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Cerebrospinal fluid α-synuclein seed amplification assay positivity is associated with increased plasma neurofilament light chain in Alzheimer's disease

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ABSTRACT

Background: Synucleinopathy is the most common co-pathology in Alzheimer's disease (AD) and can be detected in vivo using cerebrospinal fluid (CSF) alpha-synuclein seed amplification assays (synSAA). CSF synSAA positivity has been linked to worse clinical outcome. This study investigated whether synSAA positivity is also associated with levels of plasma neurofilament light chain (NfL), a sensitive, unspecific marker of neurodegeneration.

Methods: We retrospectively analyzed a cohort of 240 individuals across the AD clinical continuum who had undergone clinical, neuropsychological, and biomarker assessments including CSF synSAA. Analyses involving plasma NfL were conducted in the subgroup with available plasma samples collected at the time of lumbar puncture ($n = 166$). One-way and two-way ANOVA were used to compare log-transformed NfL levels among AD stages, according to synSAA status. Logistic regression models examined associations between log-transformed NfL and synSAA status, adjusting for age and AD stage.

Findings: No significant age difference was found between synSAA-positive and negative groups. Plasma NfL levels were significantly higher in synSAA-positive individuals. This association remained significant after adjusting for age and AD clinical stage.

Interpretation: In AD patients, CSF synSAA positivity is associated with increase of plasma NfL levels along the AD clinical continuum. This finding supports the knowledge that synuclein co-pathology represents a contributive factor of neurodegeneration in AD patients.

1. Research in context

1.1. Evidence before this study

We searched PubMed up to May 2025 for studies examining the relationship between α-synuclein co-pathology (detected via cerebrospinal fluid α-synuclein seed amplification assay, synSAA) and neurofilament light chain (NfL) levels in patients with Alzheimer's disease (AD). Search terms included "Alzheimer's disease", "AD", "α-synuclein", "seed amplification assay", "NfL", "neurofilament light chain", and "biomarkers." We included original studies on human participants with confirmed AD biomarker profile, with no language restrictions. We found that few recent studies have examined synSAA relationship with NfL. Notably, two previous works have reported elevated CSF or plasma NfL in synSAA-positive (S+) subgroups (including Down syndrome–associated AD and biomarker-defined T+ subgroups), but none provided a comprehensive analysis with a systematic evaluation of plasma NfL levels in a well-characterized cohort of AD patients stratified by synSAA status.

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1.2. Added value of this study

This study, carried out in a large, biologically characterized cohort of AD patients along the whole clinical continuum (preclinical, prodromal and dementia stages), shows that CSF synSAA positivity, indicating the presence of Lewy Body co-pathology, is associated with the increase of plasma NfL levels.

1.3. Implications of all the available evidence

Our results support the evidence that α-synuclein co-pathology contributes to neurodegeneration in AD, as reflected by plasma NfL levels. This observation reinforces the proposal to expand the A/T/N system including the marker of synucleinopathy ("S"). Clinically, stratifying AD patients by CSF synSAA status may improve prognostic assessment and allow for personalized therapeutic strategies.

2. Introduction

Lewy body (LB) disease is the most common co-pathology in Alzheimer's disease (AD), being documented on a neuropathological level in nearly 33 % of cases (DeTure and Dickson, 2019). Advances in α-synuclein seed amplification assays (synSAA) have enabled reliable detection of α-synuclein aggregation in cerebrospinal fluid (CSF), providing a highly sensitive and specific test for identifying LB disease in vivo (Bellomo et al., 2022; Concha-Marambio et al., 2023; Arnold et al., 2022). The identification of individuals with AD who also exhibit LB co-pathology is becoming increasingly important, with the availability of disease-modifying therapies. CSF synSAA positivity in AD patients has been detected in a percentage in line with what observed in neuropathological series (approx. 1/3 of cases) and has been found to be associated with a worse cognitive outcome (Bellomo et al., 2024; Pilotto et al., 2023; Tosun et al., 2024a). Neurofilament light chain (NfL), a structural axonal protein released during any neuronal damage, has emerged as a sensitive, non-specific biomarker of neurodegeneration (Gaetani et al., 2019; Khalil et al., 2024), measurable in both CSF and plasma, with strong correlation between the two matrices (Jack et al., 2024; Sheth et al., 2025). Thus, it might be expected that plasma NfL levels may reflect the impact of LB co-pathology in AD patients.

In this study, we measured plasma NfL in a large cohort of patients from the University of Perugia, comprising patients with AD at preclinical stage (preAD), mild cognitive impairment due to AD (MCI-AD), and dementia due to AD (AD-dem) with available CSF synSAA status (Bellomo et al., 2024). Our aim was to investigate whether CSF synSAA positivity in AD is associated with increased plasma NfL levels.

3. Patients and methods

3.1. Study population

All subjects included in this study were enrolled at the Neurology Clinic of the University Hospital of Perugia (Italy). We previously assessed synSAA positivity prevalence in AD patients and its association with clinical features and outcome (Bellomo et al., 2024). We here retrospectively included n. 240 subjects within the AD continuum. As previously reported, for all subjects, CSF had been tested for core AD biomarkers, namely the β-amyloid (Aβ)1–42/Aβ1–40 ratio (Aβ42/40 ratio), phosphorylated tau protein at threonine 181 (p-tau181), and total tau (t-tau), and for synSAA. AD was diagnosed according to the CSF biomarker profile A+/T+, independent of the clinical stage (Jack et al., 2018). No patient was clinically diagnosed with neurodegenerative diseases other than AD, and in particular no cases of primary synucleinopathies (e.g., Parkinson's disease, dementia with Lewy bodies, multiple system atrophy) were present. Combining the CSF profile (A+/T+) with neuropsychological evaluation and functional assessment, AD patients were grouped as preAD (preclinical AD), prodromal, that is, MCI-AD and AD-dem (AD in dementia stage). All participants underwent a standardized diagnostic work-up including medical history, physical and neurological examination, brain imaging (computed tomography or magnetic resonance imaging), lumbar puncture, venipuncture, and comprehensive neuropsychological assessment. None of the patients had a medical history of kidney diseases. The choice of plasma for NfL quantification was driven by matrix availability within this retrospective cohort. Plasma aliquots were available for 168 patients; two were excluded due to the presence of subacute cerebral hemorrhage, a condition known to substantially affect plasma NfL concentrations, resulting in a final study sample of 166 subjects ($n = 11$ preAD, $n = 92$ MCI-AD and $n = 63$ CE-dem) of which 38 % ($n = 46$) had a positive synSAA status.

3.2. Consent statement

All the procedures involving human subjects were performed following the Declaration of Helsinki. All participants gave written informed consent to use medical data and biomaterials for research purposes. The privacy rights of all human subjects involved in this study have been respected. The study was approved by the local Ethics Committee (Comitato Etico Aziende

Sanitarie Regione Umbria, protocols 19,369/AV and 20,942/21/OV).

3.3. CSF collection and biomarkers analysis

Lumbar puncture was performed according to international guidelines (Teunissen et al., 2009); 10 to 12 mL of CSF were collected in sterile polypropylene tubes (Sarstedt, cat#62.610.210) and centrifuged for 10 min (2000 ×g), at room temperature. Aliquots of 0·5 mL were frozen at −80C° in polypropylene tubes (Sarstedt, cat#72.730.007). CSF samples were analyzed on the fully automated chemiluminescent platform Lumipulse G600-II (Fujirebio Inc) for Aβ42, Aβ40, t-tau, and p-tau181 levels in the Laboratory of Clinical Neurochemistry of the University of Perugia. All CSF samples were analyzed directly in their 0.5 mL storage tubes. To determine the A+ and T+ statuses, internally generated cutoffs (Bellomo et al., 2021) were employed.

3.4. CSF synSAA

The synSAA protocol developed by Concha-Marambio and colleagues (Ma et al., 2024) was performed at Amprion Inc., as previously reported (Bellomo et al., 2024). Briefly, CSF samples were evaluated in triplicate (40 μL/well) in a clear bottom 96-well plate containing a reaction mix consisting of 0.3 mg/mL recombinant α-Syn (Amprion, cat# S2020), 100 mM PIPES pH 6.50 (Sigma, cat# 80,635), 500 mM NaCl (Lonza, cat# 51.202), 10 μM ThT (Sigma, cat# T3516), and two 1/8-in. Si_3N_4 beads (Tsubaki Nakashima). The assay was performed in a BMG FLUOstar Omega shaker/reader at 42 °C, with 15-min shaking/incubation cycles. Maximum fluorescence (Fmax) from three replicates was used for result determination; if all three replicates present Fmax higher than 3000 RFU, the sample is deemed positive (S+). If only two cross the 3000 RFU threshold, the sample is considered inconclusive. If one or no replicate presents Fmax higher than 3000 RFU, the sample is considered negative (S-).

3.5. Plasma collection and NfL analysis

Blood samples were withdrawn at the time of lumbar puncture, following international guidelines and standard operating procedures (Teunissen et al., 2009). Briefly, blood samples were taken in BD Vacutainer™ anti-coagulated ethylene-diamine-tetra-acetic acid (EDTA). Blood samples were centrifuged within 3 h after draw at 1800 rpm × 10 min, aliquoted into polypropylene tubes (Sarstedt, cat#72.730.007), and stored at −80 °C. Plasma samples were analyzed

on the fully automated chemiluminescent platform Lumipulse G1200 (Fujirebio Inc) for NfL levels in the Laboratory of Clinical Neurochemistry of the University of Perugia. All plasma samples were analyzed directly in their 0.5 mL storage tubes.

3.6. Statistical analysis

Continuous variables were summarized with their median values and interquartile range (IQR). For subgroups in which IQR could not be quantified, minimum and maximum values were reported. Given the known association between age and NfL (Khalil et al., 2020; Vermunt et al., 2022), we first evaluated whether age differed between S+ and S- individuals to ensure that any observed group differences in NfL were not confounded by age. Visual inspection of histograms and Q-Q plots indicated a non-normal distribution for age, observation supported by Shapiro-Wilk tests, which showed a significant deviation from normality for raw values ($p < 0.001$). Log_{10} transformation did not adequately normalize the distribution (Shapiro-Wilk test $p < 0.001$). Therefore, group comparisons were performed using the non-parametric Mann-Whitney *U* test. In addition, we tested for potential sex imbalance between S^+ and S^- individuals using chi-square test conducted in the entire cohort and within each diagnostic group. Fisher's exact test was used for the smallest subgroup.

Raw plasma NfL values also showed a non-normal distribution, as suggested by visual inspection of histograms and Q-Q plots, with an approximately normal distribution following log_{10} transformation (Supplementary fig. 1). These observations were supported by Shapiro-Wilk test, which showed a significant deviation from normality for raw values ($p < 0.001$) and no significant deviation for log-transformed values ($p = 0.49$). Mean log-transformed plasma NfL levels were compared across disease stages and S+/S– groups using one-way ANOVA. A two-way ANOVA was conducted to parallelly examine the effects of synSAA status and AD stage on log-transformed plasma NfL levels, including potential interaction effects. Exploratory within-stage comparisons between S+ and S− individuals in the MCI-AD and AD-dementia groups were performed using simple-effect contrasts derived from the two-way ANOVA model applied to log-transformed NfL values, with *p*-values corrected using the Benjamini–Hochberg procedure.

A multivariable logistic regression model was used, as sensitivity analysis, to assess whether log-transformed plasma NfL levels predicted synSAA positivity after adjustment for age and disease stage.

3.7. Role of the funding source

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

4. Results

Demographic details, MMSE scores, CSF synSAA status, and plasma NfL levels of the AD subjects are reported in Table 1.

Age did not significantly differ between synSAA–positive (S+) and synSAA–negative (S-) AD individuals ($p = 0.46$, Mann-Whitney *U* test). No significant differences in sex distribution were observed in the entire cohort and within groups. As expected, plasma NfL concentration significantly increased with disease stage (one-way ANOVA, $F(2,163) = 23.11$, $p < 0.001$), as shown in Fig. 1. In the whole AD group, higher plasma NfL levels were found in S+ compared to S- AD subjects (one-way ANOVA, $F(1,164) = 5.44$, $p = 0.021$; Fig. 2A). By applying two-way ANOVA, despite the strong effect of disease stage ($F(2,160) = 23.10$, $p < 0.001$), synSAA status had still a significant effect on plasma NfL levels (Fig. 2B, $F(1,160) = 7.00$, $p = 0.009$), indicating that S+ individuals have higher plasma NfL concentrations compared to S- individuals, independently from the disease stage. The interaction between synSAA status and disease stage was not statistically significant ($F(2,160) = 2.30$, $p = 0.104$). An exploratory comparison within MCI-AD and AD-dementia stages, showed no significant difference in plasma NfL levels between S+ and S− individuals in the MCI-AD group ($p = 0.74$). In AD-dementia, S+ individuals exhibited higher NfL levels ($p = 0.025$), although this difference did not remain significant after Benjamini–Hochberg correction (p_FDR = 0.051). Considering the limited sample size of preAD subjects, post-hoc tests were not applied for this group. As a sensitivity analysis, we fitted a multivariable logistic regression model including log-transformed plasma NfL, age, and disease stage. Only plasma NfL was significantly associated with synSAA positivity ($\beta = 2.58$, $SE = 1.21$, $p = 0.033$), whereas age ($p = 0.884$) and disease stage (MCI-AD vs AD-dem: $p = 0.975$; preAD vs AD-dem: $p =$

Table 1
Demographic features, MMSE score, CSF synSAA status and plasma NfL values in AD patients. Age, MMSE and NfL are reported as median (interquartile range). For the S+ preAD subgroup (n. 3), only median, minimum and maximum concentrations of NfL are reported.

	AD-total	preAD	MCI-AD	AD-dem
N	166	11	92	63
age (y)	74 (70–77)	74 (69.5–76.5)	73 (70–76)	75 (70.5–77)
sex (M/F)	52/114	4/7	28/64	20/43
MMSE	22 (18–26)	29 (27–29)	24 (21–26)	16 (12−20)
NfL (pg/mL)	23.5 (17.3–30.2)	14·9 (11.4–16.1)	21.5 (16.1–26.9)	28.5 (23–36.1)
N S+	46	3	23	20
age S+ (y)	74.5 (72–77)	77 (75–80)	74 (72–76)	74.5(69.8–77)
NfL S+ (pg/mL)	27.1 (20.6–35)	23.5 (19.2–24.4)	21.4 (17.7–27.7)	34.9 (28.2–42)
N S-	120	8	69	43
age S- (y)	73.5 (70–76)	73 (69.3–74.8)	73 (70–76)	75 (70–78)
NfL S- (pg/mL)	23 (16.1–28.4)	12.4 (11.1–15.2)	21.6 (16–26)	27.2 (22.8–33.9)

Abbreviations: S+ = positive CSF synSAA; S- = negative CSF synSAA, preAD = preclinical AD, MCI-AD = mild cognitive impairment due to AD; AD-dem = AD at the dementia stage.

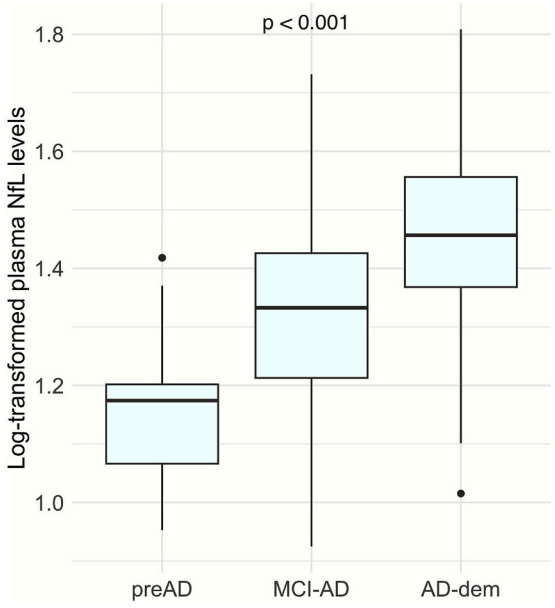

Fig. 1. Boxplots of log-transformed plasma NfL concentrations in AD patients according to disease stage. The reported *p*-value refers to the effect of AD stage on plasma NfL levels in one-way ANOVA. Boxes represent the interquartile range, the horizontal lines within boxes represent the medians, and whiskers reflect the first/third quartile ±1·5 times the interquartile range. *Abbreviations: preAD = preclinical AD, MCI-AD = mild cognitive impairment due to AD; AD-dem = AD at the dementia stage.*

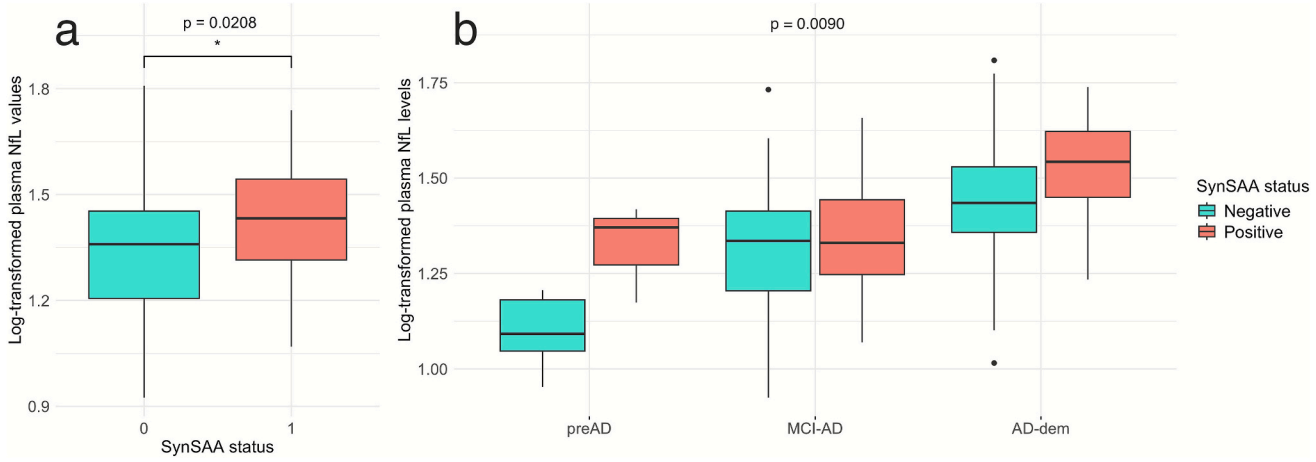

Fig. 2. Boxplots of log-transformed plasma NfL concentrations in AD individuals according to synSAA status and disease stage. A) Boxplot of log-transformed plasma NfL concentrations in patients with negative and positive synSAA. The reported *p*-value results from the comparison of log-transformed NfL values in S+ and S- AD individuals by one-way ANOVA. B) Boxplot of log-transformed plasma NfL concentrations in subjects grouped by both synSAA status and AD stage; the reported p-value reflects the main effect of synSAA status on NfL levels across AD stages in two-way ANOVA. Boxes represent the interquartile range, the horizontal lines within boxes represent the medians, and whiskers reflect the first/third quartile ±1·5 times the interquartile range. *Abbreviations: preAD = preclinical AD, MCI-AD = mild cognitive impairment due to AD; AD-dem = AD at the dementia stage.*

0.495) did not contribute to the model. Model fit indices were: null deviance = 195.9, residual deviance = 190.0, AIC = 200.

5. Discussion

In this study, we investigated the association between CSF synSAA and concentration of plasma NfL in a series of AD individuals belonging to the whole clinical spectrum of the disease. We provide evidence that AD individuals with CSF A+/T+/S+ exhibit significantly higher plasma NfL levels compared to those with CSF A+/T+/S−.

It is well known that NfL levels increase with age and AD stage (Vermunt et al., 2022; Doecke et al., 2025), but the impact of synucleinopathy in AD patients remains unclear. In this study, we found that CSF synSAA positivity was significantly associated with elevated plasma NfL levels after adjusting for disease stage and independently of age, indicating that α-synuclein co-pathology contributes to neurodegeneration in AD. These results are consistent with previous reports linking α-synuclein co-pathology to worse clinical outcome in AD (Bellomo et al., 2024; Tosun et al., 2024b; Collij et al., 2024). Given that plasma NfL is a widely recognized unspecific biomarker of neurodegeneration, our findings support the notion that co-existing α-synuclein pathology may exacerbate neuronal injury and thereby underlie the worse clinical outcome observed in these patients.

Few recent studies have suggested a link between synuclein pathology and increased biomarkers of neurodegeneration in AD. In a recently published work (Jonaitis et al., 2025) it has been reported higher CSF NfL levels in CSF tau-positive (T+) and S+ individuals as compared to CSF T+ S- subjects. Another recent work (Bernhardt et al., 2025) evidenced that, in patients affected by Down syndrome associated to AD and positive to CSF synSAA (S+), plasma NfL levels were significantly higher than in S- cases. Finally, a series of AD patients with clinical impairment exceeding their biological staging showed a higher percentage of CSF synSAA positivity and exhibited significantly higher mean plasma NfL levels (Jack et al., 2024). Indeed, plasma NfL represents a non-specific but sensitive marker of neurodegeneration across various neurological conditions (Gaetani et al., 2019; Khalil et al., 2024), while CSF total tau is a well-established biomarker of neurodegeneration closely linked to amyloid pathology and downstream tau-related processes (Mattsson et al., 2016; Teunissen and Parnetti, 2016; Giuffrè et al., 2023). It is worth of note that, in a previous study (Bellomo et al., 2024), we did not find a significant association between synSAA positivity and CSF t-tau levels. The lack of association between CSF t-tau levels and CSF synSAA status supports the hypothesis that synucleinopathy may act as a parallel, amyloid-independent mechanism of neuronal injury, that can be reflected by plasma NfL.

Our findings give further support to the recently proposed extension of the A/T/N framework to include α-synucleinopathy ("S") as an additional element of AD biomarker profiling (Jack et al., 2024). With the advent of disease-modifying therapies, particularly anti-amyloid agents, it is plausible that the presence of α-synuclein co-pathology could influence therapeutic response. However, evidence on this point is currently lacking, and our data do not directly address treatment outcomes. Therefore, while synuclein-related neurodegeneration may represent an additional, independent process, its impact on responsiveness to anti-amyloid therapy remains speculative and warrants investigation in future studies.

As a strength of our study, the large, well-characterized cohort spanning the full clinical continuum of AD with comprehensive biomarker assessment can be acknowledged. As limitations, the small size of some subgroups (e.g. S+ preAD individuals), and the cross-sectional design of the study, which precludes conclusions on temporal dynamics between synSAA positivity and NfL increase, should be acknowledged. Furthermore, we recognise that cerebrovascular burden and systemic comorbidities potentially affecting plasma NfL were not comprehensively assessed, although major intracranial pathologies and renal insufficiency were excluded. Moreover, postmortem neuropathological confirmation was not available in our cohort; nevertheless, a high concordance between α-synuclein SAA positivity and Lewy body pathology, even in Alzheimer's disease cases, has been reported in the literature (Arnold et al., 2022).

In conclusion, our findings provide evidence that neuronal α-synuclein pathology, as detected by CSF synSAA, is associated with elevated plasma NfL levels in AD, underscoring the contribution of synucleinopathy to neurodegeneration.

CRediT authorship contribution statement

Giovanni Bellomo: Writing – review & editing, Writing – original draft, Visualization, Supervision, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Andrea Toja:** Writing – review & editing, Writing – original draft, Supervision, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Lorenzo Gaetani:** Writing – review & editing, Supervision. **Giovanna Nardi:** Writing –

review & editing, Methodology, Investigation. **Federico Paolini Paoletti:** Writing – review & editing, Supervision. **Davide Chiasserini:** Writing – review & editing, Supervision. **Lucilla Parnetti:** Writing – review & editing, Supervision, Resources, Conceptualization.

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Declaration of competing interest

The authors declare the following competing financial interests: G. Bellomo and L. Gaetani completed paid consultancies for Fujirebio Europe. L. Parnetti served as an Advisory Board Member for Fujirebio and Novo Nordisk.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nbd.2025.107234.

Data availability

Data will be made available from the corresponding author upon reasonable request.

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