α-Synuclein heteromers with ß-amyloid and tau decreased in red blood cells of Alzheimer's disease and Lewy Body dementia patients.

Simona Daniele
University of Pisa
Filippo Baldacci
University of Pisa
Rebecca Piccarducci
University of Pisa
Giovanni Palermo
University of Pisa
Linda Giampietri
University of Pisa
Laura Manca
University of Pisa
Deboarh Pietrobono
University of Pisa
Daniela Frosini
University of Pisa
Valentina Nicoletti
University of Pisa
Gloria Tognoni
University of Pisa
Filippo Sean Giorgi
University of Pisa
Annalisa Lo Gerfo
University of Pisa
Lucia Petrozzi
University of Pisa
Chiara Cavallini
University of Pisa
Ferdinando Franzoni
University of Pisa
Roberto Ceravolo

University of Pisa
Gabriele Siciliano
University of Pisa
Maria Letizia Trincavelli
University of Pisa
Claudia Martini (claudia.martini@unipi.it)
University of Pisa https://orcid.org/0000-0001-9379-3027
Ubaldo Bonuccelli
University of Pisa

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Abstract

Background Red blood cells (RBC) account for more than 99% of α-syn concentrations in blood representing an interesting in vivo model for studying peripheral pathological alterations proved in neurodegeneration. The aim of the current study was to investigate the diagnostic value of total α-syn, Aβ 1-42 , tau and their heteroaggregates in RBCs of Lewy Body Dementias (LBDs) and Alzheimer's disease (AD) patients compared to and healthy controls (HCs).

Methods With a "home-made" sandwich enzyme-linked immunosorbent assay (ELISA) system, RBCs levels of total α-syn, Aβ 1-42 , tau and their heteroaggregates (α-syn/Aβ 1-42 and α-syn/tau) were measured in 27 subjects with LBDs (PDD, n = 17; DLB, n = 10), 51 subjects with AD (AD dementia, n = 37, prodromal AD, n = 14), and HC (n = 60).

Results Compared with HC, total α-syn and tau concentrations as well as α-syn/tau heterodimers were significantly lower in LBDs group ($p = 0.009$, $p = 0.009$, and $p < 0.001$, respectively) and in AD group ($p = 0.011$, $p = 0.003$, and $p < 0.001$, respectively), whereas the heteroaggregates α-syn/Aβ 1-42 were significantly lower in AD dementia group ($p < 0.001$) only. RBC α-syn/tau heterodimers had the higher diagnostic accuracy for differentiating patients with LBD vs controls (AUROC = 0.80).

Conclusion RBC α-syn heteroaggregates may be useful for differentiating between neurodegenerative dementias (LBD and AD) and healthy control. In particular, RBC α-syn/tau heterodimers have demonstrated good diagnostic accuracy for differentiating LBDs from HC. However, they are not consistently different between LBD and AD. Our findings also go beyond the clinical setting, suggesting that α-syn, Aβ 1-42 , and tau interact in vivo to promote the aggregation and accumulation of each other presumably accelerating cognitive dysfunction.

Background

Alzheimer disease (AD), and Lewy bodies dementias (LBD), encompassing Dementia with Lewy body (DLB) and Parkinson disease dementia (PDD), are the most prevalent dementia-related neurodegenerative disorders (NDs) in general population [1–3], with AD representing more than 50% of dementia diagnoses [4, 5]. DLB is the second most common neurodegenerative dementia in older adults, with an incidence rate of 31.6 per 100,000 person-years in the 65-and-older population [6]. In Parkinson Disease (PD) as well, late stage patients usually manifest overt cognitive decline, and full-blown dementia, known as PDD, is reported with a point prevalence close to 30% of cases [7].

Since these NDs share comparable pathophysiological mechanisms, namely misfolded protein accumulation, they are also collectively referred to as proteinopathies [8, 9]. Far from being strictly independent diseases, concomitant occurrence of multiple misfolded proteins has been commonly found. Thus, together with misfolded α-synuclein (α-syn) aggregates – LBD pathologic hallmark – tau and beta-amyloid copathologies are common [10–12]. Similarly, various degrees of abnormal deposition of α-syn are seen in nearly half of AD individuals [4, 13]. AD patients have shown high levels of

Cerebrospinal Fluid (CSF) α-syn levels [14, 15], whereas a reduction has been reported in PD and DLB patients [16]. Of note, there is now ample evidence, including several longitudinal studies, indicating the robust prognostic value of low CSF β-amyloid 1–42 ($A\beta_{1-42}$) in terms of the development of cognitive impairment in PD and DLB [17, 18].

Therefore, a substantial overlap of pathophysiological mechanisms involving Aβ, tau, and α-syn metabolisms characterizes the development and progression of these NDs [19, 20]. Beyond co-occurrence, these different pathologies seem to mutual influence and interact each other leading to hybrid oligomers ("heteroaggregates" of α-syn, tau, and $A\beta_{1-42}$) in patients' brains and cellular models [21–27].

As a consequence, a contemporary evaluation of these pathophysiological pathways is mandatory for every biomarker-based study having the objective to identify patients within the AD and LBDs spectrum [28].

Due to accessibility and cost reasons, research attention is focusing on peripheral biomarkers moving from CSF to blood [16, 24]. Pathological alterations in blood proteins have been suggested to reflect CSF changes due to simple diffusion or barrier impairment that characterizes neurodegeneration [24, 29]. Red Blood Cells (RBCs) contain 99% of the circulating α-syn, and plasma measurements raised some concerns for possible contamination due to hemolysis [30]. Moreover, RBCs are involved in the accumulation and clearance of the misfolded proteins [31–33] and may represent an interesting peripheral fluid reflecting neurodegeneration. Previous works on RBCs reported the presence of α-syn "heteroaggregates" in healthy subjects [25], PD patients [34], and AD patients [35].

The aim of the present study was 1) to compare $A\beta_{1-42}$, total tau (tau), total α-syn (α-syn), α-syn/$A\beta_{1-42}$ heterodimers, and α-syn/tau heterodimers in RBCs of patients with AD-dementia or prodromal AD (AD group) and patients with PDD or DLB (LBD group), compared to cognitively healthy subjects (HC group); 2) to evaluate whether these markers will help to differentiate between HCs and both AD and LBDs, and between AD and LBD participants.

Methods

Population

Participants for this cross-sectional study included 51 subjects in AD group (37 AD-dementia patients and 14 prodromal-AD patients), 27 subjects in the LBD group (17 PDD and 10 DLB), all enrolled from the Center of Neurodegenerative Disease of the Neurology Unit at Hospital of Pisa and 60 cognitively healthy controls (HC group) enrolled from patients' families (without parental relationship) and volunteers.

All participants underwent detailed clinical and neurological assessments, including collecting history from a close relative, neurological examination and routine blood tests. All patients (AD and LBD groups) underwent an extensive cognitive evaluation, including the MiniMental State Examination (MMSE) [36],

and a Magnetic Resonance Imaging (MRI) scan to rule out secondary causes of dementia/cognitive impairment and parkinsonism.

AD patients received their diagnosed according to the IWG-2 criteria [37], either prodromal AD or AD dementia, and displayed atypical progressive amnestic phenotype, associated to evidence of AD pathophysiological process (either decreased CSF $A\beta_{1-42}$ together with increased tau or phospho-tau (p-tau), or positive cerebral amyloid PET).

The LBD group consisted of patients with either diagnosis of probable PDD or probable DLB, as defined by the Movement Disorder Society Task Force [38] and the DLB consortium [39], respectively. The study was approved by the local Ethical committee and in accordance with the Declaration of Helsinki. Each participant gave his written informed consent.

Collection of RBCs

Blood was collected into a tube containing an anticoagulant (EDTA) from AD and LBD groups and HC. Following centrifugation at 200 x g at 4 °C for 10 min [25], RBCs were separated from plasma. The isolated RBCs were centrifuged at 1000 x g for 10 min, washed with PBS and frozen at -20 °C until use. The total amount of proteins in RBCs was calculated through the Bradford method. Before to use the RBCs in the immunoenzymatic assay, these cells were suspended in 2 mM SDS to a final concentration of 40 mg of total proteins in 100 µl [35].

Quantification of $A\beta_{1-42}$ in RBCs

To quantify the amount of $A\beta_{1-42}$ in RBCs an immunoenzymatic assay was employed, as elsewhere described [25, 40]. A specific antibody direct to $A\beta_{1-42}$ (ab10148, Abcam) was used to coat the wells (60 µl-well) and it was maintained overnight at 4 °C. BSA 1% was added in each well and it was incubated for 2 h at 37 °C. RBCs (0.2 mg/100 ml) were added to each well and incubated at 25 °C for 1 h. A polyclonal antibody to $A\beta_{1-42}$ (sc-28365, Santa Cruz Biotechnology) (75 µl-well) was used to probe samples for 1,5 h at 25 °C. A donkey anti-goat-HRP antibody was incubated at 37 °C for 1 h [34, 41, 42]. The standard curve was obtained using recombinant human $A\beta_{1-42}$ solutions at different concentrations (Pesini et al., 2012, Daniele et al., 2018d, Baldacci et al., 2019°).

Quantification of tau in RBCs

To measure the levels of tau in RBCs an immunoenzymatic assay was used, as described elsewhere [25, 40]. A specific antibody direct to tau (sc-32274, Santa Cruz Biotechnology) was employed to coat the wells (60 µl-well) and it was maintained overnight at 4 °C. BSA 1% was added in each well and it was incubated for 1 h at 37 °C. RBCs (0.5 mg/100 ml) were added to each well and incubated at 25 °C for 1 h. A polyclonal antibody to tau (sc-5587, Santa Cruz Biotechnology) (75 µl-well) was used to probe samples for 2 h at 37 °C. A goat anti-rabbit-HRP antibody was incubated at 37 °C for 1,5 h. The standard curve was obtained using recombinant human tau solutions at different concentrations [25, 35].

Quantification of α-syn in RBCs

To define the quantity of α-syn in RBCs an immunoenzymatic assay was utilized, as described elsewhere [25, 40]. A specific antibody direct to α-syn (sc-10717, Santa Cruz Biotechnology) was added to coat the wells (60 µl-well) and it was maintained overnight at 4 °C. BSA 1% was added in each well and it was incubated for 1 h at 37 °C. RBCs (0.2 mg/100 µl) were added to each well and incubated at 25 °C for 2 h. A monoclonal antibody to α-syn (sc-12767, Santa Cruz Biotechnology) was employed and incubated for 2 h at 37 °C. An anti-mouse-HRP antibody was incubated at 37 °C for 1.5 h [25, 34, 43–45]. The standard curve was obtained using recombinant human α-syn solutions at different concentrations [25, 35].

Detection of α-syn/$A\beta_{1-42}$ heterocomplexes

Detection of α-syn/$A\beta_{1-42}$ heterocomplexes in RBCs was performed through “home-made” immunoenzymatic assay [34, 46, 47]. A specific antibody direct to $A\beta_{1-42}$ (ab10148, Abcam) was used to coat the wells (60 µl-well), and it was maintained overnight at room temperature. RBCs (40 mg/sample in 2 mM SDS) were added to each well and incubated at 25 °C for 2 h. BSA 1% was added in each well and it was incubated for 30 min at 37 °C. A mouse monoclonal anti-α-synuclein 211 antibody (sc-12767 Santa Cruz Biotechnology) was employed and incubated at 37 °C for 2 h. A goat anti-mouse-HRP antibody was incubated at 37 °C for 1.5 h. The concentration of α-syn-$A\beta_{1-42}$ in RBCs was measured according to a standard curve [34], using a solution of recombinant human α-syn and recombinant human $A\beta_{1-42}$ at different concentrations in SDS 2 mM. The solution, constituted of 1 mg of each protein diluted in 2 mM SDS, was prepared and incubated in parafilm-sealed tubes at 37 °C for 16 h in an “Eppendorf Thermomixer” with continuous mixing (500 rpm) [34, 35, 48].

Detection of α-syn/tau heterocomplexes

Detection of α-syn/tau heterocomplexes in RBCs was performed through “home-made” immunoenzymatic assay [25, 46, 47]. A specific antibody direct to α-syn (sc-514908, Santa Cruz Biotechnology) was used to coat the wells (60 µl-well) and it was maintained overnight at room temperature. RBCs (80 mg/sample in 2 mM SDS) were added to each well and incubated at 25 °C for 2 h. BSA 1% was added in each well and it was incubated for 30 min at 37 °C. A rabbit polyclonal anti-tau H-150 antibody (sc-5587, Santa Cruz Biotechnology) was employed and incubated at 37 °C for 2 h. A goat anti-rabbit-HRP antibody was used at 37 °C for 1.5 h [34]. The concentration of α-syn/$A\beta_{1-42}$ in RBCs was measured according to a standard curve [34], using a solution of recombinant human α-syn and recombinant human tau at different concentrations in SDS 2 mM. The solution, constituted of 1 mg of each protein diluted in 2 mM SDS, was prepared and incubated in parafilm-sealed tubes at 37 °C for 1 h in an “Eppendorf Thermomixer” with continuous mixing (500 rpm) [34, 35].

Statistical analysis

The Shapiro–Wilk test was used to test for normality. Continuous variables are presented as mean and standard deviation, with variables with a skewed distribution given as median and [interquartile range], while categorical variables are expressed as percentage.

Comparisons across groups for cross-sectional analyses of biomarker data were performed using one-way ANOVA test, and Tukey's post-hoc test was applied, where appropriate, to explore a significant difference in markers concentrations between group means. Variables with a skewed distribution were logarithmically transformed for use in ANOVA.

Then, if significant differences had been detected at post-hoc tests, the diagnostic potential of each biomarker was examined calculating the area under the receiver operating characteristic curve (AUROC) and its associated confidence intervals (CI). SPSS-IBM software, version 21, for Mac Os X were used; the statistical significance threshold level was set at $p < 0.05$.

Results

The clinical-demographic characteristics of the three groups and diagnostic subgroups are reported in Table 1. Participants included 51 subjects in AD group (37 AD-dementia patients and 14 prodromal-AD patients), 27 subjects in the LBD group (17 PDD and 10 DLB) and HC.

Table 1
Demographic and clinical data of participants divided by groups; in italics are reported values relative to diagnosis subgroups. All data, except for N, are indicated as mean ± standard deviation and median values (25th and 75th quartiles).

	AD (N = 51)	**AD-D (N = 37)**	**AD-P (N = 14)**	**LBD (N = 27)**	**PDD (N = 17)**	**DLB (N = 10)**	**HC (N = 60)**
Sex, N (F/M)	26/25	21/16	5/9	8/19	7/10	1/9	21/39
Age (years)	69.39 ± 6.62 70 (65–75)	69.94 ± 6.44 70 (67–74)	67.92 ± 6.65 67 (63–75)	72.85 ± 4.59 74 (68–77)	72.88 ± 4.17 74 (69–76)	72.80 ± 5.01 74 (68–77)	64.72 ± 8.74 65 (56–71)
MMSE	23.54 ± 3.89 24 (21–26)	22.30 ± 3.70 24 (20–25)	26.79 ± 1.98 28 (26–28)	18.26 ± 6.47 18.5 (14–23)	19.69 ± 4.93 19 (15–23)	16.00 ± 7.59 17 (9–23)	-

The levels of the measured RBC proteins (α-syn, tau, $A\beta_{1-42}$ and their heterocomplexes, i.e., α-syn/$A\beta_{1-42}$ and α-syn/tau) in the three groups are reported in Table 2.

Table 2

Red Blood Cells biomarker levels of participants divided by groups, presented as ng of the measured protein/mg of total proteins. All data, except for N, are indicated as mean (± standard deviation) and median values (25th and 75th quartiles). A one-way ANOVA analysis was performed for each biomarker (p-value, if significant, shown in table), followed by a post-hoc Tukey's test (see superscript letters for p-value).

	AD (N = 51)	LBD (N = 27)	HC (N = 60)	P-value of between group ANOVA
$A\beta_{1-42}$ (ng/mg)	16.43 ± 16.81 10.00 (4.20-24.15)	15.86 ± 10.46 13.38 (8.04–19.02)	14.53 ± 14.09 9.84 (6.01–17.57)	ns
tau (ng/mg)	4.25 ± 4.99 2.57 (1.02–6.37)	3.19 ± 5.13 0.95 (0.46–4.55)	8.43 ± 11.52 [a,b] 6.02 (2.43–8.45)	0.008
α-syn (ng/mg)	16.46 ± 15.85 11.35 (6.33–21.17)	15.31 ± 18.30 9.51 (5.50-19.42)	39.62 ± 57.19 [b,c] 19.85 (4.7-47.94)	0.003
α-syn/$A\beta_{1-42}$ (ng/mg)	1.91 ± 1.91 1.48 (0.58–2.41)	3.10 ± 2.01 2.77 (1.16–4.40)	3.80 ± 3.32 [d] 2.62 (1.52–4.79)	0.001
α-syn/tau (ng/mg)	1.04 ± 1.54 0.71 (0.37–1.06)	0.86 ± 0.10 0.44 (0.26–1.10)	2.36 ± 1.87 [d,e] 1.86 (0.66–3.88)	< 0.001

[a] P = 0.011 versus AD; [b] P = 0.009 versus LBD; [c] P = 0.003 versus AD; [d] P < 0.001 versus AD; [e] P < 0.001 versus LBD.

HC had significantly higher levels of RBC α-syn and tau with respect to AD and LBD patients. Consistent with these data, α-syn/tau heterodimers levels were significantly higher in HC than in patients with AD or with LBDs. α-syn/$A\beta_{1-42}$ concentrations were significantly higher in HC than in AD group only.

In contrast, there were no significant differences in $A\beta_{1-42}$ levels neither between the AD and HC, nor between LBDs and HC. Finally, comparable levels of every measured biomarker were evidenced between AD and LBD.

For the significant differences detected at post-hoc tests, the diagnostic potential of each biomarker was examined calculating the AUROC and its associated CI. Table 3 summarizes the accuracy of RBC biomarkers. The performance of α-syn/tau and α-syn/$A\beta_{1-42}$ in discriminating AD participants from HC was fair, whereas tau and α-syn poorly differentiated the two groups. α-syn/tau demonstrated also a good ability to discriminate LBD versus HC. Total tau might differentiate LBD patients from HC with fair

accuracy, α-syn poorly differentiated LBD from HC while RBC α-syn/$A\beta_{1-42}$ levels were unable to discriminate HC from LBD.

Table 3
Diagnostic accuracies of the RBCs biomarkers in differentiating HC from AD and LBD .

Group Comparisons	Predictors	AUROC	95% CI
HC vs AD	α-syn/tau	0.756	0.665–0.848
	α-syn/$A\beta_{1-42}$	0.715	0.620–0.810
	tau	0.656	0.552–0.759
	α-syn	0.606	0.500-0.711
HC vs LBD	α-syn/tau	0.808	0.708–0.908
	tau	0.734	0.618–0.850
	α-syn	0.626	0.508–0.744

Discussion

The main result of our study was that, among the proposed biomarkers, only tau, α-syn, and α-syn/tau managed to differentiate cognitively healthy from cognitively impaired subjects, with α-syn/$A\beta_{1-42}$ that could only discriminate between AD and HC. The most performing marker was α-syn/tau, which managed to separate with good and fair accuracy LBD and AD respectively from controls. Of note, none of them was useful for differential diagnosis between AD and LBD groups.

Emerging evidences suggest that neurodegenerative diseases are not related to the cerebral deposition of single/specific abnormal proteins, but rather to a mixed pattern of these misfolded proteins [20, 24, 35]. Several studies support the notion that $A\beta_{1-42}$, tau, and α-syn interact in vivo to promote aggregation and accumulation of each other and accelerate cognitive dysfunction [49]. Interestingly, their expression levels and aggregation processes are not restricted to the brain, but reach peripheral compartments, possibly configuring a systemic disease [35, 50]. Among peripheral cells, RBCs were demonstrated to be particularly susceptible to the oxidative stress and accumulation of misfolded proteins [25, 31, 33, 51–54]. Herein, a cohort of AD and LBDs patients and a group of HCs were recruited to quantify the RBCs concentrations of α-syn and its heterocomplexes with tau and $A\beta_{1-42}$, and to test their potential discriminatory accuracy. Indeed, literature highlights need for additional tools to distinguish LBD from AD, especially for the early-stage diagnosis, and a combination of blood biomarkers may be a more promising approach to differentiate AD and LBD from other conditions than looking for a single molecule.

In the present study, $A\beta_{1-42}$ concentrations in RBCs did not differ between HC and patients (AD and LBD). Very few data are available on the blood levels of amyloid protein. Previous cross-sectional studies confirmed that plasma Aβ of AD patients is not much different from normal controls [55], but somewhat promising results have been seen for combinations of $A\beta_{1-42}$ and $A\beta_{1-40}$. Of note, recent studies allowed us to measure very low amounts of several Aβ-related peptides in plasma using ultrasensitive assays, supporting the use of plasma Aβ42/40 ratios as surrogate biomarkers of cerebral Aβ deposition [56, 57]. Considering the remarkable role of CSF $A\beta_{1-42}$ in PD conversion to dementia [58, 59], studies on $A\beta_{1-42}$ contribution in PD are mandatory. The determination of plasmatic α-syn has yielded conflicting results so far in synucleinopathies; a recent meta-analysis concluded for higher levels in PD patients compared to controls [60], while opposite results in LBD [61–63].

Recently, we explored RBCs $A\beta_{1-42}$ levels as a potential biomarker, finding comparable levels between HC and PD subjects [34], or HC and AD [35]. In contrast, $A\beta_{1-42}$ fibrils in RBCs have been found to be significantly higher in AD patients when compared to HC [64]. However, in this study, the diagnosis was based on purely clinical criteria [65] and $A\beta_{1-42}$ fibrils were quantified by a different detection method [64]. Taken together, these data highlight the need for the investigation on peripheral $A\beta_{1-42}$, and, most importantly, for more uniformed protocols to improve comparability of results.

Doubtless, the contribution of α-syn to LBD diagnosis is more meaningful than Aβ, primary because α-syn content in blood, particularly that stored in RBCs, is much higher than in CSF [30, 66]. In our study, total α-syn concentrations were lower in AD and LBD patients compared to HC. These data confirm the previous results obtained in a different cohort of AD subjects [35] and PD subjects [30, 33, 34]. Nonetheless, α-syn concentration was not able to discriminate AD subjects from LBD ones.

RBC tau concentration was reduced in AD and LBD subjects compared to HC, without discriminating the two patient populations. To our knowledge, very few studies have assessed the RBC tau protein concentrations in NDs [25]. In a previous study, tau protein in RBCs has been found to be similar in AD and HC [35], while higher tau levels have been demonstrated in plasma of AD patients [67–70]. Further investigation on tau isoforms and distribution will certainly be of interest, considering the potential contribution of tau pathology in LBD progression, especially in the case of $A\beta_{1-42}$ copathology.

Heterocomplexes of α-syn with tau and $A\beta_{1-42}$ have been proven to occur both in cellular models and in patients' brains [21–24, 34, 35, 71]. Noteworthy, α-syn forms heterocomplexes with both $A\beta_{1-42}$ and tau proteins in brain tissues and RBCs of senescence-accelerated mice, similarly with previous data reported in human samples [24, 25, 34, 72]. In our study, both α-syn/$A\beta_{1-42}$ and α-syn/tau concentrations in RBCs were significantly lower in AD patients than HC, as previously reported [35]. Furthermore, α-syn/tau levels were also reduced in LBD subjects than in HC. By contrast, α-syn/$A\beta_{1-42}$ heterocomplexes in PD patients were higher than in controls and correlate with the disease's progression [34]. Overall, these data highlight the potential role of α-syn heteromers as biomarkers in dementia and LBD. Interestingly, both α-syn/tau

and α-syn/$A\beta_{1-42}$ heterodimers in RBCs can fairly discriminate AD from HC, and α-syn/tau heterodimers distinguish LBD from HC with good accuracy.

The strength of our study was that AD patients received a biomarker-based diagnosis and a nigrostriatal degeneration were confirmed in LBD patients. However, some caveats are needed. In particular, our sample size is relatively small hindering further stratifications (e.g. specific investigations regarding PDD and DLB subsets). Moreover, our samples are not homogeneous in terms of age, sex prevalence, and disease stages. In fact, AD group consisted of both prodromal (Mild Cognitive Impairment, MCI) and mild demented patients, whereas LBD group was composed only of demented patients and did not comprehend an MCI PD category. Furthermore, given the cross-sectional nature of our study, and the lack of adequate follow-up, it is impossible to explore the prognostic value of these biomarkers. Another limitation is not having compared biomarkers in RBCs with the relative concentrations in plasma and CSF to clarify their clearance process.

Nevertheless, due to the multifactorial etiology of NDs and the existence of multiple elements involved in NDs pathogenesis, it could be interesting to further evaluate RBCs concentrations of phosphorylated tau (specifically reflecting the presence of neurofibrillary tangles, NFTs), phosphorylated α-syn (since it represents the 90% of insoluble α-syn contained in LBs), $A\beta_{1-42}$ fibrils and aggregates (which bind RBCs in a sharply larger share of AD patients compared to HC), and oligomeric α-syn, whose dosage in RBCs has already shown significant results in PD [34].

Conclusions

Our findings go beyond the clinical setting, suggesting that α-syn, $A\beta_{1-42}$, and tau interact in vivo to promote the aggregation and accumulation of each other presumably accelerating cognitive dysfunction. In order to evaluate and expand these results, additional studies including larger cohorts of patients need to be carried out, taking into account different phenotypes in different stages of disease and evaluating the overtime changes in biomarker concentrations based on the pathogenic process evolution. It is fundamental to deepen the research on the potential role of blood biomarkers given the minimal invasiveness of a blood test compared to other diagnostic examinations. Overall, these investigations could not only serve to obtain further diagnostic confirmations and prognostic evaluations but also provide additional clarifications on the complex pathogenic mechanisms underpinning NDs.

Abbreviations

AD, Alzheimer's disease; MCI, Mild Cognitive Impairment; LBD, Lewy bodies dementias; DLB, Dementia with Lewy body; PDD, Parkinson disease dementia; NDs, neurodegenerative disorders; HC, cognitively healthy group; $A\beta_{1-42}$, total β-amyloid peptide 1
42 fraction; tau, total tau protein; α-syn, total α-synuclein; α-syn/$A\beta_{1-42}$, heterodimer α-synuclein/ β-amyloid peptide 1–42 fraction; α-syn/tau, heterodimer α-synuclein/tau protein.

Declarations

Compliance with Ethical Standards

Study procedures were approved by our Local Board and were in accordance with the provisions of the Declaration of Helsinki. All participants or their representatives gave written informed consent for the use of their clinical data for research purposes.

Consent for publication

Not applicable.

Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon request.

Competing interest

The authors declare that they have no competing interests.

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Authors' contributions

FB, SD, GT, FSG, FF, RC, GS, MLT, CM, and UB were involved with the conception, design, and interpretation of data. FB, SD, RP, GP, LG, ALG, LP, DP, and CC performed the experiments. FB, SD, RP, GP, LG, DP, MLT and UB were involved with data analysis. MLM carried out data and statistical analysis. FB, GP, LG, DF, and VN collected the clinical material. CM, MLT, GT and UB provided general overall supervision of the study, and acquired funding. All authors contributed to the drafting and critical revision of the manuscript and have given final approval of the version to be published.

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Figures

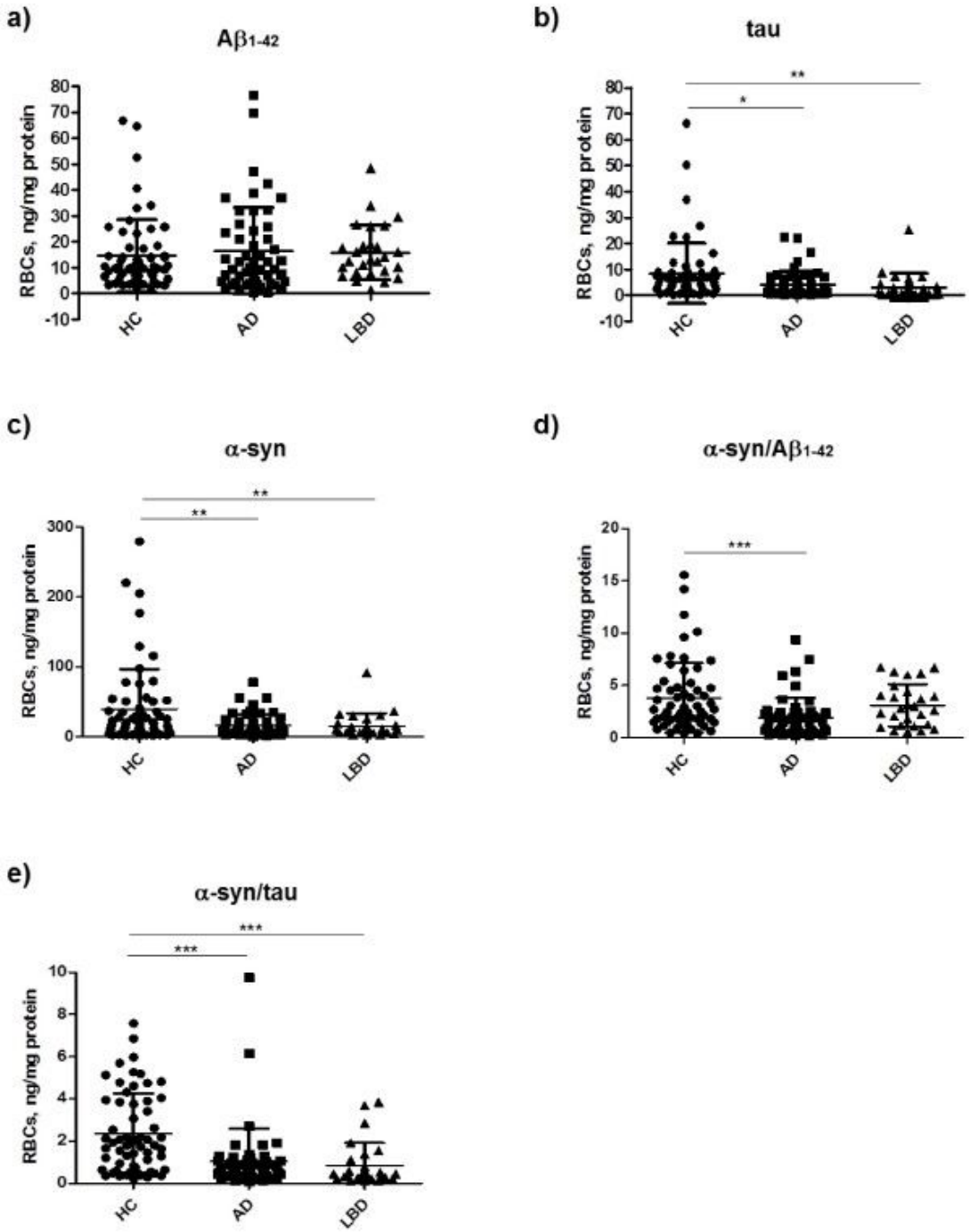

Figure 1

Scatter diagram of RBC levels of (a) Aβ1-42, (b) tau, (c) α-syn, (d) α-syn/Aβ1-42, and (e) α-syn/tau in AD, LBD, and HC, obtained by means of an immunoenzymatic assay, as described in the Method section. Data are expressed as mean +-standard deviation. Differences between the three groups were evaluated by an ANOVA analysis, followed by Tukey's post-hoc test: *p < 0.05, **p < 0.01, and ***p<0.001 versus HC.