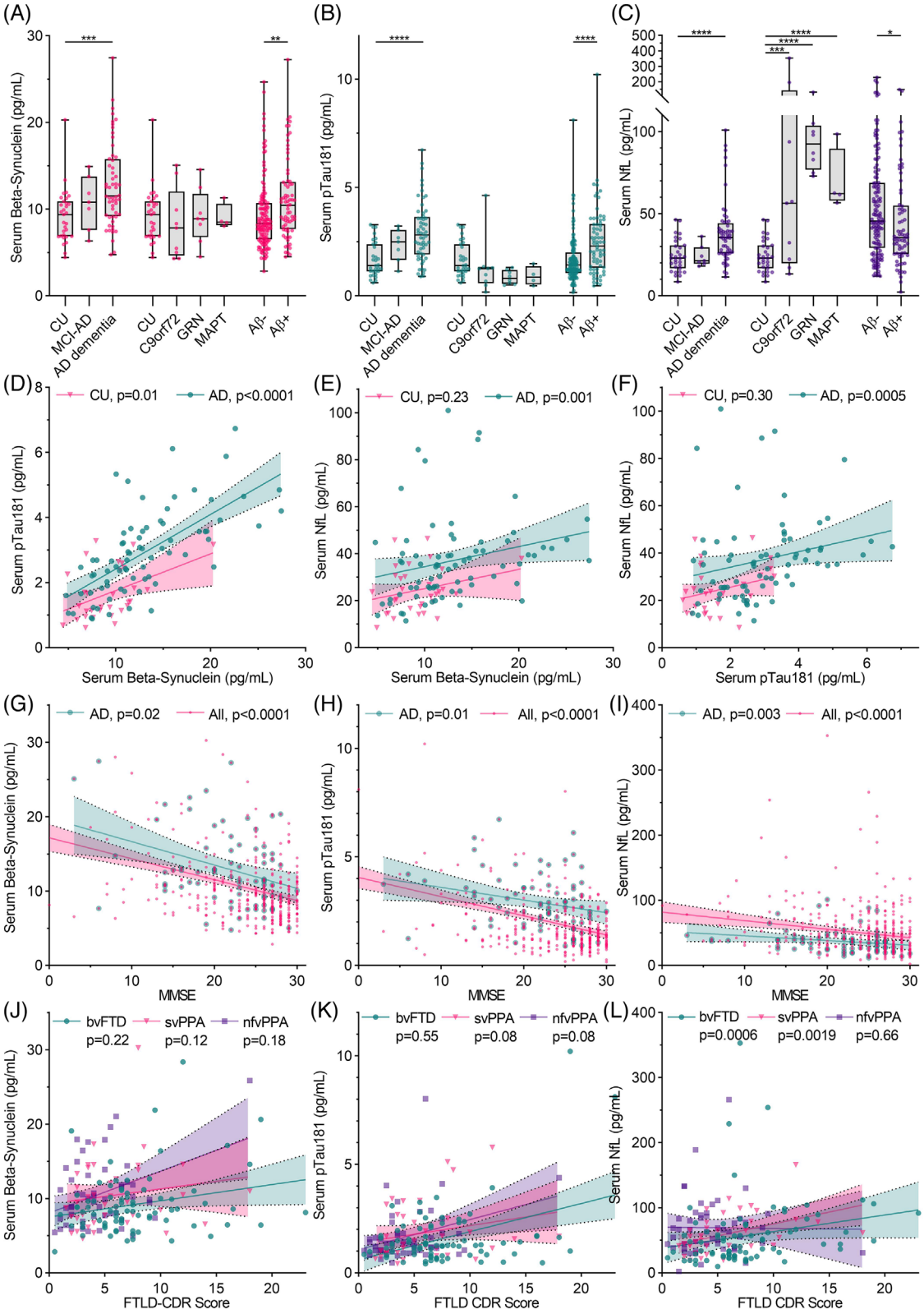

FIGURE 2 Subgroup comparison of blood biomarkers and correlation analysis. (A) Serum beta-synuclein, (B) p-tau181, and (C) NfL concentration in diagnostic subgroups including patients with mild cognitive impairment due to Alzheimer's disease (MCI-AD, $n = 7$); AD dementia ($n = 56$); genetic frontotemporal lobar degeneration (FTLD) with mutations of the *C9orf72* ($n = 9$), *GRN* ($n = 8$), and *MAPT* ($n = 4$) genes; patients with (Aβ+, $n = 70$) and without (Aβ−, $n = 135$) suspected amyloid pathology based on their CSF Aβ42 concentration, and cognitively unimpaired individuals (CU, $n = 31$). Boxes are median and interquartile range; whiskers are min and max. Groups were compared by multiple linear regression of the log2-transformed biomarker values including age and sex as covariates. $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$, $^{****}P < .0001$. (D–L) Correlation analysis using Spearman rank correlation coefficient between serum beta-synuclein, p-tau181, NfL, and clinical scores for cognitive impairment

To investigate the relationship of blood biomarkers with amyloid pathology, patients with available CSF Aβ42 levels were divided into amyloid-positive (Aβ+) and amyloid-negative (Aβ−) individuals (independent of their clinical diagnosis) using a cutoff of 550 pg/mL (Figure 2A–C). Serum beta-synuclein and p-tau181 levels were significantly higher in Aβ+ individuals (beta-synuclein 10.5 pg/mL, IQR 7.7–13.1 pg/mL, $P < .01$; p-tau181 2.31 pg/mL, IQR 1.29–3.31 pg/mL, $P < .0001$) compared with Aβ− patients (beta-synuclein 8.3 pg/mL, IQR 6.5–10.7 pg/mL; p-tau181 1.42 pg/mL, IQR 1.05–2.01 pg/mL). In contrast, NfL levels in Aβ+ individuals were significantly lower (35.4 pg/mL, IQR 25.5–54.9 pg/mL vs 45.5 pg/mL, IQR 29.1–68.8 pg/mL, $P < .05$) reflecting the more pronounced increase of NfL in FTLD syndromes.

3.6 | Correlation analyses of biomarkers

Serum NfL levels showed a strong ($r = 0.58$, $P = .001$) and p-tau181 a moderate ($r = 0.38$, $P = .03$) and significant correlation with age in CU individuals as described previously.[24,25] No significant correlation with age was observed for serum beta-synuclein values ($r = 0.21$, $P = .26$). Correlation data including 95% CIs are listed in Table S1.

Beta-synuclein and p-tau181 levels in serum correlated moderately with each other in CU ($r = 0.45$, $P = .01$) and strong in patients with AD ($r = 0.71$, $P < .0001$, Figure 2D). Both beta-synuclein and p-tau181 showed a non-significant and weak correlation with NfL levels in CU individuals ($r = 0.22$, $P = .23$ and $r = 0.19$, $P = .30$) but a moderate correlation in patients with AD ($r = 0.39$, $P = 0.001$ and $r = 0.40$, $P = .0005$, Figure 2E,F).

We used the MMSE score and FTLD-CDR score to investigate the relationship of serum biomarkers with cognitive alterations in AD and FTD patients. All three serum markers correlated significantly with the MMSE score in patients with AD (beta-synuclein $r = -0.27$, $P = .02$; p-tau181 $r = -0.29$, $P = .01$; NfL $r = -0.34$, $P = .003$) and in the whole cohort (beta-synuclein $r = -0.31$, $P < .0001$; pTau181 $r = -0.32$, $P < .0001$; NfL $r = -0.26$, $P < .0001$, Figure 2G–I). Serum beta-synuclein and p-tau181 were not correlated with the FTLD-CDR score in bvFTD ($r = 0.14$, $P = .22$ and $r = 0.07$, $P = .55$), svPPA ($r = 0.26$, $P = .12$ and $r = 0.29$, $P = .08$), and nfvPPA patients ($r = 0.19$, $P = .18$ and $r = 0.24$, $P = .08$, Figure 2J,K). In contrast, serum NfL levels correlated significantly with the FTLD-CDR score in bvFTD ($r = 0.38$, $P = .0006$) and svPPA ($r = 0.49$, $P = .0019$) but not in nfvPPA patients ($r = 0.06$, $P = .66$, Figure 2L).

3.7 | Relationship of serum biomarkers with brain atrophy

To investigate how serum biomarker levels relate to disease-associated structural changes in the brain, we performed correlation analysis with atlas-based volumetry of MRI data. Results of the correlation analyses of all brain regions, biomarkers, and diseases are listed in Table S2 and are visualized for all individuals and separately for AD and bvFTD patients in Figure 3.

In the analysis of MRI data from all individuals ($n = 374$), serum beta-synuclein levels showed a significant negative correlation, mainly with specific temporal structures (temporal gyri, fusiform, and parahippocampal gyri) but also to a lower extent with frontal (frontal gyri, middle orbitofrontal gyrus), parietal (superior parietal, postcentral, angular and supramarginal gyri, precuneus), and occipital regions (inferior and middle occipital gyri), and the cingulate gyrus, hippocampus/amygdala, and insula. Frequently, correlations were more pronounced in or even restricted to the left hemisphere (Figure 3 and Table S2). The strongest associations of serum beta-synuclein levels with regional brain atrophy were observed in patients with AD. A significant negative correlation was observed with temporal structures, mainly of the left hemisphere (temporal gyri $r = -0.33$ to -0.46, $P < .01$, fusiform gyrus $r = -0.36$, $P = .005$, parahippocampal gyrus $r = -0.27$, $P = .035$) and the gyrus rectus ($r = -0.38$, $P = .003$ and -0.30, $P = .018$), left insula ($r = -0.28$, $P = .027$) and left putamen ($r = -0.28$, $P = .029$) (Figure 3 and Table S2). In bvFTD patients, a significant negative correlation was observed only for the left inferior frontal gyrus ($r = -0.27$, $P = .02$) and right precentral gyrus ($r = -0.27$, $P = .03$, Figure 3 and Table S2). In svPPA and nfvPPA patients, where serum beta-synuclein levels showed a tendency to be elevated (Figure 1), no significant correlation with the volume of any brain region was observed (Figure 3 and Table S2).

The brain regions correlating with serum p-tau181 levels in all individuals were quite similar to beta-synuclein, with more pronounced correlation of occipital structures (superior occipital and lingual gyri) but without correlation to frontal structures and the cingulate gyrus (Figure 3 and Table S2). In patients with AD, we observed a significant negative correlation of p-tau181 levels with the insula ($r = -0.39$, $P = .002$ and $r = -0.34$, $P = .008$), putamen ($r = -0.39$, $P = .002$ and $r = -0.37$, $P = .004$), right caudate ($r = -0.29$, $P = 0.025$), left fusiform gyrus ($r = -0.27$, $P = .037$), and left gyrus rectus ($r = -0.27$, $P = .036$). Within the FTLD disease spectrum, bvFTD patients showed a significant correlation of serum p-tau181 with the right lateral orbitofrontal gyrus ($r = 0.30$, $P = .01$), left inferior occipital gyrus ($r = -0.26$, $P = .03$), right caudate ($r = 0.25$, $P = .04$), right gyrus rectus ($r = 0.28$, $P = .02$),

and severity of dementia (MMSE, Mini-Mental State examination; FTLD-CDR, FTLD-specific clinical dementia rating). All ($n = 374$), AD ($n = 74$), behavioral variant frontotemporal dementia (bvFTD, $n = 77$), semantic variant primary progressive aphasia (svPPA, $n = 38$), and non-fluent variant primary progressive aphasia (nfvPPA, $n = 51$). Dots are individual values, solid lines are linear regression and dotted lines show the 95% confidence interval (CI). Correlation coefficients and 95% CI are given in Table S1. -Aβ+/negative; Aβ42, amyloid beta 42; AD, Alzheimer's disease; bvFTD, behavioral variant frontotemporal dementia; CI, 95% confidence interval; CU, cognitively unimpaired individuals; FTLD, frontotemporal lobar degeneration; FTLD-CDR, FTLD-specific clinical dementia rating; MCI-AD, mild cognitive impairment due to Alzheimer's disease; MMSE, Mini-Mental State examination; NfL, neurofilament light chain; nfvPPA, non-fluent variant primary progressive aphasia; ptau181, phosphorylated tau 181; svPPA, semantic variant primary progressive aphasia

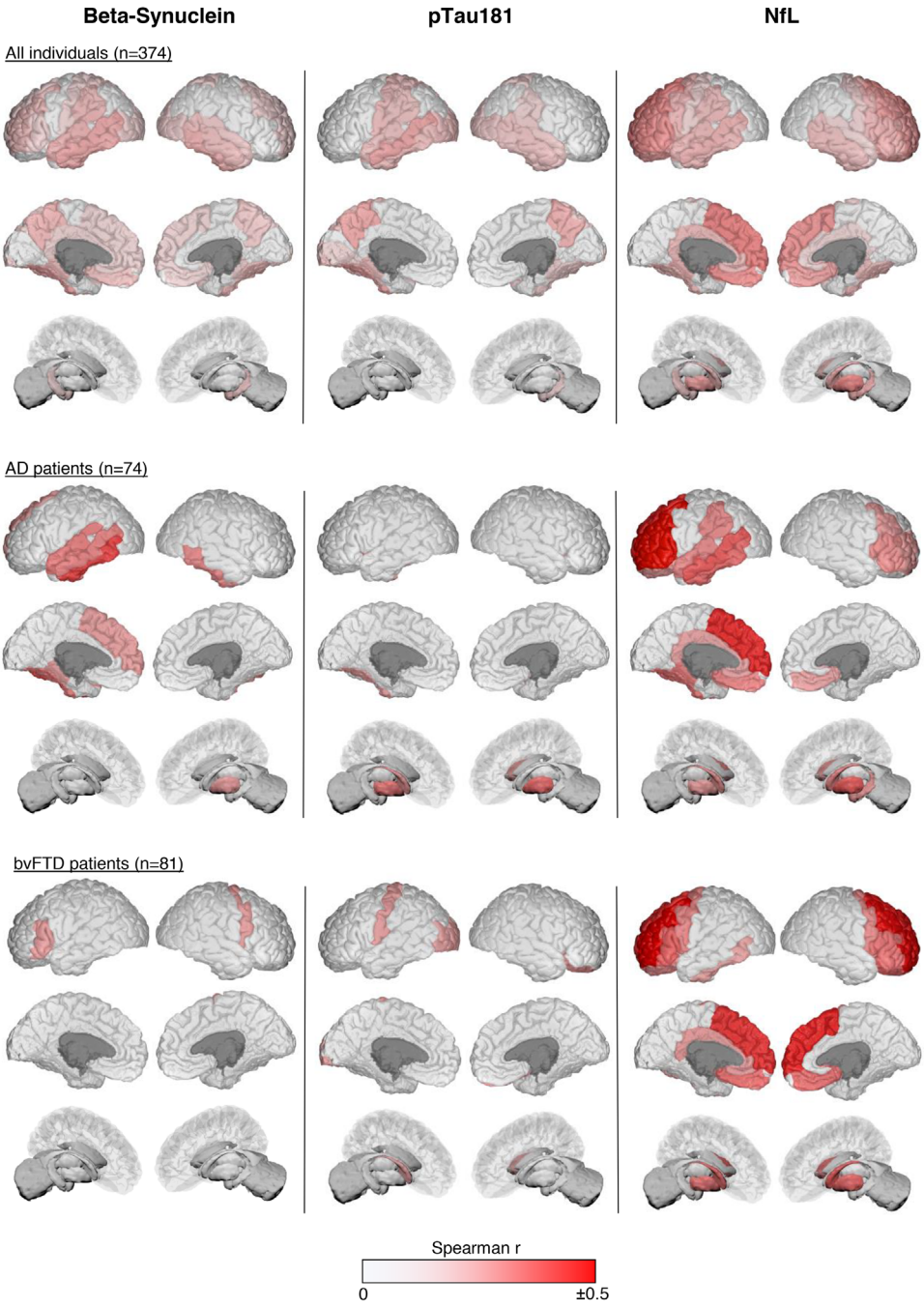

FIGURE 3 Correlation of serum beta-synuclein, p-tau181, and NfL levels with regional brain atrophy. Serum biomarker levels were correlated with the volumes of 55 cortical and subcortical brain regions based on structural MRI data in all individuals, AD, and bvFTD patients. For each patient, volumetric data were normalized to the individual total intracranial volume and correlation analysis was performed with Spearman's rank correlation coefficient. Benjamini-Hochberg FDR was used to adjust for multiple comparisons. Brain regions with a significant correlation ($P < .05$) are indicated by their strength of correlation (Spearman r value). All indicated brain regions show a negative correlation with blood markers except correlation of p-tau181 with orbitofrontal gyrus, caudate, and insula. Correlation coefficients and P-values for all brain regions are listed in Table S2. Drawings were generated by BrainPainter[22] (gyrus rectus and angular gyrus are not visualized). AD, Alzheimer's disease; bvFTD, behavioral variant frontotemporal dementia; FDR, false discovery rate; NfL, neurofilament light chain; p-tau181, phosphorylated tau 181

right insula ($r = 0.24$, $P = .04$), and left postcentral gyrus ($r = -0.27$, $P = .03$, Figure 3 and Table S2).

Serum NfL levels in all individuals showed a significant negative correlation with a higher number of brain regions than in beta-synuclein and p-tau181, with the strongest association observed for frontal structures (frontal, orbitofrontal, and precentral gyri, gyrus rectus) and the striatum but also with temporal (temporal, fusiform, and parahippocampal gyri) and parietal regions (angular, postcentral, and supramarginal gyri) and the cingulate gyrus, hippocampus/amygdala, and insula (Figure 3 and Table S2). In patients with AD, strong and

significant negative correlations were observed especially for (mainly left) frontal regions (frontal and orbitofrontal gyri, $r = -0.27$ to -0.51, $P = .036$ to 8.5e-5), but also left temporal (temporal gyri $r = -0.33$ to -0.43, $P = .009$ to 6.2e-4; fusiform gyrus $r = -0.37$, $P = .003$ and parahippocampal gyrus $r = -0.36$, $P = .005$) and left parietal structures (angular gyrus $r = -0.28$, $P = .029$; supramarginal gyrus $r = -0.33$, $P = .009$), the striatum ($r = -0.28$ to -0.43, $P = .018$ to 7.3e-4), left cingulate gyrus ($r = -0.26$, $P = .04$), left hippocampus/amygdala ($r = -0.29$, $P = .02$), and insula ($r = -0.34$–0.43, $P = .002$ to 5.1e-4) (Figure 3 and Table S2). Within the FTLD spectrum of diseases, the strongest correlations of NfL with brain atrophy was observed in bvFTD, mainly in frontal structures (frontal, orbitofrontal and precentral gyri, gyrus rectus, $r = -0.31$ to -0.53, $P = .008$ to 2.3e-6) and the striatum ($r = -0.37$ to -0.41, $P = .002$ to 3.5e-4, Figure 3, Table S2).

4 | DISCUSSION

In our study of 374 individuals, we showed increased levels of serum beta-synuclein in patients with AD but not in FTLD. Beta-synuclein levels correlated mainly with temporal brain atrophy and were associated with cognitive decline, with the strongest effects for both observed in patients with AD. Strong correlation of beta-synuclein levels with serum p-tau181 and elevated levels in Aβ+ individuals indicate direct or indirect association of beta-synuclein changes with amyloid pathology. Serum p-tau181 showed the most specific changes of all three markers in AD but the lowest correlation with structural changes, which is in agreement with its suggested relation to amyloid/tau pathology rather than to structural damage. Serum NfL was elevated in all diseases but more pronounced in FTLD, in agreement with its strongest relation to frontal brain structures.

Beta-synuclein is a synaptic protein and its measurement in blood has been suggested recently as a candidate biomarker for synaptic degeneration in AD.[1] We here confirmed increased serum beta-synuclein levels in AD in a multicentric cohort of the German FTLD Consortium and support it as a consistent finding in AD. Beta-synuclein blood concentration and discriminatory performance for AD were in the same range as in our previous study (AUC 0.74 in both),[1] supporting the robustness of the assay and findings. This is important for future applications and the definition of cutoff values. The present study extended our studies to diseases of the FTLD spectrum including bvFTD and PPAs, which are relevant in the differential diagnosis of AD. Within the group of PPAs, beta-synuclein levels were highest in patients with lvPPA, which belong to the AD continuum. Although beta-synuclein levels showed a tendency to be elevated in all PPAs, no significant changes were observed here. Previous data show that serum beta-synuclein is not changed in Lewy body–associated dementias as well,[1] and our findings in blood are consistent with the observations described for beta-synuclein in CSF.[1,2,7–9] These data support a strong relation of beta-synuclein changes to AD. The increase of serum beta-synuclein levels in AD could be evidence that synaptic degeneration is more pronounced in AD compared with other types of neurodegenerative dementias. This is supported by observations in CSF for other pre- and postsynaptic marker proteins such as neurogranin[26] and GAP-43,[27] which show the strongest changes in AD.

The predominant changes of beta-synuclein levels in AD could also originate in an association of beta-synuclein with AD-related brain regions or AD pathology. To address this question, we correlated serum beta-synuclein levels with MRI data to characterize its association with structural changes in different brain regions. Increased beta-synuclein levels were associated mainly with a lower volume of temporal structures, and the correlation was strongest in patients with AD. According to the Braak and Thal stages of AD,[28,29] the earliest neuropathological alterations in AD appear in the inferior medial and lateral temporal lobe, and our correlation data with MRI indicate that this is reflected by increased beta-synuclein levels in blood already in patients with early AD (MCI-AD) in our subgroup analysis. In addition, beta-synuclein expression seems to be higher in the temporal than the frontal lobe,[30] providing a further explanation for the more pronounced association of beta-synuclein changes in biofluids with AD than with FTD. Lower expression of beta-synuclein in frontal structures might result in lower release from degenerating synapses in FTD.

The increased beta-synuclein levels in amyloid-positive versus amyloid-negative patients, and the strong correlation with p-tau181 levels, could indicate an association of beta-synuclein with AD-related amyloid pathology. On the other hand, no changes of serum beta-synuclein were observed in other tauopathies (i.e., PSP and CBS), which makes a relation to tau pathology less likely. However, the relation of beta-synuclein levels to amyloid/tau pathology was not the main question of this study. Future studies including patients with a thorough characterization of amyloid and tau pathology using positron emission tomography (PET) or neuropathological data are required to clarify whether there is a causal relation or the association is due to coinciding mechanisms.

We compared changes of serum beta-synuclein with the established blood markers NfL and p-tau181 to display the different patterns of mechanisms in diseases and to evaluate the added value of beta-synuclein to the existing panel of blood markers. Serum p-tau181 showed a specific increase in AD and the AD-associated lvPPA, whereas serum NfL as a general neurodegeneration marker was increased in all diseases but more pronounced in FTLD. This is the expected pattern of changes for these markers as described in several other studies.[31–35] Both serum p-tau181 and NfL levels were correlated mainly with temporal and NfL also more pronounced with frontal brain structures, which is also in agreement with previous observations.[31,35] It shows that our cohort reflects changes observed by other studies, underscoring the transferability of our results. However, in AD patients, p-tau181 showed a lower association with structural changes than beta-synuclein and NfL, and only in a few regions. This is in contrast to the strong and specific increase of serum p-tau181 levels in patients with AD. However, correlation data with PET imaging indicate that p-tau181 in blood is a marker of amyloid/tau pathology.[32] This would mean that it is associated only indirectly with structural damage and explain the lower correlation with MRI data in AD compared with the structural damage markers beta-synuclein (synapse) and NfL (axon).

Serum beta-synuclein was changed predominantly in AD and AD-associated lvPPA; minor changes were observed in svPPA and nfvPPA. Thus changes in beta-synuclein across the disease spectrum examined here are clearly different from NfL, and both markers show only a weak correlation with each other. This underscores beta-synuclein as a marker reflecting pathological alterations different from NfL (e.g., synaptic degeneration). In contrast, the pattern of beta-synuclein alterations is similar to p-tau181, with the slight increase of beta-synuclein in svPPA and nfvPPA. However, as discussed earlier, beta-synuclein levels in AD show a stronger relation to structural damage than p-tau181, arguing against a common pathological origin. It is in good agreement with beta-synuclein being a marker of temporal synaptic degeneration coinciding with amyloid/tau pathology reflected by p-tau181. This could also explain the slightly higher values in svPPA and nfvPPA because both syndromes are characterized by a strong temporal involvement.[19] Additional studies are needed to further clarify these associations.

Synaptic degeneration is the neuropathological correlate of cognitive decline in AD, and synaptic markers such as beta-synuclein are, therefore, of high interest as surrogate markers to predict and monitor memory impairment. Serum beta-synuclein correlated significantly with cognitive scores in AD, supporting this hypothesis. However, the strength of correlation was similar to pTau181 and NfL which reflect other types of pathology. The cross-sectional design of our study makes it difficult to compare and evaluate the value of the three different markers for prediction and monitoring of memory impairment. A comparison of these markers will require longitudinal studies including pre-symptomatic individuals to characterize and compare the time course of the biomarker changes in relation to cognitive decline and their predictive value. In contrast to AD, only NfL correlated with cognitive scores in FTD. This is in agreement with the more AD-specific changes of serum beta-synuclein and p-tau181, whereas NfL is most affected in FTD and shows the strongest association with frontal brain atrophy.

A limitation of our study might be the diagnosis by clinical criteria only without any neuropathological assessment. FTD patients with a certain amount of AD copathology might thus not be recognized and lead to an overestimation of beta-synuclein changes in FTD subgroups. However, diagnosis and patient characterization were performed in specialized centers using harmonized procedures across the German FTLD consortium ensuring the highest quality for the diagnostic workup. In addition, the number of patients in some subgroups (e.g., MCI-AD and FTLD mutations) are low and should be handled as exploratory observations, which need to be confirmed in larger cohorts. The cross-sectional design allows only assumptions on the time-course of changes and provide a basis for the initiation of longitudinal studies.

In conclusion, our study provides evidence that serum beta-synuclein changes are related strongly to AD and reflect mainly synaptic degeneration in the temporal lobe. There is a clear difference between serum beta-synuclein and the established markers NfL and p-tau181 in regard to their changes in different diseases or their relation to structural brain changes, supporting the added value of beta-synuclein to the existing panel of blood markers. In addition, our study raises several novel questions that need to be answered. Further studies must address how beta-synuclein blood levels are related to amyloid and tau pathology using PET imaging. In addition, the investigation of blood beta-synuclein in relation to synaptic density in the brain measured by SV2A PET will be important to evaluate the hypothesized association with temporal synaptic degeneration. The study of beta-synuclein blood levels in pre-symptomatic autosomal dominant AD mutation carriers and individuals with Down syndrome will provide information about the time course of changes and—when studied in comparison with other fluid and imaging markers—show how it might contribute to improve early diagnosis. Patient samples with longitudinal follow-up data will uncover the prognostic value of blood beta-synuclein in AD. Because synaptic degeneration is observed in many diseases, beta-synuclein in blood must be studied in large and more heterogenous populations including other types of dementia but also non-neurodegenerative diseases such as psychiatric syndromes, traumatic brain injury, or stroke to address the question of specificity. Although we are just at the beginning with evaluating beta-synuclein as a biomarker, it might be used in the future as an easily accessible blood test to assess synaptic degeneration in clinical practice. This could help in early AD diagnosis to detect the beginning of degenerative processes in the brain of amyloid-positive subjects and define the point to start with disease-modifying AD treatments—when available. The beta-synuclein blood test might also be used for disease staging, prognosis, and monitoring of treatment effects in patients with AD and other diseases characterized by synaptic degeneration.

AUTHOR CONTRIBUTIONS

Conception and design of the work: Patrick Oeckl and Markus Otto. Acquisition, analysis, or interpretation of data: Patrick Oeckl, Sarah Anderl-Straub, Adrian Danek, Janine Diehl-Schmid, Klaus Fassbender, Klaus Fliessbach, Steffen Halbgebauer, Hans-Jürgen Huppertz, Holger Jahn, Jan Kassubek, Johannes Kornhuber, Bernhard Landwehrmeyer, Martin Lauer, Johannes Prudlo, Anja Schneider, Matthias L. Schroeter, Petra Steinacker, Alexander E. Volk, Matias Wagner, Juliane Winkelmann, Jens Wiltfang, Albert C. Ludolph, and Markus Otto. All authors contributed to drafting of the work or revising it critically for important intellectual content and finally approved the version to be published, and agree 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.

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interpretation of the data; writing of the report; or decision to submit the manuscript for publication.

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CONFLICTS OF INTEREST

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SUPPORTING INFORMATION

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

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