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Influence of co-pathology on CSF and plasma synaptic markers SNAP25 and VAMP2 in Alzheimer's disease and Parkinson's disease

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Abstract

Background Synaptic dysfunction is a relevant feature of Alzheimer's disease (AD) and Parkinson's disease (PD) and can be quantified through the measurement of cerebrospinal fluid (CSF) synaptic markers, such as the presynaptic proteins synaptosomal-associated protein 25 kDa (SNAP25) and vesicle-associated membrane protein 2 (VAMP2). Plasma-based assays for synaptic markers are also emerging. In neurodegenerative diseases, synaptic dysfunction can be directly driven by proteinopathies such as amyloidosis (A), tauopathy (T), and α-synucleinopathy (S), which in turn can be detected via CSF biomarkers. This observational study aimed to: (i) evaluate the concordance of SNAP25 and VAMP2 in CSF and plasma; (ii) compare SNAP25 and VAMP2 concentrations in CSF and plasma across AD, PD, and control groups; (iii) examine the impact on synaptic markers concentration of CSF α-synuclein seed amplification assay (αS-SAA) positivity (S+) in AD, and (iv) of CSF amyloid/tau (A+/T+) positivity in PD.

Methods We included 80 AD patients (preclinical, mild cognitive impairment [MCI], and dementia stages), 47 PD patients, and 41 controls with other neurological diseases (OND) and known CSF A/T/S profiles. All AD and 5/47 PD patients were CSF A+/T+, while 26/80 AD and all PD patients were CSF S+. All OND had a non-A+/T + and a S − profile. SNAP25 and VAMP2 concentrations in CSF and plasma were measured using Simoa-based immunoassays.

Results CSF and plasma SNAP25 were positively correlated, but no correlation was observed for VAMP2 in these matrices. CSF and plasma SNAP25 and CSF VAMP2 were higher in AD compared to PD and OND. Synaptic markers were elevated in preclinical AD and remained stable across MCI-AD and AD dementia stages. AD patients with CSF αS-SAA positivity showed no significant difference in synaptic markers compared to those without CSF αS-SAA positivity, independent of clinical stage. In PD, A+/T + patients had higher CSF and plasma SNAP25 (132.3 ± 41.6; 1.9 ± 0.5 pg/mL) compared to non-A+/T + PD (105.4 ± 34.2; 1.3 ± 0.3 pg/mL) ($p < 0.001$ and $p < 0.01$, respectively).

Conclusions SNAP25 reliably serves as a marker of synaptic injury when measured in both CSF and plasma, whereas VAMP2 demonstrates reliability exclusively in CSF. Both markers are primarily influenced by AD pathology and remain unaffected by α-synucleinopathy, suggesting their potential in detecting AD-related synaptic dysfunction.

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Keywords Alzheimer's disease, Parkinson's disease, Synaptic markers, SNAP25, VAMP2, Cerebrospinal fluid, Blood, Co-pathology

Background

Synaptic dysfunction and loss are relevant pathophysiological features of Alzheimer's disease (AD) and Parkinson's disease (PD) [1]. Synaptic impairment can drive the progression of symptoms in neurodegenerative diseases and affect the brain resilience to neurodegeneration [2]. The protein misfolding underlying neurodegenerative diseases can directly influence synaptic functionality. As an example, α-synuclein oligomers may affect presynaptic vesicle clustering through interactions with vesicle-associated membrane protein 2 (VAMP2) [3]. Protein misfolding associated with AD and PD can be identified in vivo through fluid biomarkers. Amyloidosis (A), tauopathy (T) and α-synucleinopathy (S) are indeed reliably detectable through cerebrospinal fluid (CSF) measurement of β-amyloid (Aβ)1–42/Aβ1–40 ratio (Aβ42/40), phosphorylated tau protein at threonine 181 (p-tau181), and through α-synuclein seed amplification assay (αS-SAA), respectively [4].

In AD patients, α-synucleinopathy is often present as a co-pathology, with CSF αS-SAA being positive in approximately 30% of AD cases [5]. At the same time, CSF Aβ42/40 and p-tau181 can frequently be found altered in PD patients, suggesting that AD co-pathology is common in PD [6]. The presence of α-synucleinopathy in AD is associated with worse disease progression and with higher frequency of atypical clinical manifestation [5, 7]. Vice versa, in PD patients AD co-pathology is associated with a higher risk of developing cognitive impairment [6].

The impact of AD pathology in PD and that of α-synucleinopathy in AD in terms of synaptic impairment is largely unexplored. Recent advances in fluid biomarkers have opened new avenues for the in vivo quantification of synaptic injury [8]. CSF biomarkers have been at the forefront of this progress, with neurogranin—a postsynaptic protein—emerging as a prominent indicator. Elevated levels of neurogranin in the CSF have been observed in patients with AD, correlating strongly with cognitive performance [9, 10]. Additionally, other synaptic proteins, including β-synuclein, synaptosomal-associated protein 25 kDa (SNAP25), and VAMP2, have shown promise as CSF biomarkers for synaptic degeneration in AD [11–14]. Despite the robust findings from CSF-based studies, the exploration of synaptic protein biomarkers in plasma remains limited. To date, plasma studies have primarily focused on β-synuclein, relying on mass spectrometry techniques that, while sensitive, pose significant challenges for widespread application [15]. The development of accessible blood-based synaptic biomarkers would represent a significant advancement in the field.

The aims of this study are to determine: (i) CSF/plasma concordance of the synaptic markers SNAP25 and VAMP2; (ii) how SNAP25 and VAMP2 in CSF and plasma change according to the clinical diagnoses of AD and PD; (iii) whether CSF αS-SAA positivity influences the concentrations of SNAP25 and VAMP2 in the CSF and plasma of AD patients; (iv) whether CSF A/T positivity influences SNAP25 and VAMP2 concentrations in PD patients.

Methods

Study population

We retrospectively analyzed paired CSF and plasma samples of 169 consecutive patients referred to the Section of Neurology, University Hospital of Perugia, Italy, between January 2016 and January 2022. The cohort included 80 patients with AD, 47 patients with PD and 42 control patients with other neurological diseases (OND). All patients underwent medical history, physical and neurological examination, a thorough neuropsychological evaluation, brain imaging (computed tomography or magnetic resonance imaging), and lumbar puncture for the measurement of CSF core AD biomarkers, namely Aβ42/40, p-tau181 and total tau (t-tau). Further, for all the patients and controls, the presence of α-synucleinopathy was tested by means of CSF α-synuclein seed amplification assay (αS-SAA), as previously described [5].

AD patients ($n = 80$) were diagnosed according to a CSF biomarker profile A+/T+, independent of the clinical stage, in line with the 2018 National Institute of Aging–Alzheimer's Association criteria [16]. Combining the CSF profile (A+/T+) with neuropsychological evaluation and functional assessment, AD patients were grouped as preclinical AD (pre-AD) ($n = 19$), mild cognitive impairment due to AD (MCI-AD) ($n = 30$), and dementia due to AD (dem-AD) ($n = 31$). 26 AD patients (32.5%) showed evidence of α-synucleinopathy (αS-SAA+) on CSF αS-SAA, being classified as A+/T+/S+.

PD patients ($n = 47$) were diagnosed according to the current diagnostic criteria [17, 18]. Based on neuropsychological assessment, PD patients were categorized as cognitively normal PD (PD-CN) ($n = 16$), PD with mild cognitive impairment (PD-MCI) ($n = 26$) or PD with dementia (PDD) ($n = 5$) [19]. All PD patients were αS-SAA+, and 5 of them (2.4%) showed evidence of AD co-pathology (CSF A+/T+/S+).

OND patients ($n = 42$) underwent lumbar puncture for CSF analysis within the diagnostic work-up for suspected neurological disturbances (headache, mononeuropathy,

psychiatric disturbances, subjective cognitive complaints). Their CSF profile was negative for AD biomarkers, and they all tested negative on CSF αS-SAA (CSF A-/T-/S-). According to the neuropsychological evaluation, these subjects were either cognitively unimpaired (OND-CN) ($n = 21$) or had mild cognitive impairment (OND-MCI) ($n = 21$). Only OND-MCI subjects with stable cognitive performances at 2-year follow-up and a negative brain ^{18}F-fluorodeoxyglucose-positron emission tomography result were included.

For all the patients included, the Mini Mental State Examination (MMSE) score at the time of lumbar puncture was available. Further, for PD patients, also Montreal Cognitive Assessment (MoCA) score and Unified Parkinson's Disease Rating Scale, part III (UPDRS-III) were available.

The main demographic and clinical features of each diagnostic group are summarized in Table 1. All patients underwent lumbar puncture and venipuncture at the Section of Neurology, University Hospital of Perugia, Italy, and paired CSF and plasma were collected, handled, and stored according to international guidelines [20]. Samples were stored at -80 °C and thawed only once prior to analysis.

CSF and plasma SNAP25 and VAMP2 measures

The immunoassay for SNAP25 was developed by ADx NeuroSciences with Homebrew Simoa assay kit components from Quanterix (#101354, Quanterix, Billerica, USA). A mouse monoclonal antibody (mAb), ADx404 [21], targeting the N-terminal end of human SNAP25, was used as the capture antibody, with bead coupling optimized according to the Homebrew Assay Development Guide. The detector antibody, RD-086, another mouse monoclonal antibody recognizing an internal epitope within the N-terminal region of SNAP25, was optimized for detector coupling. Additional optimization steps included adjusting diluents, utilizing helper beads, and determining the optimal bead number. The SNAP25 measurements were performed by using 1.50E + 07 active ADx404 coupled capture beads/mL (50% helper beads) and 0.5 mg/mL of RD-086 detector antibody in a PBS/casein-based sample diluent. A 60-minute incubation (80 cadences) in a cuvette was performed with 25 µL of the ADx404 coupled beads solution, 1:6 diluted plasma sample or 1:120 diluted CSF sample, and 20 µL of the RD-086 detector antibody solution. After several wash steps, a second incubation of 5 min and 15 s (7 cadences) was conducted where 100 µL of 50 pM Streptavidin-β-Galactosidase (SBG) solution was added to the cuvette, followed by multiple wash steps. Lastly, Resorufin β-D-galactopyranoside (RGP) substrate solution was added to the solution and loaded on to the SIMOA disc array. Signal conversion to concentrations was performed using a synthetic peptide representing the N-terminal sequence of human SNAP25.

Similarly, the immunoassay for VAMP2 is a Homebrew Simoa assay developed in accordance with the Homebrew Assay Development Guide (#101354, Quanterix, Billerica, USA). An initial version of this assay was described by Goossens et al., 2023 [22]. The capture antibody is a mouse monoclonal antibody, RD-087, while the detector antibody was replaced with a mouse monoclonal antibody, RD-0-81, from ADx NeuroSciences. The VAMP2 measurements were performed by using 2E + 07 active RD-087 coupled capture beads/mL (50% helper beads) and 0,5 µg/mL of RD-081 detector antibody. A 60-minute incubation (80 cadences) in a cuvette was performed with 25 µL of the RD-087 coupled beads solution, 1:3 diluted plasma sample or 1:16 diluted CSF sample, and 20 µL of the RD-081 detector antibody solution. After several wash steps, a second incubation of 5 min

Table 1 Demographical and clinical features of each diagnostic group

	pre-AD	MCI-AD	dem-AD	PD-CN	PD-MCI	PDD	OND-CN	OND-MCI
n	19	30	31	16	26	5	21	21
% Male	26.3%	26.7%	29%	56.3%	76.9%	100%	66.7%	33.3%
Age (y)	72.8 ± 5.9	71.4 ± 5.7	71.4 ± 7.5	64.6 ± 6.2	67.7 ± 6	75.8 ± 3.3	66.2 ± 6.4	72.8 ± 6.9
MMSE	28 ± 1.1	22.9 ± 3.3	16.5 ± 6.2	28.6 ± 1.5	25.8 ± 2.1	21 ± 3.6	28.5 ± 1	25.2 ± 2.6
MoCA	-	-	-	25.9 ± 2.6	19.3 ± 4.1	14.4 ± 1.1	-	-
UPDRS-III	-	-	-	27 ± 7.7	31.8 ± 9.8	33.5 ± 10	-	-
A- / T-	0	0	0	13	20	1	14	15
A- / T+	0	0	0	3	2	0	7	3
A+ / T-	0	0	0	0	2	1	0	3
A+ / T+	18	30	31	0	2	3	0	0
αS-SAA+	4	11	13	16	26	5	0	0

Data are expressed as mean ± standard deviation or number and percentages

Abbreviations. dem-AD: dementia due to Alzheimer's disease. MCI-AD: mild cognitive impairment due to Alzheimer's disease. MMSE: Mini Mental State Examination. MoCA: Montreal Cognitive Assessment. OND-CN: controls with other neurological diseases, cognitively normal. OND-MCI: controls with other neurological diseases and with mild cognitive impairment. PD-CN: Parkinson's disease cognitively normal. PDD: Parkinson's disease with dementia. PD-MCI: Parkinson's disease with mild cognitive impairment. pre-AD: preclinical Alzheimer's disease. UPDRS-III: Unified Parkinson's Disease Rating Scale, part III

and 15 s (7 cadences) was conducted where 100 µL of 50 pM SBG solution was added to the cuvette, followed by multiple wash steps. Lastly, RGP substrate solution was added to the solution and loaded on to the SIMOA disc array. The assay targets a region near the N-terminus of VAMP2, and a synthetic peptide encompassing the epitopes of both antibodies was used to convert signals to VAMP2 concentrations. Further characteristics of both immunoassays and the run qualifications for this study can be found in supplemental material (Supplemental Table 1), (Supplemental Table 2), (Supplemental Table 3).

Statistical analysis

All statistical analyses were performed using SPSS Statistics 29.0 (IBM Inc., Armonk, NY) and GraphPad Prism 10 (GraphPad Software, La Jolla, CA). A log10 transformation was applied to all markers to enable the use of parametric statistical tests. Correlation analyses were conducted using the Pearson correlation coefficient (r). Differences between continuous variables were assessed with Student's t-test (for two-group comparisons) and one-way analysis of variance (ANOVA) (for comparisons across multiple groups). In both correlation analyses and group comparisons, p-values were adjusted for multiple comparisons using the Bonferroni correction. Generalized linear models (GLMs) were employed to examine differences in synaptic marker levels across diagnostic groups while adjusting for age. Receiver operating characteristic (ROC) analyses were performed to evaluate the diagnostic accuracy of biomarkers, with the area under the curve (AUC) reported. An adjusted p-value < 0.05 was considered statistically significant for all analyses.

Results

Correlation of CSF and plasma synaptic markers

In the entire cohort, CSF and plasma SNAP25 levels were weakly and positively correlated ($r = 0.31$, $p < 0.001$), whereas no significant correlation was observed between CSF and plasma VAMP2 levels. Within CSF, SNAP25 and VAMP2 showed a strong positive correlation ($r = 0.87$, $p < 0.001$) (Fig. 1).

Diagnostic groups and synaptic markers

CSF SNAP25 and VAMP2 concentrations were significantly higher in AD (167.7 ± 41.3 pg/mL; 369.3 ± 148.2 pg/mL) compared to PD (109.9 ± 33.4 pg/mL; 253.5 ± 119.9 pg/mL) and OND (117.6 ± 41.6 pg/mL; 300.1 ± 182.7 pg/mL) ($p < 0.001$) (Table 2) (Fig. 2). Plasma SNAP25 was also elevated in AD (1.9 ± 0.6 pg/mL), compared to PD (1.3 ± 0.3 pg/mL) and OND (1.4 ± 0.4 pg/mL) ($p < 0.001$), while plasma VAMP2 showed no significant differences among groups (Table 2) (Fig. 2). ROC analysis showed that CSF and plasma SNAP25 were effective in discriminating AD from OND, with an AUC of 0.831 (95% CI: 0.744–0.918) for CSF SNAP25 and 0.762 (95% CI: 0.669–0.856) for plasma SNAP25 (Supplemental Fig. 1) (Supplemental Table 4). No significant differences were observed for any synaptic marker between PD and OND groups (Supplemental Table 4) (Fig. 2) (Supplemental Fig. 1).

Demographics and synaptic markers

Sex and age were not associated with any of the synaptic markers in CSF or plasma. Across diagnostic groups, AD patients were significantly older than subjects from other groups ($p < 0.001$). After adjusting for age in GLMs, AD diagnosis remained independently associated with higher levels of CSF SNAP25 (B = 0.188; 95% CI: 0.141–0.236; $p < 0.001$), CSF VAMP2 (B = 0.162; 95% CI: 0.084–0.241; $p < 0.001$), and plasma SNAP25 (B = 0.166; 95% CI: 0.118–0.214; $p < 0.001$).

Synaptic markers in AD and PD with CSF evidence of co-pathologies

Within the AD group, there were no significant differences in synaptic markers between patients with CSF αS-SAA + and αS-SAA- profiles (Fig. 3), even when

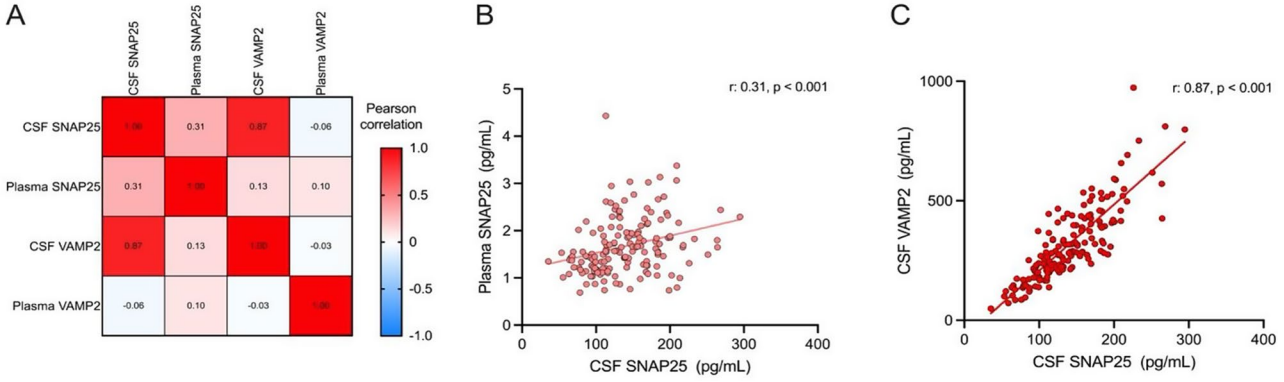

Fig. 1 Correlations between CSF and plasma SNAP25 and VAMP2. Panels show (**A**) Pearson's correlation coefficients between synaptic markers as heatmap, (**B**) scatter plot of correlation between CSF and plasma SNAP25 in the study cohort and (**C**) scatter plot of correlation between CSF SNAP25 and VAMP2 in the study cohort. CSF: cerebrospinal fluid. SNAP25: synaptosomal-associated protein 25 kDa. VAMP2: vesicle-associated membrane protein 2

Table 2 CSF and plasma synaptic markers across diagnostic groups

	CSF SNAP25 (pg/mL)	Plasma SNAP25 (pg/mL)	CSF VAMP2 (pg/mL)	Plasma VAMP2 (pg/mL)
AD (all) ***n*** **= 80**	**167.7 ± 41.3 median 164.2, IQR 41.3**	**1.9 ± 0.6 median 1.9, IQR 0.7**	**369.3 ± 148.2 median 324.6, IQR 162.1**	**35.6 ± 10.2 median 34.9, IQR 10.8**
pre-AD *n* = 19	159.6 ± 40.5 median 152.9, IQR 55.2	1.6 ± 0.4 median 1.7, IQR 0.3	379.5 ± 141.9 median 321.3, IQR 253	34.6 ± 7.7 median 36.1, IQR 11.6
MCI-AD *n* = 30	171.9 ± 45.1 median 168.8, IQR 57.2	2.2 ± 0.7 median 2.2, IQR 0.9	379.1 ± 177.6 median 324.6, IQR 198.4	35.9 ± 11.9 median 35.3, IQR 14.5
dem-AD *n* = 31	168.3 ± 38.3 median 167.7, IQR 54.1	1.9 ± 0.6 median 1.9, IQR 0.8	353.2 ± 119.2 median 329.6, IQR 151.8	35.9 ± 9.8 median 33.6, IQR 11.3
PD (all) *n* = 47	**109.9 ± 33.4 median 109.9, IQR 37.3**	**1.3 ± 0.3 median 1.2, IQR 0.4**	**253.5 ± 119.9 median 223.4, IQR 160.1**	**35.8 ± 11.6 median 33.5, IQR 14.4**
PD-CN *n* = 16	112.5 ± 29.4 median 112.5, IQR 36.9	1.3 ± 0.3 median 1.3, IQR 0.6	274.1 ± 111.1 median 269.8, IQR 158.2	34.1 ± 12.7 median 31.9, IQR 12.7
PD-MCI *n* = 26	108.1 ± 37.5 median 102.1, IQR 36.9	1.3 ± 0.3 median 1.2, IQR 0.3	250.8 ± 133.2 median 200.9, IQR 185.2	35.6 ± 11.4 median 33.8, IQR 16.6
PDD *n* = 5	111.4 ± 26 median 115.9, IQR 51.4	1.5 ± 0.6 median 1.4, IQR 0.9	209.1 ± 64.8 median 232.7, IQR 124.9	41.8 ± 10.2 median 36.1, IQR 19.3
OND (all) *n* = 42	**117.6 ± 41.6 median 106.9, IQR 60.2**	**1.4 ± 0.4 median 1.4, IQR 0.4**	**300.1 ± 182.7 median 238.9, IQR 271.6**	**34.4 ± 13.9 median 33.6, IQR 21**
OND-CN *n* = 21	123.3 ± 50.1 median 107.7, IQR 82.5	1.3 ± 0.4 median 1.3, IQR 0.4	332.3 ± 219.3 median 238.9, IQR 276.9	32.2 ± 13.8 median 33.6, IQR 21.4
OND-MCI *n* = 21	111.2 ± 29.6 median 106.9, IQR 34.7	1.5 ± 0.3 median 1.4, IQR 0.5	265.8 ± 132.1 median 235.5, IQR 216.1	36.8 ± 14.2 median 32.8, IQR 20.1
p-values	AD vs. OND: $p < 0.001$ AD vs. PD: $p < 0.001$	AD vs. OND: $p < 0.001$ AD vs. PD: $p < 0.001$ OND-CN vs. OND-MCI: $p = 0.024$	AD vs. OND: $p < 0.001$ AD vs. PD: $p < 0.001$	

Abbreviations. dem-AD: dementia due to Alzheimer's disease. IQR: interquartile range. MCI-AD: mild cognitive impairment due to Alzheimer's disease. OND-CN: controls with other neurological diseases, cognitively normal. OND-MCI: controls with other neurological diseases and with mild cognitive impairment. PD-CN: Parkinson's disease cognitively normal. PDD: Parkinson's disease with dementia. PD-MCI: Parkinson's disease with mild cognitive impairment. pre-AD: preclinical Alzheimer's disease. SNAP25: synaptosomal-associated protein 25 kDa. VAMP2: vesicle-associated membrane protein 2.

stratified by clinical stage. In the PD group, patients with a CSF A+/T + profile showed higher levels of CSF SNAP25 (132.3 ± 41.6 pg/mL) and plasma SNAP25 (1.9 ± 0.5 pg/mL) compared to those with a non-A+/T + profile (105.4 ± 34.2 pg/mL and 1.3 ± 0.3 pg/mL, respectively) ($p < 0.001$ and $p < 0.01$, respectively) (Fig. 4).

Synaptic markers and stages of cognitive and motor impairment

CSF and plasma SNAP25 were inversely correlated with MMSE scores at baseline in the entire cohort (r = -0.31, $p < 0.001$; and r = -0.35, $p < 0.001$, respectively) (Supplementary Fig. 2), while no correlation was found between MMSE and VAMP2. In the AD group, CSF and plasma markers did not significantly differ among patients with pre-AD, MCI-AD, or dem-AD (Table 2) (Fig. 5). Similarly, within the PD group, no significant differences were found among PD-CN, PD-MCI, and PDD (Table 2) (Fig. 5). Moreover, no correlation was found between synaptic markers and MoCA or UPDRS-III scores in PD subgroups. Interestingly, in the OND group, plasma SNAP25 levels were significantly higher in OND-MCI compared to OND-CN ($p = 0.024$) (Table 2) (Fig. 5).

Discussion

In this study, we explored the synaptic markers SNAP25 and VAMP2 in CSF and plasma in a well-characterized clinical cohort of AD and PD patients with comprehensive molecular profiling, including CSF biomarkers of amyloidosis, tau phosphorylation, and α-synucleinopathy.

The most relevant finding of this study is that synaptic markers are elevated in the presence of AD pathology, even when AD represents a co-pathology within other neurodegenerative diseases, such as PD. Another synaptic marker, neurogranin—which, unlike SNAP25 and VAMP2, has a postsynaptic localization—has previously been shown to be specifically increased in the CSF of patients with AD compared to those with other neurodegenerative diseases including PD, atypical parkinsonian syndromes [23], and frontotemporal dementia [24]. Furthermore, consistent with findings on another presynaptic marker such as β-synuclein [25], we observed increased CSF levels of SNAP25 and VAMP2 in AD patients, including those at the preclinical stage. These markers remained stable throughout the clinical continuum of AD, from preclinical AD to dementia, suggesting that SNAP25 and VAMP2, like other synaptic proteins [25], may serve as sensitive state markers. In plasma, the increase in SNAP25 was more pronounced in MCI-AD compared to pre-AD patients, although the difference did not reach statistical significance. This finding suggests that in plasma, the elevation of synaptic markers might become detectable later in the disease continuum.

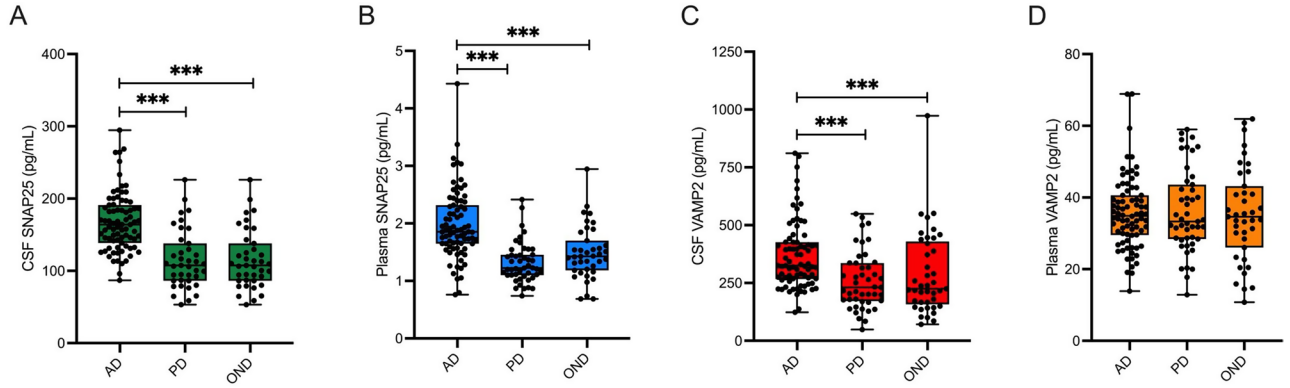

Fig. 2 CSF and plasma SNAP25 and VAMP2 across diagnostic groups. Panels show levels of (**A**) CSF SNAP25, (**B**) plasma SNAP25 (**C**) CSF VAMP2, and (**D**) plasma VAMP2 in AD ($n=80$), PD ($n=47$) and OND ($n=42$). *** $p<0.001$. AD: Alzheimer's disease. CSF: cerebrospinal fluid. OND: other neurological diseases. PD: Parkinson's disease. SNAP-25: synaptosomal-associated protein 25 kDa. VAMP2: vesicle-associated membrane protein 2

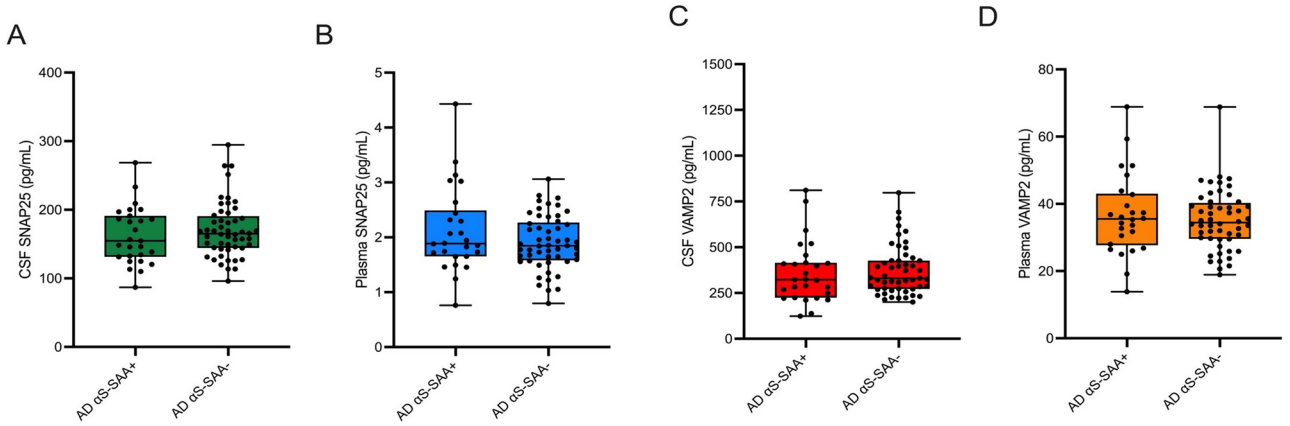

Fig. 3 CSF and plasma SNAP25 and VAMP2 in AD patients with and without in vivo evidence of α-synucleinopathy. Panels show levels of (**A**) CSF SNAP25, (**B**) plasma SNAP25 (**C**), CSF VAMP2, and (**D**) plasma VAMP2 in AD patients with (αS-SAA+) ($n=26$) and without (αS-SAA-) ($n=53$) evidence of α-synucleinopathy on CSF αS-SAA. AD αS-SAA+: patients with Alzheimer's disease and with positive cerebrospinal fluid α-synuclein seed amplification assay. AD αS-SAA-: patients with Alzheimer's disease and with negative cerebrospinal fluid α-synuclein seed amplification assay. CSF: cerebrospinal fluid. SNAP-25: synaptosomal-associated protein 25 kDa. VAMP2: vesicle-associated membrane protein 2.

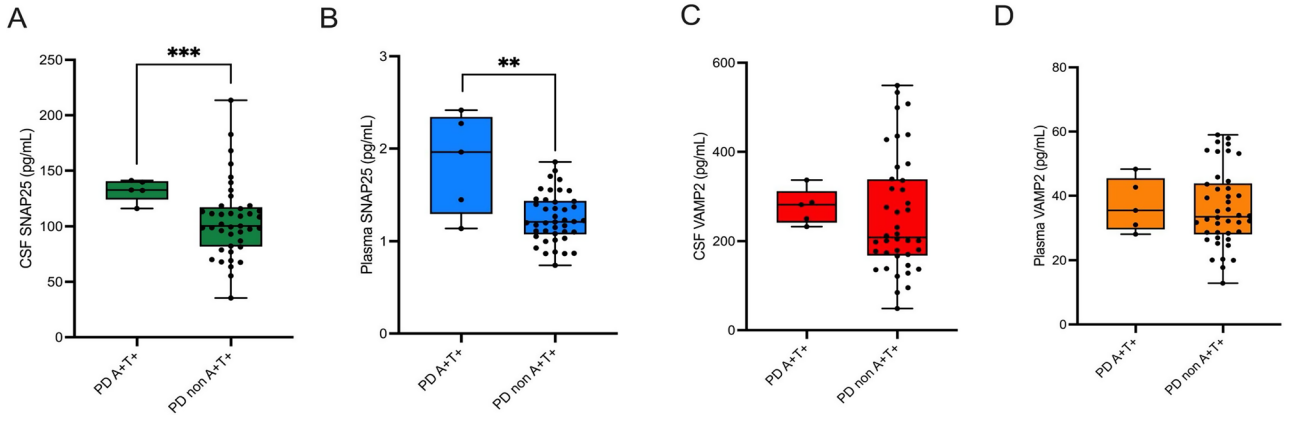

Fig. 4 CSF and plasma SNAP25 and VAMP2 in PD patients with and without in vivo evidence of AD co-pathology. Panels show levels of (**A**) CSF SNAP25, (**B**) plasma SNAP25 (**C**) CSF VAMP2, and (**D**) plasma VAMP2 in PD patients with (A+T+) ($n=5$) and without (non A+T+) ($n=42$) evidence of AD copathology on CSF biomarkers. PD A+T+: patients with Parkinson's disease and with positive cerebrospinal fluid biomarkers of amyloidosis and tau phosphorylation. PD non A+T+: patients with Parkinson's disease and with negative cerebrospinal fluid biomarkers of amyloidosis and tau phosphorylation. CSF: cerebrospinal fluid. SNAP-25: synaptosomal-associated protein 25 kDa. VAMP2: vesicle-associated membrane protein 2.

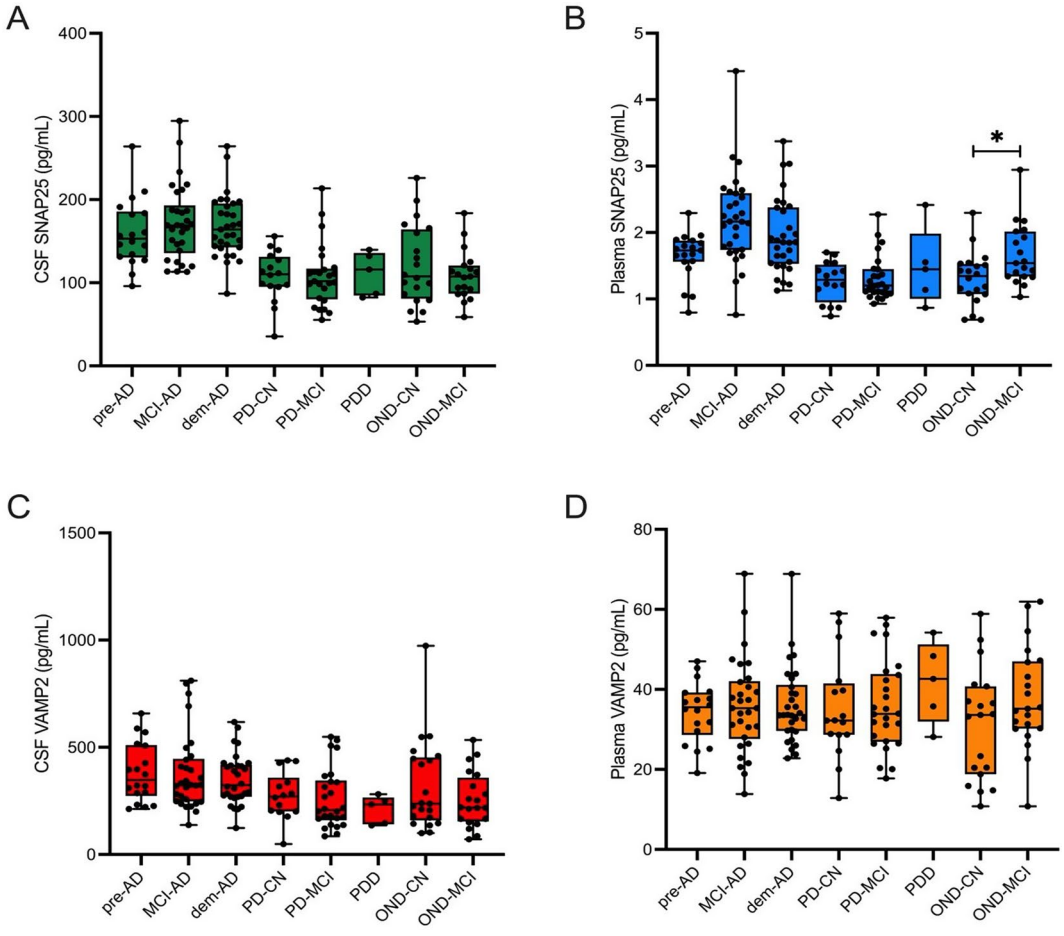

Fig. 5 CSF and plasma SNAP25 and VAMP2 in the different subgroups. Panels show levels of (**A**) CSF SNAP25, (**B**) plasma SNAP25 (**C**) CSF VAMP2, and (**D**) plasma VAMP2 in pre-AD ($n=19$), MCI-AD ($n=30$), dem-AD ($n=31$), PD-CN ($n=16$), PD-MCI ($n=26$), PDD ($n=5$), OND-CN ($n=21$) and OND-MCI ($n=21$). *$p<0.05$. CSF: cerebrospinal fluid. pre-AD: preclinical Alzheimer's disease. MCI-AD: mild cognitive impairment due to Alzheimer's disease. dem-AD: dementia due to Alzheimer's disease. OND-CN: other neurological diseases, cognitively normal. OND-MCI: other neurological diseases with mild cognitive impairment. PD-CN: Parkinson's disease, cognitively normal. PD-MCI: Parkinson's disease with mild cognitive impairment. PDD: Parkinson's disease dementia. SNAP-25: synaptosomal-associated protein 25 kDa. VAMP2: vesicle-associated membrane protein 2.

The early elevation of SNAP25 and VAMP2, detectable even in preclinical AD, highlights that synaptic injury is a very early event in the AD continuum. Interestingly, the presence of α-synucleinopathy in AD, as indicated by CSF αS-SAA positivity, did not significantly influence synaptic markers levels. This suggests that the primary driver of synaptic dysfunction in AD is specific to its pathophysiological mechanisms, rather than being modulated by the presence of α-synuclein co-pathology.

In PD patients, the presence of an AD-like CSF profile (A+/T+) was associated with significantly higher levels of SNAP25 in both CSF and plasma. This finding aligns with observations in other synaptic markers, such as β-synuclein, which is not elevated in Lewy body diseases unless AD co-pathology is present [6, 26]. These results underscore the relevance of AD co-pathology in driving synaptic dysfunction in PD.

Outside neurodegenerative diseases, synaptic markers showed potential relevance in detecting cognitive impairment. In our control cohort, cognitively impaired individuals with other neurological diseases (OND-MCI) exhibited significantly higher plasma SNAP25 levels compared to cognitively normal subjects (OND-CN). This finding suggests that SNAP25 may serve as a marker of cognitive function, like previous findings for neurogranin, another synaptic marker capable of capturing subtle cognitive impairments, also in non-AD contexts [27].

From a technical perspective, we found that plasma SNAP25 correlated with its CSF counterpart, showing similar changes across clinical groups and biomarker profiles in the study cohort. This consistency underscores the reliability of plasma SNAP25 as a surrogate marker for CNS synaptic dysfunction, providing a less invasive option for monitoring synaptic injury. In contrast,

plasma VAMP2 did not correlate with its CSF counterpart and exhibited inconsistent changes across clinical and biomarker-defined groups. This discrepancy may be partly explained by the broader extra-CNS expression of VAMP2, including endocrine, genital, urinary, and lymphoid tissue, as opposed to the CNS-selective expression of SNAP25 [28]. Consequently, plasma VAMP2 appears to be less reliable as a proxy for central synaptic damage.

This study's strengths include the use of a well-characterized cohort with comprehensive CSF and plasma biomarker profiling and the inclusion of advanced diagnostic techniques such as αS-SAA. However, limitations include the relatively small number of PD patients with confirmed AD co-pathology, which may limit the generalizability of the findings to broader PD populations.

Despite these limitations, our findings reinforce the validity of synaptic markers, particularly SNAP25, in detecting and monitoring synaptic dysfunction in neurodegenerative diseases. The ability of plasma SNAP25 to reflect CSF levels offers a promising avenue for non-invasive diagnostic and prognostic approaches. Conversely, the lack of reliability in plasma VAMP2 underscores the importance of understanding tissue-specific expression patterns when selecting biomarkers for clinical application.

Conclusions

This study provides evidence that synaptic markers are robust indicators of AD-related pathology and highlights the relevance of AD pathology in modulating synaptic dysfunction.

Abbreviations

A	Amyloidosis
Aβ42/40	β-amyloid (Aβ)1–42/Aβ1–40 ratio
AD	Alzheimer's disease
ANOVA	One-way analysis of variance
AUC	Area under the curve
CSF	Cerebrospinal fluid
dem-AD	Dementia due to AD
GLMs	Generalized linear models
MAb	Monoclonal antibody
MCI-AD	Mild cognitive impairment due to Alzheimer's disease
MMSE	Mini Mental State Examination
MoCA	Montreal Cognitive Assessment
MRD	Minimal required dilution
OND	Other neurological diseases
OND-CN	Other neurological diseases, cognitively unimpaired
OND-MCI	Other neurological diseases with mild cognitive impairment
PD	Parkinson's disease
PD-CN	Cognitively normal Parkinson's disease
PD-MCI	Parkinson's disease with mild cognitive impairment
PDD	Parkinson's disease with dementia
p-tau181	Phosphorylated tau protein at threonine 181
pre-AD	Preclinical Alzheimer's disease
ROC	Receiver operating characteristic
S	α-synucleinopathy
SNAP25	Synaptosomal-associated protein 25 kDa
T	Tauopathy
t-tau	Total tau
UPDRS-III	Unified Parkinson's Disease Rating Scale, part III
VAMP2	Vesicle-associated membrane protein 2
αS-SAA	α-synuclein seed amplification assay
αS-SAA+	Evidence of α-synucleinopathy

Supplementary Information

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Supplementary Material 1

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Author contributions

LG: Designed study, data acquisition, performed analysis, drafted manuscript. GB: Designed study, data acquisition, performed analysis, revised manuscript. DC: Data acquisition, revised manuscript. CDR: Data acquisition. JG: Data acquisition. FPP: Data acquisition. EV: Designed study, data acquisition, revised manuscript. LP: Designed study, data acquisition, performed analysis, revised manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The study was approved by the local Ethics Committee (Comitato Etico Aziende Sanitarie Regione Umbria 19369/AV and 20942/21/OV). All the patients provided informed consent to participate in the study.

Consent for publication

Not applicable.

Competing interests

LG has participated in advisory boards for and received writing or speaker honoraria and travel grants from Almirall, Biogen, Eisai, Euroimmun, Fujirebio, Lilly, Merck, Mylan, Novartis, Roche, Sanofi, Siemens Healthineers, and Teva. GB received honoraria from Fujirebio and completed paid consultancies for Parkinson's Foundation. He received travel/educational grants from Fujirebio and Alzheimer's Association. DC has nothing to disclose. CDR and JG are employee at ADx NeuroSciences NV, Ghent, Belgium. FPP has nothing to disclose. EV is co-founder of ADx NeuroSciences NV, Ghent, Belgium. LP served as Member of Advisory Boards for Fujirebio, IBL, Roche, and Merck.

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