Value of cerebrospinal fluid α -synuclein species as biomarker in Parkinson's diagnosis and prognosis

Since diagnosis of Parkinson's disease (PD) is mostly based on clinical criteria, it is almost impossible to formulate an early diagnosis, as well as a timely differential diagnosis versus other parkinsonisms. A great effort in searching reliable biomarkers both for early diagnosis and prognosis in PD is currently ongoing. Cerebrospinal fluid has been widely investigated as potential source for such biomarkers, with particular emphasis on α -synuclein (α -syn) species. We reviewed all the clinical studies carried out so far on cerebrospinal fluid quantification of α -syn species in PD. Current evidence supports the value of total and oligomeric α -syn in PD diagnosis and in the differential diagnosis of PD and other parkinsonisms. Conversely, the role of α -syn species in PD prognosis remains unsatisfactory.

Keywords: α -synuclein • cerebrospinal fluid biomarkers • cognitive impairment • disease progression • early diagnosis • motor symptoms • parkinsonisms • Parkinson's disease • prognosis

At present, the clinical diagnosis of Parkinson's disease (PD) is unsatisfactory. It largely relies on clinical criteria, which makes it almost impossible to formulate an early diagnosis, as well as an accurate and timely differential diagnosis between PD and other parkinsonisms. Moreover, when considering the heterogeneity of PD subtypes, with their different clinical and prognostic features, we definitely have to admit that for this disease, similarly to other neurodegenerative diseases, we need reliable biomarkers both for early diagnosis and for prognosis prediction. The availability of these objective indicators would allow us to appropriately and promptly use forthcoming neuroprotective drugs.

In PD patients, we observe a progressive decline in both motor and nonmotor functions.

When speaking about biomarkers in neurodegenerative diseases, cerebrospinal fluid (CSF) represents a sort of 'gold standard' as source of information on the biochemical changes taking place in the brain and reflected in the periphery. It gives the unique opportunity to catch,

at the same time, multiple indicators of the different pathophysiological cascades underlying the disease mechanisms [1]. α -synuclein (α -syn) is the main actor in the pathogenesis of synucleinopathies, being the major component of the Lewy bodies. Mutations of the α -syn gene are related with early-onset monogenic familial PD and are associated with increased risk for sporadic PD [2].

During the last 10 years, several studies have been carried out to measure CSF total α -syn levels in PD and other parkinsonisms, with the primary aim to confirm its diagnostic value as biomarker of synucleinopathy. The great majority of these studies have shown a trend toward a decrease of CSF α -syn levels in PD patients, although a large overlap between PD, other neurological diseases and controls is observed. Therefore, more recently, other α -syn species (i.e., oligomeric α -syn, phosphorylated α -syn) have been considered to improve PD diagnostic accuracy. A few studies have also addressed the issue of the possible role of α -syn as prognostic factor for motor and nonmotor symptoms [3,4].

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Cognitive impairment represents one of the most common and devastating nonmotor symptoms of this disease. The risk of cognitive impairment increases with PD duration. Results from longitudinal studies have shown that mild cognitive deficits may be documented in approximately 25% of newly diagnosed patients, and dementia occurs in up to 80% of patients over the course of the disease [5,6].

Therefore, the identification of biomarkers for cognitive decline in PD is important in order to delay or prevent dementia.

In this systematic review, all the studies carried out so far on CSF measurement of α -syn species in PD both as diagnostic and prognostic factor have been considered and reported. Issues about methodology of the CSF measurements have been also addressed.

Search strategy

References were identified by searching in PubMed databases from inception until 30 April 2015. We used the intersection of the following terms: 'cerebrospinal fluid', 'alpha-synuclein' and 'Parkinson's disease'. Search was not restricted to publications in English language. We obtained 161 records. Two reviewers have independently screened titles, abstracts and full-texts. 32 publications were considered relevant and listed in our review. In case of disagreement on the relevance of individual papers, consensus was reached after discussion. References of selected articles were also checked to detect missing pieces of literature. We selected studies without any restrictions on settings and design excluding preclinical studies, conference proceedings, reviews and research investigating post-mortem CSF. Studies were included if they assessed the role of α -syn for differentiation between PD patients from healthy subjects or patients suffering from any other neurodegenerative diseases. Studies were eligible if at least one group of PD patients was included and total and/or oligomeric and/or phosphorylated α -syn were quantified in CSF.

We have included also the results of Parkinson Progression Marker Initiative (PPMI [7]) study. The PPMI study is designed to establish a comprehensive set of clinical, imaging and biosample data that will be used to define biomarkers of PD progression. The full baseline characterization of the PPMI cohort has not yet been published; however, the paper of Kang 2013 analyzes a subset of these patients, while preliminary data on the full cohort are available in the website [8].

Quantification of α -synuclein species in cerebrospinal fluid

α -synuclein is a small (~15 kDa) protein mostly localized at the presynaptic neuronal terminals. The spe-

cific function of α -syn is still a matter of debate; however, a plethora of different roles have been suggested, including synaptic plasticity, brain lipid metabolism and regulation of vesicle dynamics and trafficking [9]. Since the discovery of α -syn mutations causative of PD [10] and the evidence that α -syn is the main component of Lewy bodies [11], intense investigation aimed at understanding the role of this protein in PD etiopathogenesis.

On the other hand, the possibility to use α -syn as a biomarker is relatively recent and derives from the discovery of its presence in biological fluids, including blood and CSF [12,13]. Given the observations on α -syn metabolism in physiological and pathological conditions, total α -syn is expected to be decreased in CSF of PD patients, possibly due to its accumulation as Lewy bodies in the brain tissue. The opposite (i.e., increase in CSF) should happen for α -syn oligomers, as they are credited with being the soluble α -syn species responsible for neurotoxicity. In recent years, most of the efforts have been focused on the measurement of α -syn species in CSF of PD patients, to understand if this protein can be used as PD biomarker, in analogy to amyloid peptides assessment in Alzheimer's disease (AD) [14].

The most common methods to measure α -syn species in biological fluids are immunochemical. The majority of the studies analyzed in this review used either in-house developed or commercially available ELISA assays to quantify the total content of α -syn (Table 1). Other α -syn species, such as oligomeric and phosphorylated α -syn, have also been measured mostly using in-house ELISA assays. ELISA setups for total α -syn are quite similar among the studies, using a monoclonal antibody for capture while a polyclonal antibody is used for detection of the analyte [15]. However, the antibodies used for capture and detection are often different. These differences caused a high variability among the published studies (Figure 1). Different groups have reported quite dissimilar ranges of total α -syn in biological fluids and especially in CSF [16,17]. With the improvement of the current assays, the majority of the recent studies have reported the total α -syn CSF levels in the low (0.5–20) ng/ml range [18–20].

ELISA assays for total α -syn are also commercially available from several manufacturers. Recently, the first interlaboratory study to establish the variability of an ELISA assay for total α -syn has been performed. The study included a round robin evaluation of a research use only (RUO) α -syn assay among 18 European laboratories experienced in CSF analysis. Several performance parameters were determined, including intra- and inter-assay variability, precision and dilution linearity. The laboratories analyzed CSF samples cen-

Figure 1. Mean values of total α -synuclein level in controls and Parkinson's disease, across the studies reviewed.

trally provided with ELISA kits from the same batch, to reduce the variability linked to the kits. Raw luminescence data were also centrally analyzed using the same procedure to extract quantification data. The results highlighted a high variability of the total α -syn absolute values among the laboratories involved, possibly due to the analytical procedures, namely interoperator variability and analytical instrumentation, calling for further optimization of the assay [21].

Oligomeric α -syn has been mostly measured with solid-phase ELISAs [22,23]. The first studies used the same monoclonal antibody for capture and detection, postulating that only oligomeric synuclein would be responsible for the signal, due to the availability of free epitopes in some of the monomers composing the aggregates [22]. A major issue with the development of an assay for oligomeric α -syn species resides in the availability of specific antibodies able to recognize only the aggregated forms and not the monomeric α -syn. Recently, several groups started to produce antibodies specific for aggregated forms, such as oligomeric or fibrillar α -syn, in order to increase the specificity of the assay, further clarifying which species are actually measured [24,25]. A recent paper described several con-

formation-specific antibodies for oligomeric and fibrillar α -syn [24]. These antibodies have a higher affinity for aggregated species and no reactivity or lower toward monomeric α -syn, thus allowing an accurate detection of the aggregated species.

The second most frequently used technique to measure α -syn is xMAP™, also called Luminex [26]. This method is based on in-suspension immunodetection and quantification of the specific analyte. In solid-phase ELISAs, the capture antibody is adsorbed on a microwell plate, which works as a solid support for the assay. Luminex assays use polystyrene beads coated with the capture antibody, which reacts with the analyte of interest. Detection uses a secondary antibody coupled to fluorescent tags. Due to the in-suspension format, the xMAP technique is theoretically capable to boost the maximum sensitivity of a solid-phase ELISA, increasing the possibility of interaction between the analyte and the coated beads suspended in the buffer. Furthermore, the technique is amenable to multiplexing, as differently color-coded beads can be coated with different capture antibodies, thus recognizing multiple targets. Hong and colleagues [26] performed the most comprehensive study using this method,

finding lower CSF levels of total α -syn in PD patients and ultimately being able to reach an assay sensitivity for α -syn down to 0.009 ng/mL. The xMAP method has also been used to quantify CSF Ser129-phosphor-

Table 1. Methods for measuring cerebrospinal fluid α -synuclein.

Study (year)	Total α -synuclein	Oligomeric α -synuclein	Phosphorylated α -synuclein	Ref.
Aasly <i>et al.</i> (2014)	ELISA in-house			[30]
Aerts <i>et al.</i> (2012)	ELISA in-house			[17]
Borghi <i>et al.</i> (2000)	Western blot			[13]
Buddhala <i>et al.</i> (2015)	ELISA commercial (Covance/ BioLegend)			[31]
Compta <i>et al.</i> (2015)	ELISA commercial (Invitrogen/ Life Technologies)	ELISA in-house		[18]
Hall <i>et al.</i> (2012)	ELISA in-house			[32]
Hong <i>et al.</i> (2010)	Luminex in-house			[26]
Hu <i>et al.</i> (2015)		ELISA commercial (CUSABIO)		[33]
Kang <i>et al.</i> (2013)	ELISA commercial (Covance/ BioLegend)			[34]
Magdalinou <i>et al.</i> (2015)	NA			[19]
Mollenhauer <i>et al.</i> (2008)	ELISA in-house			[35]
Mollenhauer <i>et al.</i> (2011)	ELISA in-house			[36]
Mollenhauer <i>et al.</i> (2013)	ELISA in-house			[37]
Mondello <i>et al.</i> (2014)	ELISA commercial (Covance/ BioLegend)			[38]
Ohrfelt <i>et al.</i> (2009)	ELISA in-house			[39]
Park <i>et al.</i> (2011)	ELISA in-house	ELISA in-house		[40]
Parnetti <i>et al.</i> (2011)	ELISA in-house			[16]
Parnetti <i>et al.</i> (2014a)	ELISA in-house	ELISA in-house		[23]
Parnetti <i>et al.</i> (2014b)	ELISA in-house	ELISA in-house		[41]
Reesink <i>et al.</i> (2010)	ELISA in-house			[42]
Parkinson Progression Marker Initiative	ELISA commercial (Covance/ BioLegend)			
Shi <i>et al.</i> (2011)	Luminex in house			[43]
Stewart <i>et al.</i> (2014)	Luminex in-house			[44]
Stewart <i>et al.</i> (2015)	Luminex in-house			[45]
Tateno <i>et al.</i> (2012)	ELISA in-house			[46]
Tokuda <i>et al.</i> (2006)	ELISA in-house			[15]
Tokuda <i>et al.</i> (2010)	ELISA in-house	ELISA in-house		[22]
Toledo <i>et al.</i> (2013)	Luminex in-house			[47]
van Dijk <i>et al.</i> (2014)	TR-FRET in-house			[29]
Wang Y <i>et al.</i> (2012)	Luminex in-house		Luminex in-house	[27]
Wang H <i>et al.</i> (2012)	ELISA commercial (Invitrogen/ Life Technologies)			[48]
Wennström <i>et al.</i> (2013)	ELISA commercial (Invitrogen/ Life Technologies)			[48]

TR-FRET: Time-resolved Förster's resonance energy transfer.

ylated α -syn in PD, which were found significantly increased [27].

The third immunoassay method, used to quantify α -syn species, is based on time-resolved Förster's resonance energy transfer [28]. This technique has been used to measure total CSF α -syn in a cohort of PD patients. Reduced levels in PD with respect to control subjects were found; the range of the analyte was between 0.5 and 3 ng/ml [29].

Results

Diagnostic value

In Table 2, the results of studies on CSF α -syn performance for distinguishing PD from controls, other parkinsonisms and other neurodegenerative diseases are reported. In a consistent proportion of them, AD biomarkers ($A\beta_{1-42}$, t-tau, p-tau) were also investigated. All the studies report univariate test statistics for group comparisons. In 10 out of 28, diagnostic accuracy measures, such as sensitivity and specificity, were also reported.

Parkinson's disease versus controls

The majority of the studies report lower CSF levels of total α -syn in PD subjects as compared with controls [15–16,22–23,26–27,29,31–32,34–38,41,43,46–47,49]. By contrast, several studies reported comparable levels of CSF total α -syn in PD patients and controls [13,17–18,27,30,39–40]. When oligomeric α -syn was quantified, in some studies, it was found to be higher in PD than controls [22,40–41], while in other reports, it did not significantly differ between groups [18,23]. The only study including phosphorylated α -syn showed higher levels in PD versus controls [27]. No significant differences were found in total α -syn levels between sporadic PD patients and LRRK2 mutation carriers [30].

Parkinson's disease versus parkinsonisms

Several studies analyzed the role of α -syn in the differential diagnosis between PD and other parkinsonisms (Table 2). PD and multiple system atrophy (MSA) patients showed similar CSF total α -syn levels in several studies [17,32,36,43], while in two reports, MSA subjects showed lower levels [27,38]. Progressive supranuclear palsy (PSP) subjects showed either similar [17,27] or higher [32,38] levels of CSF total α -syn when compared with PD. Phosphorylated α -syn was found to be higher in PD than MSA and PSP patients [27]. Corticobasal degeneration cases had similar [32] or higher [38] CSF total α -syn levels as compared with PD. No significant differences in CSF total α -syn levels were found in vascular parkinsonism versus PD [17].

Parkinson's disease versus dementias

When compared with PD, Parkinson's disease with dementia (PDD) patients showed similar levels of CSF total α -syn [18,32,49], and higher CSF levels of oligomeric α -syn [18]. Most of the studies report similarly reduced levels of CSF total α -syn in PD and dementia with Lewy bodies (DLB) cohorts [17,32,35,39,46]. In one investigation [16], lower levels of total α -syn were found in DLB and frontotemporal dementia patients, whereas Wennström *et al.* [49] found higher levels of total α -syn in DLB subjects. Remarkably, AD patients have consistently shown higher CSF levels of total α -syn as compared with PD [26,32,35–36,43]. Only in a study, no significant differences were found between AD and PD [39]. In a study, higher levels of phosphorylated α -syn were found in PD versus AD [49].

In half of the studies, at least one AD biomarker was investigated. In ten studies, $A\beta_{1-42}$, tau and p-tau were analyzed. In PD patients, α -syn levels have been found to be positively correlated with total tau levels [18–19,31,34,41], p-tau [19,34] and $A\beta_{1-42}$ [18–19,31]. Total tau/ α -syn ratio could better differentiate between PD and other neurodegenerative dementias in a study [16]. Recently, oligomeric α -syn has been shown to be positively correlated with tau [18].

Prognostic value

Correlation with clinical parameters

The results of studies on the correlation of total and oligomeric α -syn with clinical parameters are reported in Table 3. No correlation between either total or oligomeric α -syn in CSF and disease duration has been documented [16,18,23,26,30–32,34,36,38,40–42]. Only Magdalino *et al.* [19] found a positive correlation between total α -syn and disease duration, whereas oligomeric α -syn showed opposite behavior in two studies (negative association in Aasly *et al.* [30], positive association in Hu *et al.* [33]). When analyzing the relationship between α -syn concentrations at baseline and clinical rating scales, no significant correlations were found. No correlation between α -syn and Hoehn and Yahr (H&Y) stage has been found [18–19,23,26,30–31,38,41–43,49], with the exception of three studies where lower CSF levels of total α -syn [15,34] and higher oligomeric α -syn [30] correlated with a higher H&Y score. The Unified Parkinson's disease rating score (UPDRS) rates the patient's disability in both cognitive and motor domain. Most studies focus on the correlation with motor symptoms and generally no significant correlation with the levels of α -syn was found [23,27,29,44–45]. Kang *et al.* [34] found a correlation between higher UPDRS score with higher levels of total α -syn at baseline; by contrast, Stewart *et al.* [45] in one of the cohorts analyzed found a negative correlation of phosphory-

Table 2. Results of cerebrospinal fluid α -synuclein for distinguishing Parkinson's disease from controls, other parkinsonisms and other neurodegenerative diseases.

Study (year)	Groups	Total α -synuclein	Oligomeric α -synuclein	Phosphorylated α -synuclein	Ref.
Aasly <i>et al.</i> (2014)	Symptomatic PD due to LRRK2 mutations 13, asymptomatic LRRK2 mutation carriers 20, sporadic PD 35, controls 42	Sporadic PD = controls Sporadic PD = symptomatic PD due to LRRK2 mutations Sporadic PD = asymptomatic LRRK2 mutation carriers	NA	NA	[30]
Aerts <i>et al.</i> (2012)	58 PD, 47 MSA, 3 DLB, 22 vascular parkinsonism, 10 PSP, 2 CBD, 57 controls	PD = controls PD = MSA PD = DLB PD = vascular parkinsonism PD = PSP	NA	NA	[17]
Borghi <i>et al.</i> (2000)	12 PD, 10 controls	PD = controls	NA	NA	[13]
Buddhala <i>et al.</i> (2015)	77 PD, 30 controls	PD < controls	NA	NA	[31]
Compta <i>et al.</i> (2015)	21 PD, 13 controls, 20 PDD, 23 iRBD	PD = controls PD = PDD PD = iRBD	PD = controls PD < PDD PD > iRBD		[18]
Hall <i>et al.</i> (2012)	90 PD, 33 PDD, 70 DLB, 48 AD, 45 PSP, 48 MSA, 12 CBD, 107 controls	PD < controls PD = CBD PD = MSA PD = DLB PD = PDD PD < PSP PD < AD	NA	NA	[32]
Hall <i>et al.</i> (2015)	42 PD, 69 controls	PD < controls	NA	NA	[20]
Hong <i>et al.</i> (2010)	117 PD, 50 AD, 132 controls	PD < controls PD < AD	NA	NA	[26]
Kang <i>et al.</i> (2013)	63 PD, 39 controls (healthy)	PD < controls	NA	NA	[34]
Magdalinou <i>et al.</i> (2015)	31 PD, 14 CBS, 31 MSA, 33 PSP, 26 AD, 16 FTD, 30 controls		NA	NA	[19]
Mollenhauer <i>et al.</i> (2008)	8 PD, 38 DLB, 13 AD, 8 CJD, 13 controls (OND)	PD < controls PD = DLB PD < AD PD < CJD	NA	NA	[35]

=: Unchanged.

AD: Alzheimer's disease; CJD: Creutzfeldt–Jacob disease; DLB: Dementia with Lewy Bodies; CBD: Corticobasal degeneration; DLBD: Diffuse Lewy body disease; FTD: Frontotemporal dementia; iRBD: idiopathic REM-sleep behavior disorder; MS: Multiple sclerosis; MSA: Multiple system atrophy; NMO: Neuromyelitis optica; OND: Other neurological diseases; PD: Parkinson's disease; PDD: Parkinson's disease with dementia; PSP: Progressive supranuclear palsy; SCC: Subjective cognitive complaints.

Table 2. Results of cerebrospinal fluid α -synuclein for distinguishing Parkinson's disease from controls, other parkinsonisms and other neurodegenerative diseases (cont.).					
Study (year)	Groups	Total α -synuclein	Oligomeric α -synuclein	Phosphorylated α -synuclein	Ref.
Mollenhauer <i>et al.</i> (2011)	Training set: 51 PD, 29 MSA, 55 DLB, 62 AD, 76 controls (OND)	PD < controls PD < DLB PD < AD PD = MSA	NA	NA	[36]
Mollenhauer <i>et al.</i> (2013)	78 PD (drug naive), 48 controls	PD < controls	NA	NA	[37]
Mondello <i>et al.</i> (2014)	52 PD, 34 MSA, 32 PSP, 12 CBD, 22 controls	PD < controls PD < CBD PD < PSP PD > MSA	NA	NA	[38]
Ohrfelt <i>et al.</i> (2009)	15 PD, 15 DLB, 66 AD, 55 controls	PD = controls PD = DLB PD = AD	NA	NA	[39]
Park <i>et al.</i> (2011)	23 PD, 29 controls (11 symptomatic, 18 OND), 15 MS	PD = controls PD = MS	PD > controls PD > MS		[40]
Parkinson Progression Marker Initiative	423 PD, 196 controls	PD < controls	NA	NA	
Parnetti <i>et al.</i> (2011)	38 PD, 32 DLBD, 48 AD, 31 FTD, 32 controls (OND).	PD < controls PD > DLB PD > FTD	NA	NA	[16]
Parnetti <i>et al.</i> (2014a)	71 PD (8 of 44 carriers of a <i>GBA</i> mutation) and 45 controls (OND)	PD < controls	PD = controls	NA	[23]
Parnetti <i>et al.</i> (2014b)	44 PD and 25 controls (OND)	PD < controls	PD > controls	NA	[41]
Reesink <i>et al.</i> (2010)	18 PD, 63 AD, 34 SC, 35 DLB	PD = AD PD = SC PD = DLB	NA	NA	[42]
Shi <i>et al.</i> (2011)	126 PD, 32 MSA, 50 AD, 137 controls	PD < controls PD = MSA PD < AD	NA	NA	[43]
Tateno <i>et al.</i> (2012)	9 AD, 6 DLBD, 11 PD, 11 MSA, 11 controls (OND)	PD < controls PD = MSA PD = DLB PD < AD	NA	NA	[46]
Tokuda <i>et al.</i> (2006)	33 PD, 38 controls (9 healthy, 29 with OND)	PD < controls	NA	NA	[15]

=: Unchanged.
AD: Alzheimer's disease; CJD: Creutzfeldt–Jacob disease; DLB: Dementia with Lewy Bodies; CBD: Corticobasal degeneration; DLBD: Diffuse Lewy body disease;
FTD: Frontotemporal dementia; iRBD: idiopathic REM-sleep behavior disorder; MS: Multiple sclerosis; MSA: Multiple system atrophy; NMO: Neuromyelitis optica;
OND: Other neurological diseases; PD: Parkinson's disease; PDD: Parkinson's disease with dementia; PSP: Progressive supranuclear palsy; SCC: Subjective cognitive complaints.

Table 2. Results of cerebrospinal fluid α -synuclein for distinguishing Parkinson's disease from controls, other parkinsonisms and other neurodegenerative diseases (cont.).

Study (year)	Groups	Total α -synuclein	Oligomeric α -synuclein	Phosphorylated α -synuclein	Ref.
Tokuda <i>et al.</i> (2010)	32 PD, 28 controls (16 normal, 12 with OND)	PD < controls	PD > controls	NA	[22]
Toledo <i>et al.</i> (2013)	63 PD, 39 controls	PD < controls	NA	NA	[47]
van Dijk <i>et al.</i> (2014)	53 PD, 50 controls	PD < controls	NA	NA	[29]
Wang Y <i>et al.</i> (2012)	Discovery cohort: 93 PD, 26 AD, 78 controls, 33 PSP, 16 MSA. Validation cohort: 116 PD, 50 AD, 126 controls, 27 PSP, 25 MSA	PD < controls PD > MSA PD = PSP PD < AD	NA	PD > controls PD > MSA PD > PSP PD > AD	[27]
Wang H <i>et al.</i> (2012)	9 PD, 22 NMO, 18 MS, 11 controls	PD = controls PD < NMO	NA	NA	[48]
Wennström <i>et al.</i> (2013)	52 controls, 46 AD, 38 PD, 22 PDD, 33 DLB	PD < controls PD = PDD PD < DLB PD < AD	NA	NA	[49]

=: Unchanged.

AD: Alzheimer's disease; CJD: Creutzfeldt–Jacob disease; DLB: Dementia with Lewy Bodies; CBD: Corticobasal degeneration; DLBD: Diffuse Lewy body disease; FTD: Frontotemporal dementia; iRBD: idiopathic REM-sleep behavior disorder; MS: Multiple sclerosis; MSA: Multiple system atrophy; NMO: Neuromyelitis optica; OND: Other neurological diseases; PD: Parkinson's disease; PDD: Parkinson's disease with dementia; PSP: Progressive supranuclear palsy; SCC: Subjective cognitive complaints.

lated α -syn with UPDRS score. When considering cognitive symptoms, no correlation was found between the levels of total or oligomeric α -syn and neuropsychological assessment or Mini Mental State Examination scores [16,18,23,26,31–32,34,40–41,49].

Cognitive decline

The issue of a possible prognostic value of α -syn with respect to cognitive impairment in PD has been addressed in a few recent studies, giving inconsistent results (Table 4). When measured by cognitive speed testing, higher levels of total α -syn have been found to be associated with cognitive decline in PD [20], suggesting that lower α -syn can be predictive of a positive cognitive outcome [44]. In a cohort of PD patients followed-up for 4 years, no significant correlations of Montreal Cognitive Assessment and Mini Mental State Examination scores with CSF total and oligomeric α -syn were found [41].

Worsening of motor symptoms

No significant correlations between total α -syn concentrations and H&Y and UPDRS scores have been observed in some studies [43,44], while in another inves-

tigation [20], higher levels of CSF total α -syn predicted worsening in motor symptoms (Table 4).

Conclusion & future perspective

In the last years, an increasing number of studies measuring CSF α -syn species in PD has been carried out, with the aim to further highlight their possible role as biomarkers. The great majority of studies have considered the measurement of total α -syn; in a smaller proportion also oligomeric forms have been analyzed. Definitely insufficient are the data about other species (i.e., phosphorylated α -syn).

We systematically assessed the available studies in order to evaluate if the value of CSF α -syn species either as diagnostic or prognostic biomarkers in PD can be claimed.

The investigations are quite heterogeneous in terms of patients studied and methods followed for α -syn determination. Some important issues emerged in terms of α -syn determination and its utility in terms of prognostic and diagnostic marker for PD.

Technical issues on α -synuclein determination

The most common methods used for measuring CSF

Table 3. Results of correlation analyses between cerebrospinal fluid α -synuclein and clinical parameters in Parkinson's disease cohorts.

Study	Sample	Total α -synuclein	Oligomeric α -synuclein	Phosphorylated α -synuclein	Ref.
Aasly <i>et al.</i> (2014)	Symptomatic PD due to LRRK2 mutations 13, asymptomatic LRRK2 mutation carriers 20, sporadic PD 35	→Disease duration (sPD/sPD+LRRK2-PD) →H&Y (sPD/sPD+LRRK2-PD) →UPDRS (sPD/sPD+LRRK2-PD)	↓Disease duration (sPD/sPD+LRRK2-PD) ↓H&Y (sPD/sPD+LRRK2-PD) →UPDRS (sPD/sPD+LRRK2-PD)	NA	[30]
Buddhala <i>et al.</i> (2015)	77 PD	→Disease duration →H&Y →MMSE	NA	NA	[31]
Compta <i>et al.</i> (2015)	21 PD	→Disease duration →H&Y →MMSE →Semantic fluency ↑Phonetic fluency ↑VOSP O4 →other VOSP domains →BNT →RAVLT →WMI	→Disease duration →H&Y →MMSE →Semantic fluency →Phonetic fluency →VOSP →BNT →RAVLT →WMI	NA	[18]
Hall <i>et al.</i> (2012)	90 PD	→H&Y →MMSE	NA	NA	[32]
Hall <i>et al.</i> (2015)	42 PD	None			[20]
Hong <i>et al.</i> (2010)	117 PD	→H&Y →Disease duration →MMSE	NA	NA	[26]
Hu <i>et al.</i> (2015)	255 PD	NA	↑Disease duration ↑H&Y ↑UPDRS motor ↑RBDSQ score →PQSI →ESS →SCOPA-AUT →MMSE →HAMD →HAMA	NA	[33]

→: Uncorrelated; ↑: Positively correlated; ↓: Negatively correlated.

BNT: Boston naming test; CBS: Corticobasal syndrome; ESS: Epworth Sleepiness Scale; FSS: Fatigue Severity Scale; H&Y: Hoehn & Yahr stage; HAMA: Hamilton Anxiety Scale; HAMD: Hamilton Depression Scale; HVLT-R: Hopkins Verbal Learning Test-Revised; MMSE: Mini Mental State Examination; MoCA: Montreal Cognitive Assessment; MSA: Multiple Cognitive Assessment; PD: Parkinson's disease; PQSI: Pittsburgh Sleep Quality Index; PSP: Progressive supranuclear palsy; RAVLT: Rey Auditory Verbal Learning Test; RBDSQ: REM Sleep Behavior Disorder Screening Questionnaire; SCOPA-AUT: Scale for Outcomes in Parkinson's disease for Autonomic symptoms; SDMT: Symbol Digit Modalities Test; SRT: Selective Reminding Test; sPD: Sporadic PD; UPDRS: Unified Parkinson's Disease Rating Scale; VOSP: Visual object space; WMI: Working Memory Index; WMS-111 LNS: Wechsler Memory Scale third edition.

Table 3. Results of correlation analyses between cerebrospinal fluid α -synuclein and clinical parameters in Parkinson's disease cohorts (cont.).

Study	Sample	Total α -synuclein	Oligomeric α -synuclein	Phosphorylated α -synuclein	Ref.
Hu <i>et al.</i> (2015) (cont.)			→FSS		
Kang <i>et al.</i> (2013)	63 PD	→Disease duration ↓H&Y ↑UPDRS motor →MoCA →Semantic fluency →HVLt-R →SDMT →WMS-III LNS	NA	NA	[34]
Magdalino <i>et al.</i> (2015)	31 PD, 14 CBS, 31 MSA, 33 PSP	↑Disease duration →H&Y	NA	NA	[19]
Mollenhauer <i>et al.</i> (2011)	51 PD in the training set	→Disease duration	NA	NA	[36]
Mondello <i>et al.</i> (2014)	52 PD	→Disease duration →H&Y	NA	NA	[38]
Park <i>et al.</i> (2011)	23 PD	→Disease duration →H&Y →MMSE	→Disease duration →H&Y →MMSE	NA	[40]
Parnetti <i>et al.</i> (2011)	38 PD	→Disease duration	NA	NA	[16]
Parnetti <i>et al.</i> (2014a)	71 PD (8 of 44 carriers of a <i>GBA</i> mutation)	→Disease duration →H&Y →UPDRS	→Disease duration →H&Y →UPDRS	NA	[23]
Parnetti <i>et al.</i> (2014b)	44 PD	→Disease duration →H&Y	→H&Y →Disease duration	NA	[41]
Reesink <i>et al.</i> (2010)	18 PD	→Disease duration →H&Y	NA	NA	[42]
Shi <i>et al.</i> (2011)	39 PD	→UPDRS →H&Y	NA	NA	[43]
Stewart <i>et al.</i> (2014)	304 PD	→UPDRS total →UPDRS motor →SDMT →SRT →New Dot test	NA	NA	[44]

→: Uncorrelated; ↑: Positively correlated; ↓: Negatively correlated.

BNT: Boston naming test; CBS: Corticobasal syndrome; ESS: Epworth Sleepiness Scale; FSS: Fatigue Severity Scale; H&Y: Hoehn & Yahr stage; HAMA: Hamilton Anxiety Scale; HAM-D: Hamilton Depression Scale; HVLt-R: Hopkins Verbal Learning Test-Revised; MMSE: Mini Mental State Examination; MoCA: Montreal Cognitive Assessment; MSA: Multiple Cognitive Assessment; PD: Parkinson's disease; PQSI: Pittsburgh Sleep Quality Index; PSP: Progressive supranuclear palsy; RAVLT: Rey Auditory Verbal Learning Test; RBDSQ: REM Sleep Behavior Disorder Screening Questionnaire; SCOPA-AUT: Scale for Outcomes in Parkinson's disease for Autonomic symptoms; SDMT: Symbol Digit Modalities Test; SRT: Selective Reminding Test; sPD: Sporadic PD; UPDRS: Unified Parkinson's Disease Rating Scale; VOSP: Visual object space; WMI: Working Memory Index; WMS-111 LNS: Wechsler Memory Scale third edition.

Table 3. Results of correlation analyses between cerebrospinal fluid α -synuclein and clinical parameters in Parkinson's disease cohorts (cont.).

Study	Sample	Total α -synuclein	Oligomeric α -synuclein	Phosphorylated α -synuclein	Ref.
Stewart <i>et al.</i> (2015)	304 PD (95 in the DATATOP cohort, 199 in a multicentre collaborative cohort) and 30 subjects with LRRK2 mutations	NA	NA	→UPDRS motor (multi-centre collaborative cohort) ↓UPDRS motor (DATATOP cohort)	[45]
Tokuda <i>et al.</i> (2006)	304 PD	↓H&Y	NA	NA	[15]
van Dijk <i>et al.</i> (2014)	53 PD	→Disease duration →H&Y →UPDRS	NA	NA	[29]
Wang Y <i>et al.</i> (2012)	Discovery cohort: 93 PD. Validation cohort: 116 PD	→Disease duration →H&Y →UPDRS	NA	→Disease duration → H&Y ↑UPDRS	[27]
Wennström <i>et al.</i> (2013)	38 PD	→H&Y →MMSE	NA	NA	[49]

→: Uncorrelated; ↑: Positively correlated; ↓: Negatively correlated.

BNT: Boston naming test; CBS: Corticobasal syndrome; ESS: Epworth Sleepiness Scale; FSS: Fatigue Severity Scale; H&Y: Hoehn & Yahr stage; HAMA: Hamilton Anxiety Scale; HAMD: Hamilton Depression Scale; HVLT-R: Hopkins Verbal Learning Test-Revised; MMSE: Mini Mental State Examination; MoCA: Montreal Cognitive Assessment; MSA: Multiple Cognitive Assessment; PD: Parkinson's disease; PQSI: Pittsburgh Sleep Quality Index; PSP: Progressive supranuclear palsy; RAVLT: Rey Auditory Verbal Learning Test; RBDSQ: REM Sleep Behavior Disorder Screening Questionnaire; SCOPA-AUT: Scale for Outcomes in Parkinson's disease for Autonomic symptoms; SDMT: Symbol Digit Modalities Test; SRT: Selective Reminding Test; sPD: Sporadic PD; UPDRS: Unified Parkinson's Disease Rating Scale; VOSP: Visual object space; WMI: Working Memory Index; WMS-111 LNS: Wechsler Memory Scale third edition.

α -syn species are immunoenzymatic, using a wide variety of assays, either in-house developed or commercially available. Based on the current evidence, it can be claimed that, in CSF of PD patients, levels of total α -syn are lower than in control subjects. This result is consistently reported in the literature, including two recent meta-analyses [50,51]. In most of the studies assessing oligomeric α -syn, this species has been found to be higher in CSF of PD patients than in controls. The only available study investigating phosphorylated α -syn showed that also this species shows higher levels in PD as compared with controls.

However, several technical issues are to be solved before CSF α -syn can be considered as a useful PD biomarker. Studies aimed at understanding the influence of preanalytical and analytical factors on α -syn determination are warranted. In fact, it has been shown that variables such as type of tubes, storage temperature, centrifugation, time before storage, freeze/thaw cycles have a significant effect on the determination of AD biomarkers [52]. Systematic studies about the influence of preanalytical factors on α -syn CSF levels are lacking; a recent work highlighted how the high interassay vari-

ability of α -syn levels may be related to batch-to-batch differences of the immunoassays used [21].

A fundamental preanalytical variable for the measurement of α -syn in CSF is blood contamination, as α -syn is particularly abundant in erythrocytes. Hemoglobin has been proposed as specific marker of blood contamination [26], but no specific cut-off value to be used as an exclusion/inclusion parameter has been univocally determined. Therefore, further studies are needed to clarify this issue, which may have decisive implications for quality control of the CSF samples.

In future, international efforts and a close collaboration between the researchers and immunoassay vendors will become essential, in order to qualify standard operating procedures for CSF collection and storage suitable for α -syn, but also reference materials for quality control and kit manufacturing.

Diagnostic & prognostic performance

With respect to PD clinical staging, the spectrum of patients varies across studies, ranging from preclinical stages to *de novo* PD cases or patients in advanced phases. Clinical symptoms of the disease have been assessed

Table 4. Results of using cerebrospinal fluid α -synuclein for tracking progression of clinical parameters in Parkinson's disease.

Study (year)	Sample	Total α -synuclein	Oligomeric α -synuclein	Phosphorylated α -synuclein	Ref.
Hall <i>et al.</i> (2015)	42 PD	↑H&Y ↑UPDRS motor ↑TUG →MMSE →ADAS-cog item 3 →Arm hand left+right ↑AQT →Animal fluency	NA	NA	[20]
Parnetti <i>et al.</i> (2014b)	44 PD	→MMSE →MoCA	→ MMSE → MoCA	NA	[41]
Shi <i>et al.</i> (2011)	39 PD	→UPDRS →H&Y	NA	NA	[43]
Stewart <i>et al.</i> (2014)	304 PD	→H&Y →UPDRS →SRT ↓SDMT ↓New Dot test	NA	NA	[44]
Stewart <i>et al.</i> (2015)	304 PD (95 in the DATATOP cohort, 199 in a multicenter collaborative cohort) and 30 subjects with LRRK2 mutations	NA	NA	→ UPDRS motor	[45]

→: Uncorrelated; ↑: Positively correlated; ↓: Negatively correlated.
 DAS-cog: Alzheimer's Disease Assessment Scale-cognitive; AQT: A Quick Test of cognitive speed; H&Y: Hoehn & Yahr stage; MMSE: Mini Mental State Examination; MoCA: Montreal Cognitive Assessment; PD: Parkinson's disease; SDMT: Symbol Digit Modalities Test; SRT: Selective Reminding Test; TUG: Timed Up and Go; UPDRS: Unified Parkinson's Disease Rating Scale.

using different tools for measuring motor and cognitive domains. Several studies refer to small groups of patients.

Several studies analyzed the role of α -syn species in the differential diagnosis between PD and other parkinsonisms (MSA [53], PSP, corticobasal degeneration, vascular parkinsonism) with inconsistent findings. When compared with PD, both PD with dementia and DLB patients did not significantly differ with respect to CSF levels of α -syn species. It is of interest that AD patients show higher CSF total α -syn levels when compared with PD cases, reflecting the different pathophysiological involvement of α -syn in these diseases.

With respect to the role of CSF α -syn species as prognostic biomarkers, the majority of the studies showed no correlation with disease duration, clinical rating scales and neuropsychological assessment. The few available longitudinal studies gave inconsistent findings both for motor and cognitive domains.

We can conclude that the combined CSF assessment of total and oligomeric α -syn enables us to improve our diagnostic accuracy in distinguishing PD from other

neurological diseases and controls, while their prognostic value is questionable.

Future perspective

In order to improve our knowledge in this field, thus getting closer to a clinical use of CSF biomarkers in PD diagnosis, we do need to improve the standardization of the available assays, thus reducing the large interlaboratory variability.

Also, international collaborative networks following homogeneous protocols for enrolling large clinically well-characterized PD cohorts are highly needed.

In the future, CSF biomarkers might also serve as theragnostic markers after disease-modifying treatments.

Although the use of CSF biomarkers in PD is limited by the lack of optimal candidates, some clinical trials have incorporated CSF analysis as a measure of efficacy or target engagement, or for exploratory analysis [54]. To this purpose, CSF α -syn is now being explored as a surrogate marker of treatment efficacy and/or target engagement in patients with PD or other synucleino-

pathies. Active immunization with α -syn in patients with PD is also being investigated in Phase I trials [54].

Finally, the need to create a composite CSF biomarkers profile reflecting different neurodegenerative processes taking place in PD brain as dynamic phenomena along the course of the disease should be implemented. In order to be able to detect the disease in its premotor phase – as a *conditio sine qua non* for a rationale use of disease-modifying agents, the strategy represented by the combination of different markers is mandatory.

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Executive summary

Methodological issues about measurement of α -synuclein species in cerebrospinal fluid

- ELISA is the prominent method of measurement.
- Measurements carried out since 2000 are highly heterogeneous.
- The results of a recent international validation study highlighted the high variability of the total α -synuclein (α -syn) absolute value among laboratories.

Diagnostic value of cerebrospinal fluid α -synuclein species

- Total α -syn levels are lower in Parkinson's disease (PD) compared with controls.
- Oligomeric α -syn levels are higher in PD as compared with controls.
- Combination of total and oligomeric α -syn helps in differential diagnosis between PD and other diseases.
- Few studies investigated the prognostic value of cerebrospinal fluid α -syn species in PD, generating inconsistent data.

Future perspective

- Need for improvement of assay standardization.
- Research community should spend more effort in larger studies aiming at creating a composite cerebrospinal fluid profile including multiple neurodegeneration biomarkers.

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