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Review article

Plasma and serum alpha-synuclein as a biomarker in Parkinson's disease: A meta-analysis

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ABSTRACT

Background: Reliable biomarkers for Parkinson's disease (PD) diagnosis are urgently needed. Alpha-synuclein (α-syn) and its proteoforms play a key role in PD pathology but *in vivo* measurements have raised conflicting results, and whether α-syn in blood could distinguish PD patients from healthy controls is still controversial.
Methods: A systematic literature search yielded 35 eligible studies for meta-analysis reporting the concentration of total, oligomeric or phosphorylated α-syn in plasma and/or serum of PD patients and healthy controls. Standardized mean differences (SMD) were pooled using multivariate/multilevel linear mixed-effects models. Meta-regression analyses were conducted to investigate possible modifiers.
Results: A meta-analysis of 32 articles involving 2683 PD patients and 1838 controls showed a significant overall effect of PD on total α-syn levels (SMD = 0.85, p = 0.004). Meta-regression showed that increased SMD of total α-syn in PD was significantly associated with lower age, shorter disease duration, mild motor impairment, and Immunomagnetic Reduction assay for protein quantification. In contrast, no significant differences were observed for oligomeric or phosphorylated α-syn between PD and controls but increased oligomeric α-syn was significantly associated with shorter disease duration. The heterogeneity among studies was high (>98%).
Conclusions: These findings suggest that increased total plasma/serum α-syn levels in PD primarily occur in early phases of the disease. The evidence obtained from a small number of studies measuring plasma/serum concentrations of oligomeric and phosphorylated species of α-syn shows no difference. The clinical applicability of measuring plasma or serum α-syn species for differentiating PD from healthy control warrants further studies with better clinical profiling of PD patients.

1. Introduction

Parkinson's disease (PD) is the most common motor neurodegenerative disease (prevalence of 1% of the population >60 years) [1]. The exact mechanisms mediating the neurodegeneration are currently unknown. The major neuropathological finding in *postmortem* brains of PD patients is the presence of Lewy bodies, which are intracytoplasmic inclusions of protein aggregates whose primary component is alpha-synuclein (α-syn) [2]. Therefore, α-syn is considered the hallmark protein involved in PD pathology. Alpha-synuclein can be detected as an extracellular protein in the cerebrospinal fluid (CSF), peripheral fluids and cells, such as plasma, serum, erythrocytes, and platelets. As a result, measuring α-syn levels in biofluids *in vivo* might be a potential biomarker for PD diagnosis. Previous meta-analyses showed that the CSF α-syn concentration was significantly decreased in PD patients compared to healthy controls [3] but its diagnostic utility is still controversial [4]. As lumbar puncture is an invasive procedure, differences in α-syn levels have been explored in more accessible biofluids, like blood. In this regard, several studies have showed contradictory results, some authors finding increased levels of plasma or serum α-syn in PD patients [5–21], whereas others found no changes [22–35] or even reduced levels [36,37]. The analysis of different proteoforms of α-syn, like oligomeric (o-syn) and phosphorylated (p-syn) forms, has raised equally variable results [13,22,26,28,32,38–40].

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PD is a progressive disorder characterized by resting tremor, bradykinesia, and muscle rigidity. These motor symptoms are essential for clinical PD diagnosis, but usually they are accompanied by different degrees of cognitive, sensory, autonomic, and neuropsychiatric symptoms. Therefore, PD is a heterogeneous disorder and clinically different subtypes of PD patients can be distinguished based on patterns across motor and non-motor domains [41]. However, previous studies measuring blood α-syn and its proteoforms have barely considered the large phenotypic variability among PD patients and could have been a major confounding factor in outcome assessment. This study aimed to compile the evidence regarding α-syn levels in plasma and serum of PD patients compared to healthy controls and to perform 3 meta-analyses to quantitatively examine differences in α-syn proteoforms (total, o-syn and p-syn). Furthermore, we explored whether demographic or clinical variables influenced α-syn levels by means of meta-regression.

2. Methodology

We performed a systematic review, meta-analysis, and meta-regression. The review protocol was prepared according to the Preferred Reporting Items for Systematic reviews and Meta-Analysis Protocols (PRISMA-P) 2015 Statement. The protocol was registered with PROSPERO (registration number CRD42021288210). Our study only included anonymized data and no personal information was handled or any procedure applied to human beings, therefore, the ethical approval was not required.

2.1. Data sources and search strategy

We conducted a literature search of the MEDLINE database (via Pubmed) and ISI Web of Science (WOS) for articles published from inception to September 31, 2021. The search terms included "Parkinson's disease", "synuclein", "plasma", "serum", "total", "phosphorylated", and "oligomeric". The full search strategy is included in the Supplementary material (sTable 1). We further scanned the reference lists of all eligible studies and reviews derived from the protocol-driven search for identifying further possible studies [42]. Two independent researchers (M.Z. and G.I.) screened all titles and abstracts and whole manuscripts were reviewed for article selection based on eligibility criteria. Discrepancies in article selection were addressed either via discussion or with the involvement of a third researcher (T.M-H.).

2.2. Eligibility criteria

Studies were considered eligible if they determined the levels of α-syn in blood plasma or serum of PD patients and healthy controls. For PD patients, the clinical diagnostic criterion was the internationally agreed consensus criteria (i.e., United Kingdom PD Society Brain Bank criteria). Studies were required to report at least 1 of the following outcomes: total α-syn, o-syn and/or p-syn. Studies were excluded if they were performed in animals or cells, the study population was not PD, lacked a control group, protein levels were not explicitly mentioned, sample size was not reported, and proteins were measured in other fluids or tissues. In longitudinal studies, baseline assessments were only considered.

2.3. Data extraction

Data were extracted from all eligible studies by two independent investigators (M.Z. and G.I.) using a standardized data extraction form. The database was checked independently by one author (A.M.) and included the following information: First author's surname, year of publication, the mean and standard deviation of plasma/serum concentration of α-syn proteoforms (total, phosphorylated, oligomeric) in PD patients and healthy controls, the blood component (plasma or serum), the analytical method and supplier, age, sex (male percentage), disease duration (years), PD motor severity (Unified Parkinson Disease Rating Scale, part III score and Hoehn & Yahr scale), and cognitive test scores (Mini-Mental State Examination (MMSE) and/or Montreal Cognitive Assessment (MoCA)). When necessary, we contacted study authors to gather information.

2.4. Risk of bias assessment

The selected studies for the systematic review were checked against the Quality Assessment for Diagnostic Accuracy Studies (QUADAS-2) criteria to evaluate the quality and risk of bias [43]. Two researchers (M. Z. and G.I.) performed quality assessment and, in case of discrepancy, a consensus among the reviewers was achieved. Additional details are included in the Supplementary material (sTable 2).

2.5. Data synthesis and statistics

Meta-analysis was performed in R software (version 1.4.1717). We examined the differences of α-syn concentration (total, o-syn, p-syn) between the PD and healthy controls. Pooled standardized mean differences and 95% CIs were calculated using multivariate/multilevel linear mixed-effects models. Meta-regression analyses were conducted to investigate whether age, gender, disease duration, motor impairment severity, cognitive status, methodological assay, or blood fraction were acting as modifiers. For further details, see the Supplementary information.

3. Results

The systematic search identified 251 results. After title and abstract screening, 56 articles were considered potentially eligible. 35 articles were selected after full text review (Fig. 1). 32 articles reported total α-syn levels, from which 8 articles divided PD patients into subgroups for comparisons, resulting in 52 observations. 5 articles reported o-syn levels where 7 experimental comparisons were made between PD patients and controls, and p-syn levels were reported in 4 studies. All articles reporting oligomeric or phosphorylated α-syn levels, except three, also reported total α-syn levels. A descriptive analysis of each individual study is provided in Table 1.

3.1. Study characteristics

In total, 2683 PD patients and 1838 controls were enrolled for total α-syn measurements, 415 PD and 230 controls for o-syn, and 378 PD and 214 controls for p-syn. The publication year ranged from 2006 to 2021. The pooled mean ± SD age was 65.1 ± 12.5 years for patients and 62.5 ± 13.3 for healthy controls. 43.9% of PD patients and 46.2% of controls were women (sTable 3). The average disease duration was 5.8 ± 2.6 years, ranging from 0.9 to 10.8 years, although 18.9% of studies did not report this value. Hoehn & Yahr stage (available in 49% of studies) ranged from 1.5 to 4.0 and the mean UPDRS III score (available in 56.6% of studies) was 26.2 ± 6.1. Cognitive status was primarily assessed with Mini-Mental State Examination (49% of studies) or Montreal Cognitive Assessment (15.1% of studies), with mean ± SD scores of 25.0 ± 3.5 and 25.6 ± 3.1, respectively. Of 32 studies measuring total α-syn, 16 used Enzyme-Linked Immunosorbent Assay (ELISA), 10 Immunomagnetic Reduction (IMR) assay and 6 used alternative methods, including Single-Molecule Array (SiMoA), Electrochemiluminescence (ECL), Luminex or Surface Plasmon Resonance (SPR). All studies measuring p-syn or o-syn used ELISA, except for one study measuring p-syn that used IMR assay.

Nine articles divided PD population into subgroups, according to their cognitive status (Normal Cognition [NC], Mild Cognitive Impairment [MCI] or PD dementia [PDD]), disease stage (early, moderate, advanced), genetic status (LRRK2 vs. non-LRRK2) or accompanying symptomatology (presence or not of Rapid Eye Movement sleep behavior disorder).

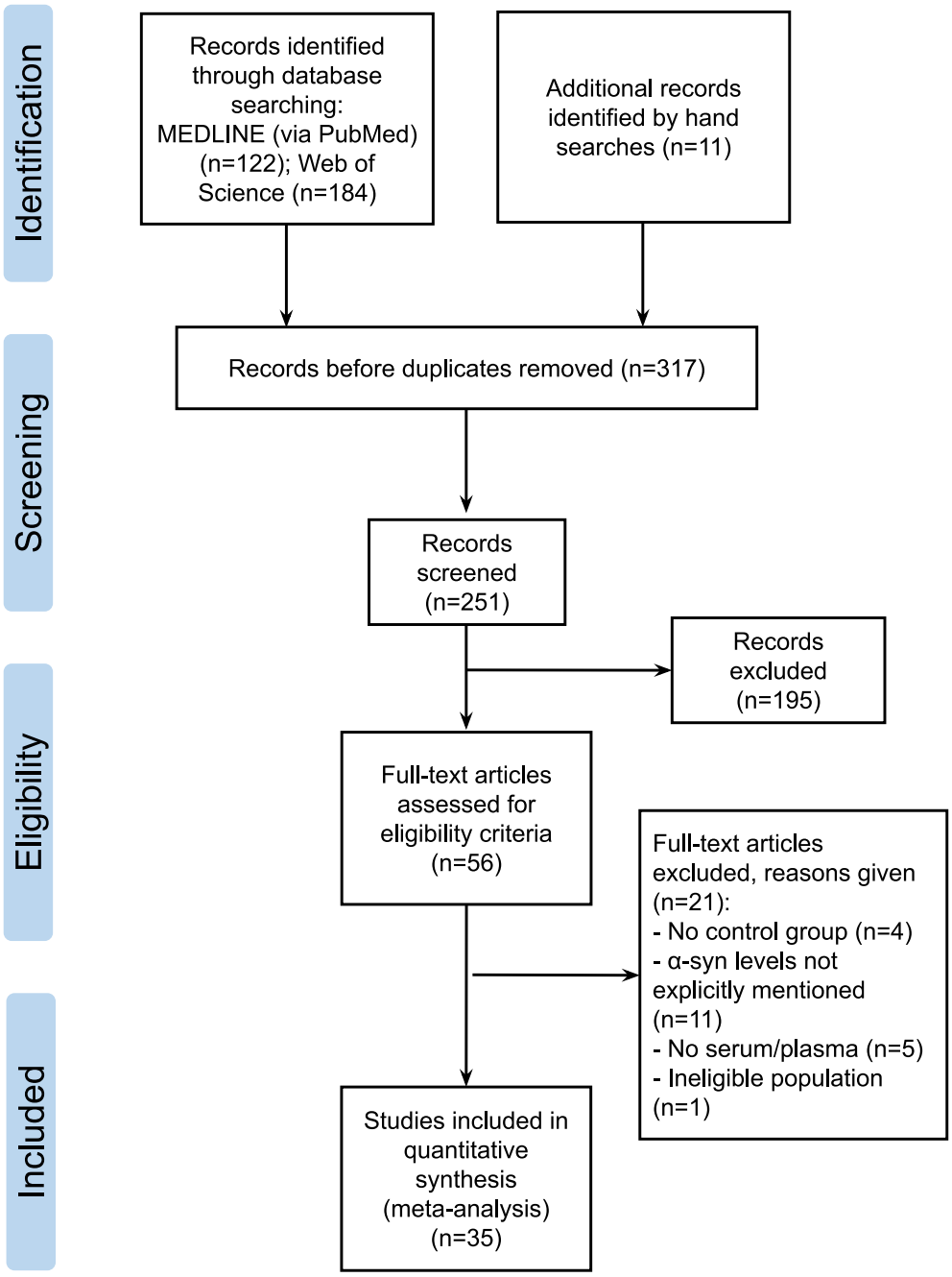

Fig. 1. PRISMA flow diagram for inclusion and exclusion of studies.

The overall quality of included articles was rated as high. The risk of bias in Patient Selection was deemed unclear in most studies (74.3%), as authors did not report the sampling method, and high in 2 studies (5.7%) because an inappropriate patient exclusion was also suspected. Regarding the Index Test domain, all articles were rated as low bias because prior knowledge of the clinical status of participant (PD or healthy control) should not bias the objective results of α-syn measurements. Most of the articles (n = 31, 88.6%) had low bias regarding Reference Standard, but risk was unclear in 4 studies (11.4%). With respect to Flow and Timing domain, one study was deemed unclear risk of bias because it did not cover the time interval between clinical diagnosis and index test, and two studies were deemed high risk of bias, as not all patients were included in the analysis (sTable 4 and sFigure 1).

3.2. Total α-syn levels in PD vs. controls

Meta-analysis indicated that total α-syn in blood was significantly increased in PD patients compared to controls (SMD 0.85; 95% CI 0.27 – 1.42; p = 0.004; Fig. 2). However, heterogeneity was extremely high (I^2 = 97.79%). About 32.9% of the total variance was estimated to be due to between-cluster heterogeneity, with the remaining 64.9% due to within-cluster heterogeneity. We detected two influential studies [20,21]. We repeated the analysis excluding one or both studies and corroborated that total α-syn levels continued to be significantly higher in PD patients than healthy controls (k = 50, SMD 0.62; 95% CI 0.10 – 1.15; p = 0.002). Also, the effect was significant when studies with SMD higher than 5 were excluded from the analysis (k = 46, SMD 0.67; 95% CI 0.22 – 1.11; p = 0.004) [12,15]. The increase in total α-syn levels in PD was further confirmed after excluding articles at high risk of bias (k = 43, SMD 0.77;

Table 1
Characteristics of PD samples included in the meta-analysis and meta-regression.

Study	Proteoform	Quantification Method	PD subgroup	n PD	n HC	Age (years)	Male (%)	Disease Duration	HY scale	UPDRS III	MMSE	MoCA
Plasma												
Bougea 2020	α-syn	ELISA (in-house)	PD	30	30	60.4 ± 10.2	50	5.2 ± 3.7	2.17 ± 0.44	13 [8-17]	28.5 [27-29]	–
			PDD	18	30	68.9 ± 10.7	38.9	9.5 ± 6	2.80 ± 0.49	27.5 [24-33]	18 [15-22]	–
Caranci 2013	α-syn	ELISA (Invitrogen)	Early PD	69	110	64.6 ± 9.3	42	10.8 ± 7.3	–	19.7 ± 12.36	–	–
Chahine 2020	α-syn	ELISA (BioLegend)	Early PD	18 (15)†	20	62.9 ± 9.9	77.7	10.6 ± 8.6 *	1.61 ± 0.50	19.7 ± 9.4	–	27.0 ± 2.5
			Moderate PD	20 (16)†	20	59.3 ± 6.3	75	40.6 ± 16.1 *	1.71 ± 0.47	26.3 ± 11.2	–	27.5 ± 1.8
			Advanced PD	21 (16)†	20	67.0 ± 7.9	57.1	114.5 ± 52.5 *	2.32 ± 0.48	32.9 ± 11.5	–	26.5 ± 3.1
Chang 2019	α-syn	IMR (Santa Cruz)	PD	48	40	67.2 ± 9.8	50	9.1 ± 6.5	–	–	23.9 ± 5.8	–
Chen CH 2020	α-syn	IMR (MagQu)	PD-NC	42	12	60.8 ± 12.5	57.1	4.5 ± 3.1	1.6 ± 0.8	14.3 ± 7.5	29.5 ± 0.5	–
			PD-MCI	66	12	66.2 ± 7.9	48.5	6.7 ± 7.8	1.9 ± 0.7	18.8 ± 7.7	27.2 ± 0.8	–
			PDD	50	12	75 ± 7.8	48	7.1 ± 4	2.7 ± 1.2	25.6 ± 14.5	20.6 ± 4.3	–
Chen NC 2020	α-syn	IMR (MagQu)	Early PD	60	28	62.8 ± 9.1	35	1 [0–3]	2 (0–2.5]	20 (16–34]	26 (22–27]	
Ding 2017	α-syn	ELISA (Senbeijia)	PD	73	26	67.6 ± 8.7	74	4.5 ± 3.5	–	23.3	28.2 ± 4.4	–
Emelyanov 2016	α-syn o-syn	ELISA (Invitrogen)	Drug-naive PD	17	18	62.4 ± 9.7	35.3	–	–	–	–	–
Fan 2020	α-syn	ECL (Mesoscale)	Early PD	43	24	58.4 ± 1.4	55.2	2.3 ± 0.3	1.9 ± 0.1	31.4 ± 2.1	–	–
Foulds 2013	α-syn p-syn	ELISA (Santa Cruz)	PD	189	91	61.9 ± 9.7	63.0	5.1 ± 4.1	–	–	–	–
Goldman 2018	α-syn	ELISA (BioLegend)	PD	115	88	68.2 ± 6.4	62.6	8.3 ± 3.1	2.2 ± 0.7	39.1 ± 13.2	–	26.8 ± 2.6
Gorostidi 2012	α-syn o-syn	ELISA (Santa Cruz)	non-LRRK2	134	109	69 ± 10.6	57.4	6.2 ± 5.3	–	–	–	–
			LRRK2	32	109	68 ± 10.1	40.6	7.5 ± 6.3	–	–	–	–
Lee 2006	α-syn	ELISA (Amersham Biosciences)	PD	105	51	64.5 ± 11.4	42.9	3.7 ± 3.1	2.4 ± 0.9	–	–	–
Lin CH 2017	α-syn	IMR (MagQu)	PD-NC	30	34	60.7 ± 10.6	48.3	4.6 ± 2.3	2.3 ± 1.1	27.7 ± 12.8	28.7 ± 0.9	–
			PD-MCI	21	34	68.9 ± 11.1	57.2	8.1 ± 6.3	2.5 ± 0.9	32.8 ± 15.3	25.3 ± 1.8	–
			PDD	29	34	79.9 ± 5.5	57.8	9.2 ± 7.3	3.3 ± 1.1	38.3 ± 12.5	17.8 ± 5.4	–
Lin CH 2018	α-syn	IMR (MagQu)	PD-NC	37	35	62.0 ± 10.5	49.7	4.6 ± 2.3	2.3 ± 1.2	25.8 ± 10.2	28.7 ± 0.9	–
			PD-MCI	29	35	66.5 ± 11.2	56.8	8.1 ± 6.3	2.4 ± 1.2	25.1 ± 10.6	25.3 ± 1.8	–
			PDD	36	35	75.6 ± 9.1	51.7	9.2 ± 7.3	3.8 ± 1.9	33.6 ± 12.6	17.8 ± 5.4	–
Lin CH 2019	α-syn p-syn	IMR (MagQu)	PD	122	68	69.3 ± 10.1	46.7	6.9 ± 3.7	–	24.5	26.4 ± 2.3	–
Lin CH 2020	α-syn	IMR (MagQu)	PD-NC	57	97	62.4 ± 11.2	54.4	2.6 ± 1.1	–	–	28.3 ± 0.9	–
			PD-MCI	29	97	66.5 ± 11.8	68.9	5.6 ± 1.6	–	–	26.8 ± 1.1	–
			PDD	87	97	72.8 ± 8.9	60.9	9.6 ± 3.2	–	–	20.8 ± 4.3	–
Lin WC 2020	α-syn	IMR (MagQu)	PD-NC	21	33	61.9 ± 10.6	47.7	–	–	–	27.5 ± 1.8	–
			PD-MCI	24	33	62.8 ± 10.2	41.7	–	–	–	26.5 ± 1.6	–
			PDD	25	33	66.2 ± 6.9	24	–	–	–	20.4 ± 4.1	–
Malec-Litwinowicz 2017	α-syn	ELISA (Invitrogen)	PD	53	38	68.4	56.9	–	2.4 ± 0.9	29.51 ± 13.12	26.6 ± 4.1	–
Mata 2011	α-syn	Luminex (in-house)	PD	86	78	66.3 ± 9.4	76.7	–	–	–	–	–
Ng 2019	α-syn	SiMoA (Quanterix)	PD	170	51	66.6 ± 9.5	58	5 ± 5	–	24.8 ± 12.5	25.4 ± 3.7	–
Shi 2010	α-syn	Luminex (Qiagen)	PD	126	122		73.8	8.3 ± 6.7	–	–	–	–

(*continued on next page*)

Table 1 (*continued*)

Study	Proteoform	Quantification Method	PD subgroup	n PD	n HC	Age (years)	Male (%)	Disease Duration	HY scale	UPDRS III	MMSE	MoCA
						63.7 ± 10.6						
Shim 2020	α-syn	ELISA (Abcam/in-house)	PD	20	20	71.4 ± 8.8	65	–	2 [2-3]	–	–	–
Wang L 2019	α-syn	ELISA (Santa Cruz)	PD	45	45	61.8 ± 9.6	60	5.1 ± 3.2	1.98 ± 0.75	25.3 ± 16	–	20 ± 6.6
Wang X 2020	p-syn o-syn	ELISA (Santa Cruz)	PD	40	40	–	60	–	–	–	–	–
Serum												
Bougea 2020	α-syn	ELISA (in-house)	PD	30	30	60.4 ± 10.2	50	5.2 ± 3.7	2.17 ± 0.44	13 [8-17]	28.5 [27-29]	–
			PDD	18	30	68.9 ± 10.7	38.9	9.5 ± 6	2.80 ± 0.49	27.5 [24-33]	18 [15-22]	–
Bu 2015	α-syn	ELISA (Invitrogen)	PD	131	141	67 ± 10	51.9	3.6 ± 3.4	–	–	–	–
Chahine 2020	α-syn	ELISA (BioLegend)	Early PD	17	20	62.9 ± 9.9	77.7	10.6 ± 8.6 *	1.61 ± 0.50	19.7 ± 9.4	–	27.0 ± 2.5
			Moderate PD	20	20	59.3 ± 6.3	75	40.6 ± 16.1 *	1.71 ± 0.47	26.3 ± 11.2	–	27.5 ± 1.8
			Advanced PD	20	20	67.0 ± 7.9	57.1	114.5 ± 52.5 *	2.32 ± 0.48	32.9 ± 11.5	–	26.5 ± 3.1
Chang 2019	α-syn	IMR (Santa Cruz)	PD	48	40	67.2 ± 9.8	50	9.1 ± 6.5	–	–	23.9 ± 5.8	–
Chatterjee 2020	α-syn p-syn	ELISA (Thermofisher)	PD	27	15	62.5 (7.7)	51.9	6.7 (3.4)	–	25.8 (15.1)	26 (4.3)	–
Gupta 2015	α-syn	ELISA (Invitrogen)	PD	97	97	56.3 ± 9.6	71.1	37.6 ± 32.9 *	–	–	–	–
Hu 2012	α-syn	ELISA (Invitrogen)	PD	110	136	59.9 ± 10.5	55.5	3.1	–	–	–	–
Hu 2015	o-syn	ELISA (Invitrogen)	PD-RBD (–)	156 (110)†	31	59.8 ± 10.4	53.9	2 [1-3]	1.5 [1.5–2.5]	25.9 ± 12.6	28 [26-30]	–
			PD-RBD (+)	69 (42)†	31	61.2 ± 11.8	47.8	3 [1.4–6]	2 [1.5–2.5]	29.1 ± 15.6	27 [22.5–29]	–
Nasirzadeh 2021	o-syn	ELISA (Shanghai Crystal Day Biotech Co)	PD	40	40	61.0 ± 9.3	70	4.8 ± 2.3	–	–	–	–
Schulz 2021	α-syn	ELISA (BioLegend)	PD	151	20	69.4 ± 9.6	66	–	3 [1-5]	30 ± 17	23 ± 5.6	–
Singh 2018	α-syn	SPR (Santa Cruz)	PD	38	33	65.2 ± 4.3	84.2	6.5 ± 4.9	–	22.7 ± 12.1	–	–
Singh 2019	α-syn	SPR (Santa Cruz)	PD	68	68	65.5 ± 4.7	86.8	6.1 ± 4.7	–	23.3 ± 19.8	–	–
Wang HL 2019	α-syn	IMR (Santa Cruz)	PD	59	60	62 (1.8)	49.2	–	–	–	–	–

Data are provided as mean ± SD, median [IQR] or mean (SEM). Disease duration is reported in years, except for those indicated with an asterisk (*) that represent months. Demographics and clinical variables correspond to PD sample. *Abbreviations:* HY, Hoehn & Yahr; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; NC, normal cognition; PD, Parkinson's disease; PDD, PD dementia; RBD, Rapid Eye Movement Sleep Behavior Disorder; UPDRS III, Unified PD Rating Scale part III (motor examination). † Numbers in parenthesis correspond to the number of participants included for α-synuclein measurement.

95% CI 0.29 – 1.26; p = 0.002).

To reveal potential sources of heterogeneity across studies, meta-regression analyses were performed. According to meta-regression results, increased SMD of total α-syn levels was significantly associated with lower age (SMD -0.04; 95% CI -0.07 – -0.02; p = 0.002), shorter disease duration (SMD -0.06; 95% CI -0.11 – -0.01; p = 0.032), and mild motor impairment (SMD 0.97; 95% CI 0.42 – 1.51; p < 0.001), whereas male sex was not. Similarly, measuring total α-syn in either plasma or serum was not associated with the outcome. Better scores in general cognition tests were significantly associated with increased total α-syn (SMD 0.06; 95% CI 0.03 – 0.10; p < 0.001), but the significance was lost after controlling for the effect of age (SMD 0.55; 95% CI -0.26 – 0.14; p = 0.185). Regarding the quantification method, PD patients showed increased plasma/serum α-syn levels compared to controls with all techniques except for Luminex (SMD range from 0.29 with SiMoA to 0.99 with SPR), but it was only significant for IMR method (SMD 2.84; 95% CI 2.08 – 3.61; p < 0.0001).

3.3. Oligomeric α-syn levels in PD vs. controls

The concentration of plasma or serum o-syn between PD patients and controls was not statistically different (k = 7, SMD 1.40; 95% CI -1.52 – 4.33; p = 0.347, Fig. 3). The heterogeneity between the 7 experimental comparisons was high (I^2 = 99.5%). We identified the study of Wang et al., 2020 [38] as an outlier, and after omitting it from the meta-analysis, the estimated SMD for o-syn decreased to −0.02 (k = 6, 95% CI -0.62 – 0.59; p = 0.954), although the heterogeneity continued to be high (I^2 = 88.6%). The results remained similar when the article at high risk of bias was excluded from the meta-analysis (k = 5, SMD 1.65; 95% CI -2.10 – 5.40; p = 0.389).

Blood component in which o-syn was measured was not a significant moderator. Mean age and percentage of males were reported in 6 out of 7 experiments and were not significant moderators of differences in o-syn. However, disease duration, which was reported in 5 out of 7 observations, resulted to be inversely associated with SMD difference of o-syn (SMD -0.17; 95% CI -0.31 – -0.03; p = 0.015). Motor severity and the analytical method were constant across studies and therefore could not be tested as moderators. Only 2 out of 7 experimental comparisons reported cognitive scores and, thus, were not included in meta-regression.

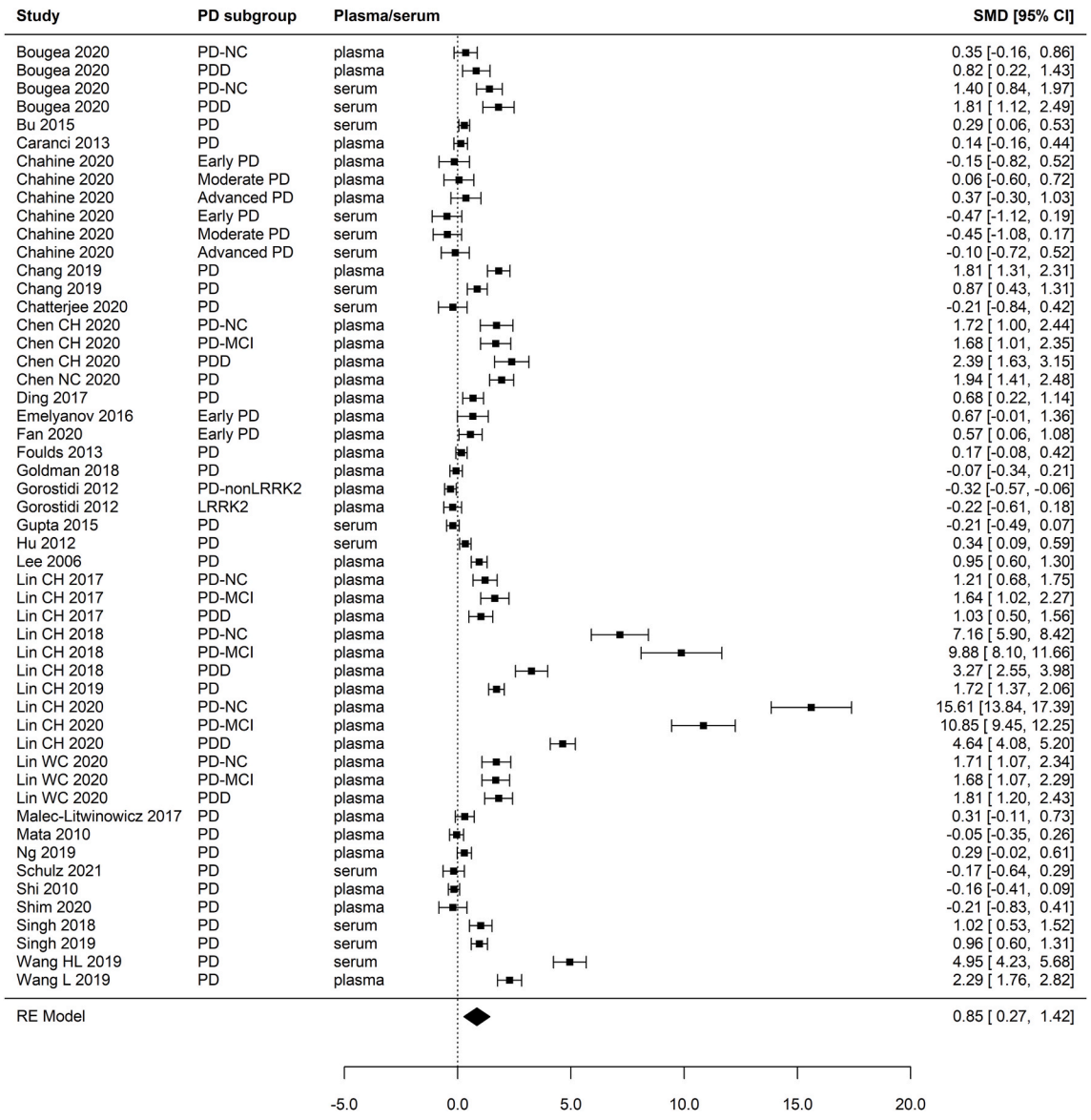

Fig. 2. Meta-analysis for plasma and serum total α-syn levels in PD vs. healthy control. Some studies differentiated PD subgroups, in which case it is specified in the second column. A positive SMD indicates greater protein concentration in PD vs. healthy control. *Abbreviations:* CI, confidence interval; MCI, mild cognitive impairment; NC, normal cognition; PD, Parkinson's disease; PDD, Parkinson's disease dementia; SMD, standardized mean difference.

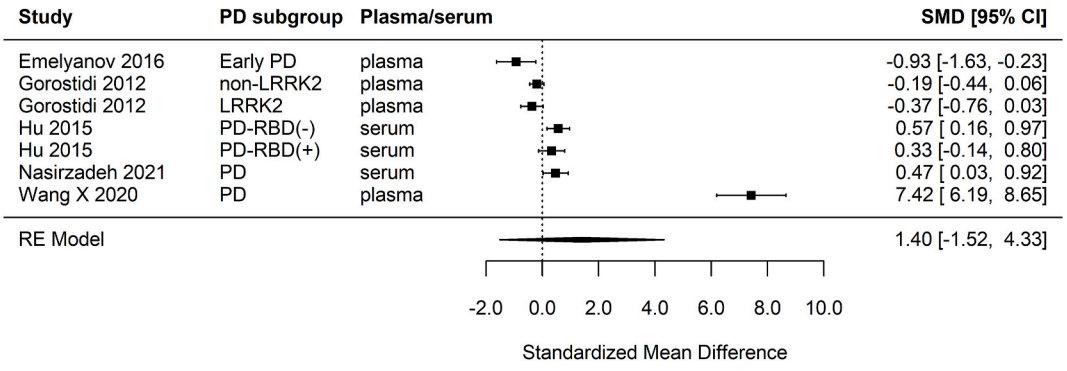

Fig. 3. Meta-analysis for plasma and serum oligomeric α-syn levels in PD vs. healthy control. Some studies differentiated PD subgroups, in which case it is specified in the second column. A positive SMD indicates greater protein concentration in PD vs. healthy control. *Abbreviations:* CI, confidence interval; RBD, Rapid Eye Movement Sleep Behavior Disorder; SMD, standardized mean difference.

3.4. Phosphorylated α-syn levels in PD vs. controls

The SMD difference of plasma/serum p-syn concentration between PD patients and controls was 2.56 (95% CI -1.49 – 6.61) but this difference was not statistically significant (p = 0.216) (Fig. 4). Heterogeneity was high across studies (I^2 = 99.7%). The study of Wang et al., 2020 [38] was identified as influential, and the estimate of SMD considerably decreased when omitting it in sensitivity analysis (SMD 0.57; 95% CI -0.64 – 1.77; p = 0.925) but heterogeneity continued to be high (96.8%). No meta-regression was performed due to the small number of studies (n = 4), and even fewer studies reporting proportion of men/women, cognitive scores, and motor impairment (2 out of 4).

4. Discussion

In this systematic review and meta-analysis, we found that total α-syn levels in blood plasma and/or serum were increased in PD patients compared to healthy controls with similar age and sex distribution. Although some influential studies could be driving this trend, a sensitivity analysis revealed that the result was robust. Meta-regression analysis identified that younger age and better clinical status of PD patients, as well as IMR analytical method, were significantly associated with increased SMD of total α-syn levels in PD patients. However, sex and blood fraction (plasma vs. serum) were not associated with the outcome. On the other hand, we observed that the concentration of plasma and/or serum p-syn and o-syn were not different between PD patients and healthy controls, although the number of studies was small. Based on these insights, we suggest that total plasma/serum α-syn levels are increased in early phases of PD, phenotypically presented with shorter disease duration and milder motor impairment, and the differences in the levels of total α-syn between PD and healthy controls become smaller as the disease progresses.

Finding non-invasive and objective biomarkers for early detection of PD is in the forefront of PD research. For most late-onset idiopathic PD, biochemical biomarkers obtained from blood represent a convenient choice, due to its easy implementation in clinical practice and in population-based screenings. Even though, shortcomings exist with respect to detecting and quantifying α-syn in blood, including methodological issues and the clinical variability of PD phenotype. One allegedly source of heterogeneity for α-syn measurements could be red blood cell (RBC) contamination of other blood fractions [44]. About 99% of α-syn in human blood is present in RBCs and any transference to plasma/serum or RBC lysis could explain the huge heterogeneity of the observed results. Platelet contamination in plasma has also been suggested as a source of heterogeneity [7,31]. In this meta-analysis, we included studies performed in plasma and serum samples, and we did not find blood component to be associated with a different outcome. In addition, different signal sensing technologies have been used across studies. According to our results, using IMR assay was significantly associated with greater α-syn plasma/serum levels in PD patients compared to healthy controls, whereas other quantification techniques showed a similar but non-significant trend. The principle behind the IMR assay considerably differs from the more widely used ELISA or Luminex immunoassays, and it could be that measuring magnetic properties provides more sensitive and reliable readouts than measuring fluorescence intensity.

To the best of our knowledge, only one previous systematic review performed meta-analysis to evaluate blood α-syn levels in PD patients [45]. In line with our results, Bougea et al. [6] found that plasma α-syn levels were significantly higher in PD patients than in healthy controls and that increasing age was associated with decreased α-syn levels. Contrary to our findings, they reported that male sex was associated with decreased levels of total α-syn, whereas disease duration and disease severity were not. Such discrepancies could be attributed to differences in the inclusion/exclusion criteria or the extracted information, and the updated evidence in the present meta-analysis. Our work included not only plasma but also experiments performed in serum, increasing 4-fold the number of participants, and we included more clinical variables for meta-regression. However, it should be mentioned that most of the significant effects of demographic and clinical modifiers showed small values of SMD, between −0.06 and 0.06, except for mild motor impairment that showed a large effect size (SMD = 0.97), but the effect size for more advanced motor stages was small and non-significant. In that sense, large changes in clinical scales would represent small changes in plasma or serum α-syn levels or would be comparable to healthy controls with increasing scores in clinical motor scales, which may limit the clinical applicability of total α-syn measurements for disease monitoring. Nonetheless, it could be useful for PD diagnosis, as we demonstrated that clinical characteristics compatible with earlier disease stages are associated with larger differences in plasma and serum total α-syn concentrations between PD and healthy controls.

Post-translational modifications of α-syn have a direct impact on its aggregation and toxicity. In fact, p-syn accounts for more than 90% of α-syn found in Lewy bodies [46] and it has been suggested to promote oligomer formation [47]. The studies measuring p-syn and o-syn in CSF have consistently shown increased levels in PD patients and have been claimed to be more reliable biomarkers for distinguishing PD patients from controls [23,48–54]. Regarding this, the evidence in peripheral fluids so far is limited. According to this meta-analysis, plasma and serum p-syn and o-syn levels were not statistically different, although the magnitude of effect was large (1.42 and 2.56, respectively). Probably, the reduced number of studies and the large heterogeneity between them provide wide confidence intervals, and thus the evidence is still scarce to draw reliable conclusions. Even though this limitation and similar to what we observed for total α-syn findings, disease duration was significantly and negatively associated with o-syn concentrations, suggesting that o-syn level differences are larger in early stages of disease progression. A similar observation was done by Aasly et al. [55] in CSF. Supporting this idea, other authors have found an inverse correlation between CSF o-syn levels and HY [54] or UPDRS III scales [49]. Unfortunately, it was not possible to evaluate the association between motor impairment and o-syn levels in the current meta-analysis because all studies reported a constant median HY score of 2.

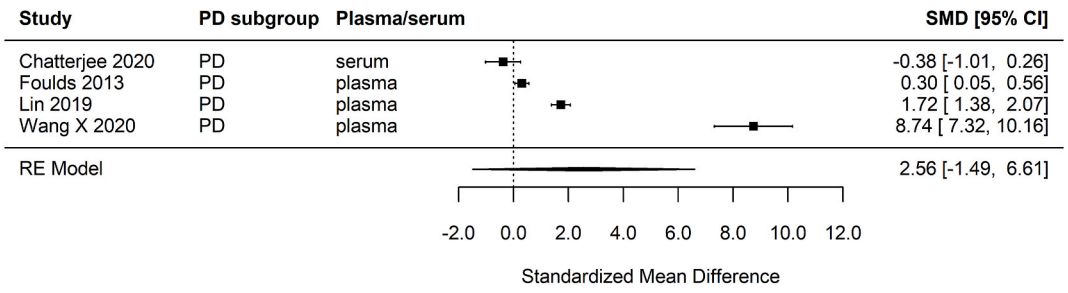

Fig. 4. Meta-analysis for plasma and serum phosphorylated α-syn levels in PD vs. healthy control. A positive SMD indicates greater protein concentration in PD vs. healthy control. *Abbreviations:* CI, confidence interval; PD, Parkinson's disease; SMD, standardized mean difference.

There are some limitations of the current meta-analysis. First, dopamine induces conformational α-syn changes in primary neuronal cultures [56], suggesting that dopaminergic drugs could act as a modifier of α-syn levels, which was not tested in the meta-regression analyses. Only one of the selected studies explored drug-naïve PD patients, and future studies including this cohort might yield less biased results. Second, the number of studies measuring plasma and serum o-syn and p-syn was low and therefore meta-regression could not be systematically applied for these proteoforms. Third, pooled analysis demonstrated substantial heterogeneity, affecting the general interpretability of the estimates. Meta-regression analyses showed that the heterogeneity might have been attributable to the analytical assay and clinical aspects of PD patients. Indeed, most studies did not provide sufficient clinical profiling of PD patients, like the presence/absence of genetic mutations. It is widely known that some genetic forms of PD present little to no α-syn inclusions, whereas other mutations show the opposite [57,58]. Also, late-onset patients have a greater risk of suffering vascular parkinsonism, and therefore may present lower expression levels of α-syn. Besides, another important source of heterogeneity is that, in most studies, the number of controls is considerably lower than that of the PD cases. Finally, we restricted the selection of research articles to those focused on the comparison between PD and healthy controls, which may hamper the direct application of our results in clinical practice, where the aim is not only to detect individuals with PD but also to distinguish PD from clinical syndromes presenting PD-like symptoms.

One clear contribution of the current meta-analysis is that we systematically tested the differences in α-syn levels and its proteoforms between PD patients and controls in the most widely used blood components for bioanalytical methods. Our results also highlight the need for reporting descriptive demographic and clinical variables of the study population when interpreting the differences in the concentrations of α-syn, as we demonstrated that results may largely depend on the clinical stage of PD patients. Moreover, we took into account that articles published within each research group tend to be correlated beyond what would be anticipated for measurement between different research groups through nested random effects, providing more accurate inference on fixed effects.

Aiming to develop diagnostic biomarkers for PD, future studies should address the aforementioned shortcomings and report demographic and clinical variables to test the validity of plasma and serum measurement of α-syn and its proteoforms. We suggest that mean age of the patients, presence of genetic mutations, percentage of males/females, disease duration, motor severity, and preferably, cognitive status of patients should be included. We also recommend future studies to report their results by separating early-stage or drug-naïve PD patients from more advanced ones. Also, more studies comparing o-syn and p-syn between PD patients and healthy controls are needed to increase statistical power and more precisely determine the effect sizes. By facing these limitations, the consistency and reliability of future research would improve. Still, it seems that the diagnostic utility of blood α-syn measurements is limited and research should focus on finding other more promising biomarkers to aid in the clinical assessment of PD.

Author contributions

A.M. and T.M-H. contributed to the conception and organization of the research project. M.Z. and G.I. contributed to the execution of the research project. A.M. performed the statistical analysis and drafted the manuscript. J.G-E. and T.M-H. critically reviewed the manuscript for important intellectual content. All authors approved the final version of the manuscript before submission.

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Conflict of interest statement and financial disclosures

JC.G.-E. has received honoraria from UCB Pharma and Zambon Group in the last 12 months. The remaining authors have not received any funding from any institution, including personal relationships, interests, grants, employment, affiliations, patents, inventions, honoraria, consultancies, royalties, stock options/ownership, or expert testimony for the last 12 months.

Data availability Statement

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parkreldis.2022.06.001.

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