Mini-review for Park Rel Dis of the Chicago IAPRD congress lecture: “Other biomarkers (skin, csf, blood, etc.)”

TITLE – **“FLUID AND TISSUE BIOMARKERS IN PARKINSON’S DISEASE: IMMUNODETECTION OR SEED AMPLIFICATION? CENTRAL OR PERIPHERAL?”**

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ABSTRACT

Over the last two decades there have been meaningful developments on biomarkers of neurodegenerative diseases, extensively (but not solely) focusing on their proteinopathic nature. Accordingly, in Alzheimer's disease determination of levels of total and phosphorylated tau (τ and p-τ, usually p-τ181) along with amyloid-beta1-42 (Aβ1-42) by immunodetection in cerebrospinal fluid (CSF) and currently even in peripheral blood, have been widely accepted and introduced to routine diagnosis. In the case of Parkinson's disease, α-synuclein as a potential biomarker (both for diagnosis and progression tracking) has proved more elusive under the immunodetection approach. In recent years, the emergence of the so-called seed amplification assays is proving to be a game-changer, with mounting evidence under different technical approaches and using a variety of biofluids or tissues, yielding promising diagnostic accuracies. Currently the least invasive but at once more reliable source of biosamples and techniques are being sought. Here we overview these advances.

1. INTRODUCTION

Biomarkers are objectively measurable clinical, biochemical, or imaging features that can assist diagnosis and differential diagnosis, but also provide prognosis information and be surrogate endpoints in clinical trials and eventually indicators in clinical practice that a given treatment is working or not[**1,2**].

Clinical biomarkers mostly come from clinical history, exam features or scores of scales. While valuable as easily accessible and deliverable, their subjective nature and heterogeneity are hurdles.

Imaging biomarkers in the field of neurodegeneration range from structural imaging (mostly MRI), which in turn can also consist of specific radiological signs obtained by visual inspection (and hence again limited by its subjectiveness) or complex quantification approaches (hampered by standardization issues), to functional techniques. The latter have evolved from initially allowing for measuring neurochemical deficits (such as presynaptic nigrostriatal dopaminergic deficiency as per dopamine transporter imaging in all neurodegenerative parkinsonisms in contrast to normality in secondary parkinsonisms) to currently detecting in vivo the abnormal aggregated protein brain deposition, as in the case of PET amyloid and tau imaging in Alzheimer's disease. However, α-synuclein detection by nuclear medicine approaches, despite recent progress, is still not available[**3**].

This takes us to the topic of the current topical review: biochemical biomarkers in biofluids or tissues for the diagnosis assessment in Parkinson's disease (PD) and its differentiation from its usual lookalikes (chiefly atypical parkinsonisms). These type of biomarkers in PD cannot be addressed without putting it in context of other degenerative conditions. Hence, for several years now, immunoassays (by ELISA and more recently automated CLEIA platforms/Simoa) of τ, phosphorylated-τ (p-τ181), amyloid-beta1-42 (Aβ1-42) and Ab42/40 in cerebrospinal fluid (CSF) have proven a reliable biomarker of Alzheimer's disease (AD)[**4**]. The success with immunoassays to detect the main AD proteins set the path and determined the subsequent struggle to measure α -synuclein in PD, and in hindsight it demonstrated that a winning strategy in one disease does not unequivocally apply to another, however related they might be. Therefore, over several years immunoassay-based studies of different variants of α-synuclein significantly contributed to the knowledge in vivo of the synucleinopathy of PD, but without enough accuracy to be a reliable biomarker for the clinic. Nowadays, however, immunoassay determination (mainly by ELISA) of other proteins (including τ, p-τ, and Aβ1-42 themselves) in CSF has also its role in PD, while the new technique

of seed amplification assays (SAAs) and other immunological techniques, such as immunoprecipitation, are improving the detection of α-synuclein in PD. In this vein, the source where all these biomarkers are being determined is another critical feature. Whereas CSF has been traditionally used, due to its intimate anatomical and functional relationship with brain parenchyma and the difficulty to measure certain proteins in peripheral blood owing to low concentrations, its rather invasive obtention has led to seek alternative peripheral sources with more sensitive techniques (blood, skin, others)[**5**].

2. FLUID BIOMARKERS

2. 1 CEREBROSPINAL FLUID

As mentioned in the Introduction, the attempt to obtain biochemical biomarkers in PD started using immunoassay techniques in CSF following the success in AD. Historically, different types of α-synuclein have been measured. Total α-synuclein (t-α-syn) is the one more extensively studied. Many studies have pointed its reduction in PD, including its spectrum with PD-dementia (PDD) and dementia with Lewy bodies (DLB), as well as multiple system atrophy (MSA), another synucleinopathy (but with predominant oligodendroglial inclusions). However, all these studies showed a remarkable overlap with not only non-neurological controls but also with other parkinsonisms and degenerative conditions, resulting in modest discriminant ability, unacceptable for diagnostic purposes in clinical practice[**6-8**]. Adding to this concern, several other studies did not even find statistically significant differences between synucleinopathies and non-synucleinopathies[**9,10**]. Yet, intriguing information was obtained from those studies. As an example, both cross-sectionally and longitudinally, higher (instead of lower) CSF t-α-syn levels were found to correlate with worse cognitive measures and progression, higher CSF t-τ levels and greater cortical thinning in posterior cortical areas in MRI[**10-12**]. Another variant of α-synuclein studied in CSF is oligomeric α-synuclein (o-α-syn). This approach is based on using the same antibody for coating and detection, thus not being able to mark single (monomeric) α-synuclein molecules, as these have the antibody epitope blocked by the coating antibody, thus not binding to the detection antibody. Conversely, oligomers have one or more epitopes available for binding with the detection antibody once bound to the coating one. The first study with this technique showed the opposite as t-α-syn: increase in CSF o-α-syn levels relative to non-neurological and diseased-controls with remarkable discriminant ability, yet some overlap[**13**]. This significant trend to elevation of CSF o-α-syn has been more consistently observed across studies, including association of even

higher levels with the presence of dementia (be it PD-dementia [PDD] or DLB)[**10,14,15**]. However, in a single study increased levels were not found among people with idiopathic REM sleep behaviour disorder (iRBD), considered a prodromal PD symptom and usually included in studies as a proxy of prodromal PD[**10**]. Another studied α-synuclein species is phosphorylated α-synuclein (p-α-syn). In different studies CSF p-α-syn were increased in PD[**16**] and other synucleinopahies, with a trend to a U-shape association with disease duration, that is, lower levels correlated with worse clinical state in early disease, but the opposite in more advanced and severe disease[**17,18**].

During these years of significant, yet insufficient in terms of clinical application, findings using immunoasays for α-synuclein, the existing evidence of AD co-pathology in PDD [**19**] and of other molecular pathways identified in genetic studies but with relevance beyond familial cases, such as the case of glucocerebrosidase (GBA)[**20**], led to study these proteins in CSF in sporadic PD too. Hence, again both cross-sectional and longitudinal studies showed increase of CSF τ and p-τ and decrease of CSF Aβ1-42 levels in PDD and DLB, and association between lower CSF Aβ1-42 levels at baseline in PD patients without dementia with progression to PDD at follow-up, and also with longitudinal measures of cortical thinning in posterior cortical areas[**21-24**]. Studies measuring PET probes for Aβ1-42 have shown association between increased PET uptake and low CSF Aβ1-42 levels in PDD and again their link with longitudinal progression to dementia among patients without dementia at baseline[**25,26**]. Likewise, an association of lowered CSF enzyme activity of glucocerebrosidase in PD cases with and without an underlying GBA mutation with PD, particularly in earlier stages has been reported[**27**]. Also mirroring the findings using CSF markers of AD, a longitudinal study has shown that lower CSF glucocerebrosidase activity is also a statistically significant longitudinal predictor of progression to PDD [**28**].

Coming back to the α-synuclein story, the mounting evidence that this protein can spread throughout the brain following a prion-like mechanism, prompted researchers to take advantage of techniques relying on the self-aggregating behaviour of prion protein (PrP) to show with a fluorescence dye (Thioflavin-T) the increase of fluorescence of CSF samples from prion disease patients (with pathological PrP) incubated with recombinant (normal) PrP. Two such techniques, real-time quaking-induced conversion (RT-QuIC) and protein misfolding cyclic amplification (PMCA) had shown the ability to amplify the aggregation and fluorescence reaction of samples with minute (even attomolar, that is 10^{-18} molar) amounts of PrP. Following this success with PrP, two studies (using RT-QuIC and PMCA one each) applied this approach to α-synuclein showing an almost perfect sensitivity and specificity not only

compared to controls but also to other degenerative conditions and even in iRBD cases again included as proxies of prodromal PD[**29,30**]. Even though the authors named the techniques differently, both RT-QuIC and PMCA to detect α-synuclein aggregates operate similarly; therefore, currently the nomenclature is increasingly being unified under the name SAAs[**31**]. Other studies have also shown a more rapid amplification reaction (in terms of time in hours) to be associated with worse disease progression, both on motor and cognitive grounds[**32**].

Although challenging to set up across different sites, as reaction parameters can widely vary among labs limiting the standardization of SAAs, these methods have rapidly generated numerous published studies and are already used in routine clinical practice at several hospitals worldwide. However, there are several challenges and caveats: quantification of results, while addressed in some studies, remains an unmet need, with results still being largely merely dichotomic (positive vs negative curves, usually with 3 or 4 replicates for sample, requiring a minimum of 2 or 3 respectively for a positive result); also, sensitivity for the MSA synucleinopathy has been overall disappointing, around 20%[**33,34**]. In this latter regard, few studies have reported higher figures[**30,35**] and there are attempts to increase the sensitivity but not at the expense of losing specificity for PD, with which MSA can be easily misdiagnosed. Hence, a different lag phase (time to start the increase of the amplification curve) and a lower maximum fluorescence (relative to PD/PDD/DLB) have been pointed as differential traits of positive SAA reaction for α-synuclein in PD vs. MSA (**Figure 1**). Alternatively, adapting SAAs to the different Lewy/MSA synuclein strains might eventually overcome this limited sensitivity[**36**].

In the meantime, some authors have used the combination of α-synuclein SAA in CSF with other markers, like CSF levels of neurofilament light chain (NfL), and quantitative MRI measures, to assist the differentiation between PD and MSA and other atypical parkinsonisms. Thus, in a study the discriminant ability between PD and MSA of CSF α-syn RT-QuIC alone was improved when combining it with CSF NfL levels[**37**], and another study[**34**] showed that further combining these two measures with the planimetric measures of midbrain and pons surfaces allowed for differentiating not only PD and MSA but also tauopathies (progressive supranuclear palsy [PSP] and corticobasal degeneration [CBD]), since positive CSF α-syn RT-QuIC, low CSF NfL and normal midbrain and pons planimetry segregated with PD, negative CSF α-syn RT-QuIC, increased CSF NfL and reduced pons planimetry with MSA and negative CSF α-syn RT-QuIC, increased CSF NfL and reduced midbrain planimetry with PSP and CBD.

Despite its lower levels in PD relative to atypical parkinsonisms, higher CSF NfL levels in PD have also been associated with dementia-risk (PDD).

The rapid increase in studies and application of α-syn SAAs has led to show that its diagnostic ability for PD overcomes that of clinical and dopamine transporter SPECT[**38**], but also to identify its heterogeneity within the universe of PD patients, showing lesser positive results in LRRK2 monogenic variant of PD in association with lower smell test scores and lesser reduction of uptake in dopamine transporter SPECT, in contrast to a pattern more consistent with sporadic PD in cases carrying GBA mutations[**39**]. These differences between sporadic and GBA-related PD on the one hand with LRRK2-related PD on the other are consistent with the shared Lewy-type pathology in the two former and lesser Lewy-type pathology in the latter.

Despite all these advances and great promise of α-syn SAAs, the aforementioned variability across sites and also of the reduced yet still existing cases of false positive and negative results, remain areas for further improvement of the technique. In this vein, there might be diverse analytes in CSF from different individuals with seeding enhancing or inhibition properties. Improved knowledge of such molecules, like high density lipoprotein (HDL)[**40**] and others yet to be discovered, can prove crucial to further improve the already great diagnostic ability of these tests. These also might give insights of phenotypic differences within the same disease, be it a synucleinopathy or a tauopathy (high and low seeders)[**41**], and provide an avenue for identifying and testing in vitro aggregation-inhibitory molecules with potential therapeutic interest.

Recently, three independent studies have highlighted the role of CSF levels of DOPA decarboxylase (DDC) as a diagnostic biomarker of dopaminergic dysfunction[**42-44**]. DDC efficiently discriminated Lewy-body disorders (LBD) and atypical parkinsonisms from controls and other non-parkinsonian neurodegenerative disorders (such as AD)[**42-44**]. Encouraging results in prodromal LBD were also found[**43,44**]. If these results are confirmed, DDC could act as a viable alternative to DAT-scan with the advantage of being acquired through lumbar puncture, a method that is used for the assessment of other biomarkers and likely to be more cost-effective. Conversely, as setbacks, CSF levels of DDC (just as DAT-scan itself) will not foreseeably be useful to differentiate among degenerative parkinsonisms (PD vs. atypical parkisonisms and those among themselves), and its validity as a progression biomarker remains unexplored too.

2.2 BLOOD

Both immunodetection and SAAs of α-synuclein have been extensively sought in peripheral blood as a source being easily accessible and less invasive relative to CSF. However, the

immunodetection techniques have struggled with the limitation of the important content of α-synuclein in red blood cells, with SAAs having faced the issue of inhibition of seeding by the protein-rich content of peripheral blood. However recently some studies using immunoprecipitation to concentrate α-syn from serum allowed for detecting pathogenic α-syn seeds even discriminating among patients of PD, DLB and MSA synucleinopathies[**45**].In addition, the study of extracellular vesicles also enables either immunodetection or SAA of α-synuclein in exosomes extracted from peripheral blood[**46,47**]. There is even preliminary experience with differential immunoprecipitation of neuronal vs. oligodendroglial exosomes from peripheral blood for differentiating α-synuclein coming from PD neurons or MSA oligodendrocytes, albeit still with remarkable overlap between groups[**48**].

Peripheral blood mononuclear cells (PBMC) constitute another potential source of biomarkers for PD and could serve as a valuable model for investigating impairment in biological pathways linked with PD pathogenesis[**49-51**].

Moreover, NfL can nowadays through single molecule array (SIMOA) be detected in blood, with several studies reporting a significant correlation between CSF and plasma or serum determination of NfL with ELISA and SIMOA respectively[**52**]. Nevertheless, these correlations are moderate or even modest, despite their statistical significance, suggesting that, for the time being, blood can be considered a screening test, with CSF remaining the gold standard.

Finally, markers previously studied as progression predictors of risk of PDD in CSF are beginning to be measurable in blood. Hence, plasma levels of Aβ, t-τ and α-synuclein itself have been successfully determined by an ultra-sensitive immunomagnetic reduction-based immunoassay in a study[**53**]. There, the authors reported significant increase of both t-τ and α-synuclein and significant reduction of Aβ1-40 (but not those of Aβ1-42) levels in plasma in PD vs. controls. Moreover, both binary logistic regressions and the respective receiver operating characteristic curves analyses showed significant association of these biomarkers in plasma with the presence of cognitive impairment. Still, these findings are still to be taken with a grain of salt since the areas under the curve, the specificities (Aβ and α-synuclein) and the sensitivities (t-τ) were modest, due to noticeable overlap among groups. Finally, transcriptome and micro-RNA studies are other approaches studied in peripheral blood. Hence, changes in pathways related to focal adhesion, mTOR, adipocytokine, neuron projection and others have been found in idiopathic and LRRK2-related PD[**54-57**].

3. TISSUE BIOMARKERS

The hypothesis that at least in part of PD patients the disease process starts in the peripheral nerve system, which has a huge extension in the human body only considering enteral and cutaneous terminal nerves, has led to also seek the above reviewed biomarkers in peripheral tissues. This has been pursued by means of skin, salivary and enteral biopsies, but also with nasal mucosa swabs and collection of saliva or even teardrops.

The most extensively studied have been the skin and the colon via biopsies, historically only with immunohistochemistry, currently already with SAAs including comparative data between immunohistochemistry and SAAs.

3.1 SKIN

Depending on the method used and the biopsy skin site, immunopositivity for α-syn has been reported to vary widely, ranging from less than <10%[**58,59**] to 100%[**60,61**]. However, consistently, PD patients have shown higher rate of α-syn positivity when comparing to healthy controls[**61**] and, although much less extensively investigated, also when compared with non α-syn parkinsonism[**62**].

Regarding α-syn SAA in skin, studies have shown this technique presents high sensitivity, as highlighted in a recent meta-analysis[**63]**.

A comparative study including PD and MSA cases and performing immunohistochemistry in skin and RT-QuIC in skin and CSF for α-syn[**64**] showed high but incomplete agreement between these techniques . However, some of the patients underwent up to 8 punches for skin biopsy and among MSA cases there was remarkable disagreement and lower reliability .

Another study also claimed that α-syn RT-QuIC accuracy was comparable in skin and CSF among iRBD patients. The skin biopsy protocol consisted of 6 biopsies per patient with the matched accuracy between skin and CSF being the result of the combined sensitivity of all the biopsies; when considering the performance of each biopsy separately, the sensitivity ranged from 58 to 69% as opposed to 75% in CSF[**65**]. Also in iRBD, a recent study [**66**] showed higher diagnostic accuracy (89% vs. 70%) in detecting pathological α-syn in skin by IF (n=81) in comparison to SAA (n=40). CSF-based α-syn -SAA was also explored yielding suboptimal results compared to those previously reported in the literature by other labs including studies specifically focusing on iRBD [**33,38,65**]. This discrepancy could be attributed to potential suboptimization of the SAA, hindering a reliable comparison between CSF and skin assays.

In this vein, a further study comparing iRBD with PD found the sensitivity of α- syn RT-QuIC in skin to be higher in the former (97%) than the latter (87%), with shorter lag phase and higher maximum fluorescence in iRBD[**67**]. Therefore, more studies are needed to fully elucidate which sample and technique are more accurate.

3.2 OTHER TISSUES BIOMARKERS

Regarding other peripheral fluids and tissues, methods need still further development for saliva and colon tissue, whereas for submandibular gland despite moderate accuracy with immunohistochemistry and high sensitivity and specificity with RT-QuIC, the performance of biopsy in terms of valid amount of tissue has been often suboptimal while carrying remarkable side effects[**5**]. In fact, a report from the 4S consortium (Systemic Synuclein Sampling Study) has recently showed that negative results in controls and positive tests in mild, moderate and advanced PD are more frequently obtained with CSF α-syn SAA compared to CSF t-α-syn by immunoassays but also to immunohistochemistry and SAA of α-syn in submandibular gland[**68**].

In the olfactory mucosa, immunoreactivity for α-syn has not demonstrated sufficient accuracy in previous reports [**69**]. Nevertheless, in a recent study investigating this aspect as a secondary objective, it was found that oligomeric α-syn was elevated in the PD group compared to the healthy control group [**70**]. On the other hand, studies utilizing SAAs have reported moderate accuracy [**71**], albeit with a lower sensitivity of 0.64 (0.49-0.76) compared to CSF and skin (p = 0.02, 0.01, respectively), as indicated by recent meta-analyses [**72**].

Other approaches are proteome studies in easily accessible fluids such as saliva and tear, which have shown increase or decrease in proteins related to the inflammatory processes, immune response, lipid metabolism, exosome formation, adipose tissue formation and oxidative stress in PD patients [**73,74**]. Still, these fluids while attractive due to their easy collection, can vary in their composition which can hamper the interpretation and standardization of these approaches.

4. CONCLUSIONS

PD biomarkers of α-synuclein have evolved from immunobased to seed amplification assays, but currently immunoprecipitation and other approaches seem to enter the scene

again in a renewed attempt to obtain reliable biomarkers in peripheral blood and other fluids and tissues more accessible than CSF.

Different biomarkers may apply to specific settings: for example, Aβ, t-τ and glucocerebrosidase activity in either CSF or blood as dementia-risk biomarkers, and NfL as a progression marker too and furthermore in the differential diagnosis with atypical parkinsonisms.

Despite the mounting studies in peripheral fluids and tissues, CSF remains the gold standard and other techniques until improved detection and accuracy can be considered as screening currently, although recent published data in skin particularly are promising. Still, if there are truly brain- vs. body-first PD cases, perhaps the use of CSF vs. peripheral samples could be critical for diagnostic accuracy. Also, for differentiation from atypical parkinsonisms the extent to which α-synuclein and τ are present or absent in peripheral tissues in MSA and PSP-CBD can also be a critical factor for the reliability of peripheral vs. CSF determination of these biomarkers. Hence, in a hypothetical future with the possibility of SAA panels for different proteins, like α-syn for PD and MSA and 4R-τ for PSP and CBD, if the seeding ability of peripheral tissues only proves to be reliable for PD, perhaps the CSF will be the preferred sample for testing the different proteins at once. Further development and assessment of the performance of SAAs for these other proteins such as 4R-τ in different fluids and tissues will likely answer this important question.

Another important issue is that of false positives and negatives, and to what extent and in which cases those correspond to technique failure, co-pathology or even cross-seeding, and the presence of inhibitors of aggregation.

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REFERENCES

1. A.W. Michell, S.J. Lewis, T. Foltynie, R.A. Barker, Biomarkers and Parkinson's disease, Brain. 127(2004) 1693-705. doi: 10.1093/brain/awh198.

2. A. Siderowf, A.E. Lang, Premotor Parkinson's disease: concepts and definitions, Mov Disord. 27 (2012) 608-16. doi: 10.1002/mds.24954.

3. U. Saeed, A.E. Lang, M. Masellis, Neuroimaging Advances in Parkinson's Disease and Atypical Parkinsonian Syndromes, Front Neurol. 11 (2020). doi: 10.3389/fneur.2020.572976.

4. M. Bjerke, S. Engelborghs, Cerebrospinal Fluid Biomarkers for Early and Differential Alzheimer's Disease Diagnosis, J Alzheimers Dis. 62 (2018) 1199-1209. doi: 10.3233/JAD-170680.

5. D.G. Coughlin, D.J. Irwin, Fluid and Biopsy Based Biomarkers in Parkinson's Disease, Neurotherapeutics, 20 (2023) 932-954. doi: 10.1007/s13311-023-01379-z.

6. B. Mollenhauer, J.J. Locascio, W. Schulz-Schaeffer, F. Sixel-Döring, C. Trenkwalder, M.G. Schlossmacher, α-Synuclein and tau concentrations in cerebrospinal fluid of patients presenting with parkinsonism: a cohort study, Lancet Neurol. 10 (2011) 230-40. doi: 10.1016/S1474-4422(11)70014-X.

7. L. Parnetti, D. Chiasserini, G. Bellomo, D. Giannandrea, C. De Carlo, M.M. Qureshi, M.T. Ardah, S. Varghese, L. Bonanni, B. Borroni, N. Tambasco, P. Eusebi, A. Rossi, M. Onofrj, A. Padovani, P. Calabresi, O. El-Agnaf, Cerebrospinal fluid Tau/α-synuclein ratio in Parkinson's disease and degenerative dementias, Mov Disord. 26 (2011) 1428-35. doi: 10.1002/mds.23670.

8. J.H. Kang, D.J. Irwin, A.S. Chen-Plotkin, A. Siderowf, C. Caspell, C.S. Coffey, T. Waligórska, P. Taylor, S. Pan, M. Frasier, K. Marek, K. Kieburtz, D. Jennings, T. Simuni, C.M. Tanner, A. Singleton, A.W. Toga, S. Chowdhury, B. Mollenhauer, J.Q. Trojanowski, L.M. Shaw; Parkinson's Progression Markers Initiative, Association of cerebrospinal fluid β-amyloid 1-42, T-tau, P-tau181, and α-synuclein levels with clinical features of drug-naive patients with early Parkinson disease, JAMA Neurol. 70 (2023) 1277-87. doi: 10.1001/jamaneurol.2013.3861.

9. M.B. Aerts, R.A. Esselink, W.F. Abdo, B.R. Bloem, M.M. Verbeek, CSF α-synuclein does not differentiate between parkinsonian disorders, Neurobiol Aging. 33 (2012) 430.e1-3. doi: 10.1016/j.neurobiolaging.2010.12.001.

10. Y. Compta, T. Valente, J. Saura, B. Segura, Á. Iranzo, M. Serradell, C. Junqué, E. Tolosa, F. Valldeoriola, E. Muñoz, J. Santamaria, A. Cámara, M. Fernández, J. Fortea, M. Buongiorno, J.L.

Molinuevo, N. Bargalló, M.J. Martí, Correlates of cerebrospinal fluid levels of oligomeric- and total-α-synuclein in premotor, motor and dementia stages of Parkinson's disease, J Neurol. 262 (2015) 294-306. doi: 10.1007/s00415-014-7560-z.

11. T. Stewart, C. Liu, C. Ginghina, K.C. Cain, P. Auinger, B. Cholerton, M. Shi, J. Zhang; Parkinson Study Group DATATOP Investigators, Cerebrospinal fluid α-synuclein predicts cognitive decline in Parkinson disease progression in the DATATOP cohort, Am J Pathol. 184 (2014) 966-975. doi: 10.1016/j.ajpath.2013.12.007.

12. S. Hall, Y. Surova, A. Öhrfelt, H. Zetterberg, D. Lindqvist, O. Hansson, CSF biomarkers and clinical progression of Parkinson disease, Neurology. 84 (2015) 57-63. doi: 10.1212/WNL.0000000000001098.

13. T. Tokuda, M.M. Qureshi, M.T. Ardah, S. Varghese, S.A. Shehab, T. Kasai, N. Ishigami, A. Tamaoka, M. Nakagawa, O.M. El-Agnaf, Detection of elevated levels of α-synuclein oligomers in CSF from patients with Parkinson disease, Neurology. 75 (2010) 1766-72. doi: 10.1212/WNL.0b013e3181fd613b.

14. M.J. Park, S.M. Cheon, H.R. Bae, S.H. Kim, J.W. Kim, Elevated levels of α-synuclein oligomer in the cerebrospinal fluid of drug-naïve patients with Parkinson's disease, J Clin Neurol. 7 (2011) 215-22. doi: 10.3988/jcn.2011.7.4.215.

15. O. Hansson, S. Hall, A. Ohrfelt, H. Zetterberg, K. Blennow, L. Minthon, K. Nägga, E. Londos, S. Varghese, N.K. Majbour, A. Al-Hayani, O.M. El-Agnaf, Levels of cerebrospinal fluid α-synuclein oligomers are increased in Parkinson's disease with dementia and dementia with Lewy bodies compared to Alzheimer's disease, Alzheimers Res Ther. 6 (2014) 25. doi: 10.1186/alzrt255.

16. P. Eusebi, D. Giannandrea, L. Biscetti, I. Abraha, D. Chiasserini, M. Orso, P. Calabresi, L. Parnetti, Diagnostic utility of cerebrospinal fluid α-synuclein in Parkinson's disease: A systematic review and meta-analysis, Mov Disord. 32 (2017) 1389-1400. doi: 10.1002/mds.27110.

17. Y. Wang, M. Shi, K.A. Chung, C.P. Zabetian, J.B. Leverenz, D. Berg, K. Srulijes, J.Q. Trojanowski, V.M. Lee, A.D. Siderowf, H. Hurtig, I. Litvan, M.C. Schiess, E.R. Peskind, M. Masuda, M. Hasegawa, X. Lin, C. Pan, D. Galasko, D.S. Goldstein, P.H. Jensen, H. Yang, K.C. Cain, J. Zhang, Phosphorylated α-synuclein in Parkinson's disease, Sci Transl Med. 4 (2012). doi: 10.1126/scitranslmed.3002566.

18. T. Stewart, V. Sossi, J.O. Aasly, Z.K. Wszolek, R.J. Uitti, K. Hasegawa, T. Yokoyama, C.P. Zabetian, J.B. Leverenz, A.J. Stoessl, Y. Wang, C. Ginghina, C. Liu, K.C. Cain, P. Auinger, U.J. Kang, P.H. Jensen, M. Shi, and J. Zhang, Phosphorylated α-synuclein in Parkinson's disease: correlation depends on disease severity, Acta Neuropathol Commun. 3 (2015) 7. doi: 10.1186/s40478-015-0185-3.

19. Y. Compta, L. Parkkinen, S.S. O'Sullivan, J. Vandrovcova, J.L. Holton, C. Collins, T. Lashley, C. Kallis, D.R. Williams, R. de Silva, A.J. Lees, T. Revesz, Lewy- and Alzheimer-type pathologies in Parkinson's disease dementia: which is more important? Brain. 134 (2011) 1493-1505. doi: 10.1093/brain/awr031.

20. E. Sidransky, M.A. Nalls, J.O. Aasly, J. Aharon-Peretz, G. Annesi, E.R. Barbosa, A. Bar-Shira, D. Berg, J. Bras, A. Brice, C.M. Chen, L.N. Clark, C. Condroyer, E.V. De Marco, A. Dürr, M.J. Eblan, S. Fahn, M.J. Farrer, H.C. Fung, Z. Gan-Or, T. Gasser, R. Gershoni-Baruch, N. Giladi, A. Griffith, T. Gurevich, C. Januario, P. Kropp, A.E. Lang, G.J. Lee-Chen, S. Lesage, K. Marder, I.F. Mata, A. Mirelman, J. Mitsui, I. Mizuta, G. Nicoletti, C. Oliveira, R. Ottman, A. Orr-Urtreger, L.V. Pereira, A. Quattrone, E. Rogaeva, A. Rolfs, H. Rosenbaum, R. Rozenberg, A. Samii, T. Samaddar, C. Schulte, M. Sharma, A. Singleton, M. Spitz, E.K. Tan, N. Tayebi, T. Toda, A.R. Troiano, S. Tsuji, M. Wittstock, T.G. Wolfsberg, Y.R. Wu, C.P. Zabetian, Y. Zhao, S.G. Ziegler, Multicenter analysis of glucocerebrosidase mutations in Parkinson's disease, N Engl J Med. 361 (2009) 1651-1661. doi: 10.1056/NEJMoa0901281.

21. Y. Compta, M.J. Martí, N. Ibarretxe-Bilbao, C. Junqué, F. Valldeoriola, E. Muñoz, M. Ezquerra, J. Ríos, E. Tolosa, Cerebrospinal tau, phospho-tau, and beta-amyloid and neuropsychological functions in Parkinson's disease, Mov Disord, 24 (2009) 2203-2210. doi: 10.1002/mds.22594.

22. G. Alves, K. Brønnick, D. Aarsland, K. Blennow, H. Zetterberg, C. Ballard, M.W. Kurz, U. Andreasson, O.B. Tysnes, J.P. Larsen, E. Mulugeta, CSF amyloid-beta and tau proteins, and cognitive performance, in early and untreated Parkinson's disease: the Norwegian ParkWest study, J Neurol Neurosurg Psychiatry. 81 (2010) 1080-1086. doi: 10.1136/jnnp.2009.199950.

23. A. Siderowf, S.X. Xie, H. Hurtig, D. Weintraub, J. Duda, A. Chen-Plotkin, L.M. Shaw, V. Van Deerlin, J.Q. Trojanowski, C. Clark, CSF amyloid beta 1-42 predicts cognitive decline in Parkinson disease, Neurology. 75 (2010) 1055-1061. doi: 10.1212/WNL.0b013e3181f39a78.

24. Y. Compta, J.B. Pereira, J. Ríos, N. Ibarretxe-Bilbao, C. Junqué, N. Bargalló, A. Cámara, M. Buongiorno, M. Fernández, C. Pont-Sunyer, M.J. Martí, Combined dementia-risk biomarkers in

Parkinson's disease: a prospective longitudinal study, Parkinsonism Relat Disord, 19 (2013) 717-724. doi: 10.1016/j.parkreldis.2013.03.009.

25. M. Buongiorno, F. Antonelli, Y. Compta, Y. Fernandez, J. Pavia, F. Lomeña, J. Ríos, I. Ramírez, J.R. García, M. Soler, A. Cámara, M. Fernández, M. Basora, F. Salazar, G. Sanchez-Etayo, F. Valldeoriola, J.R. Barrio, M. Marti MJ, Cross-Sectional and Longitudinal Cognitive Correlates of FDDNP PET and CSF Amyloid-β and Tau in Parkinson's Disease, J Alzheimers Dis. 55 (2017) 1261-1272. doi: 10.3233/JAD-160698.

26. C. Buddhala, M.C. Campbell, J.S. Perlmutter, P.T. Kotzbauer, Correlation between decreased CSF α-synuclein and $A\beta_{1-42}$ in Parkinson disease, Neurobiol Aging. 36 (2015) 476-484. doi: 10.1016/j.neurobiolaging.2014.07.043.

27. L. Parnetti, D. Chiasserini, E. Persichetti, P. Eusebi, S. Varghese, M.M. Qureshi, A. Dardis, M. Deganuto, C. De Carlo, A. Castrioto, C. Balducci, S. Paciotti, N. Tambasco, B. Bembi, L. Bonanni, M. Onofrj, A. Rossi, T. Beccari, O. El-Agnaf, P. Calabresi, Cerebrospinal fluid lysosomal enzymes and alpha-synuclein in Parkinson's disease, Mov Disord. 29 (2014) 1019-1027. doi: 10.1002/mds.25772.

28. L. Oftedal, J. Maple-Grødem, I. Dalen, O.B. Tysnes, K.F. Pedersen, G. Alves, J. Lange, Association of CSF Glucocerebrosidase Activity With the Risk of Incident Dementia in Patients With Parkinson Disease. Neurology, 100 (2023). doi: 10.1212/WNL.0000000000201418.

29. G. Fairfoul, L.I. McGuire, S. Pal, J.W. Ironside, J. Neumann, S. Christie, C. Joachim, M. Esiri, S.G. Evetts, M. Rolinski, F. Baig, C. Ruffmann, R. Wade-Martins, M.T. Hu, L. Parkkinen, A.J. Green, Alpha-synuclein RT-QuIC in the CSF of patients with alpha-synucleinopathies, Ann Clin Transl Neurol. 3 (2016) 812-818. doi: 10.1002/acn3.338.

30. M. Shahnawaz, T. Tokuda, M. Waragai, N. Mendez, R. Ishii, C. Trenkwalder, B. Mollenhauer, C. Soto, Development of a Biochemical Diagnosis of Parkinson Disease by Detection of α-Synuclein Misfolded Aggregates in Cerebrospinal Fluid, JAMA Neurol. 74 (2017) 163-172. doi: 10.1001/jamaneurol.2016.4547.

31. M.J. Russo, C.D. Orru, L. Concha-Marambio, S. Giaisi, B.R. Groveman, C.M. Farris, B. Holguin, A.G. Hughson, D.E. LaFontant, C. Caspell-Garcia, C.S. Coffey, J. Mollon, S.J. Hutten, K. Merchant, R.G. Heym, C. Soto, B. Caughey, U.J. Kang, High diagnostic performance of independent alpha-synuclein seed amplification assays for detection of early Parkinson's disease, Acta Neuropathol Commun. 9 (2021) 179. doi: 10.1186/s40478-021-01282-8.

32. H. Ning, Q. Wu, D. Han, T. Yao, J. Wang, W. Lu, S. Lv, Q. Jia, X. Li, Baseline concentration of misfolded α-synuclein aggregates in cerebrospinal fluid predicts risk of cognitive decline in Parkinson's disease, Neuropathol Appl Neurobiol. 45 (2019) 398-409. doi: 10.1111/nan.12524.

33. M. Rossi, N. Candelise, S. Baiardi, S. Capellari, G. Giannini, C.D. Orrù, E. Antelmi, A.G. Hughson, G. Calandra-Buonaura, A. Ladogana, G. Plazzi, P. Cortelli, A. Caughey, P. Parchi, Ultrasensitive RT-QuIC assay with high sensitivity and specificity for Lewy body-associated synucleinopathies, Acta Neuropathol. 140 (2020) 49-62. doi: 10.1007/s00401-020-02160-8.

34. Y. Compta, C. Painous, M. Soto, M. Pulido-Salgado, M. Fernández, A. Camara, V. Sánchez, N. Bargalló, N. Caballol, C. Pont-Sunyer, M. Buongiorno, N. Martin, M. Basora, M. Tio, D.M. Giraldo, A. Pérez-Soriano, I. Zaro, E. Muñoz, M.J. Martí, F. Valldeoriola, Combined CSF α-SYN RT-QuIC, CSF NFL and midbrain-pons planimetry in degenerative parkinsonisms: From bedside to bench, and back again, Parkinsonism Relat Disord. 99 (2022) 33-41. doi: 10.1016/j.parkreldis.2022.05.006.

35. I. Poggiolini, V. Gupta, M. Lawton, S. Lee, A. El-Turabi, A. Querejeta-Coma, F. Sixel-Döring, A. Foubert-Samier, A. Pavy-Le Traon, G. Plazzi, F. Biscarini, J.F. Montplaisir, J. Gagnon, R.B. Postuma, E. Antelmi, W.G. Meissner, B. Mollenhauer, Y. Ben-Shlomo, M.T. Hu, L. Parkkinen, Diagnostic value of cerebrospinal fluid alpha-synuclein seed quantification in synucleinopathies, Brain. 145 (2022) 584-595. doi: 10.1093/brain/awab431.

36. M. Shahnawaz, A. Mukherjee, S. Pritzkow, N. Mendez, P. Rabadia, X. Liu, B. Hu, A. Schmeichel, W. Singer, G. Wu, A.L. Tsai, H. Shirani, K.P. Nilsson, P.A. Low, C. Soto, Discriminating α-synuclein strains in Parkinson's disease and multiple system atrophy, Nature. 578 (2020) 273-277. doi: 10.1038/s41586-020-1984-7.

37. C. Quadalti, G. Calandra-Buonaura, S. Baiardi, A. Mastrangelo, M. Rossi, C. Zenesini, G. Giannini, N. Candelise, L. Sambati, B. Polischi, G. Plazzi, S. Capellari, P. Cortelli, P. Parchi, Neurofilament light chain and α-synuclein RT-QuIC as differential diagnostic biomarkers in parkinsonisms and related síndromes, NPJ Parkinsons Dis. 7 (2021) 93. doi: 10.1038/s41531-021-00232-4.

38. L. Concha-Marambio, S. Weber, C.M. Farris, M. Dakna, E. Lang, T. Wicke, Y. Ma, M. Starke, J. Ebentheuer, F. Sixel-Döring, M.L. Muntean, S. Schade, C. Trenkwalder, C. Soto, B. Mollenhauer, Accurate Detection of α-Synuclein Seeds in Cerebrospinal Fluid from Isolated Rapid Eye Movement Sleep Behavior Disorder and Patients with Parkinson's Disease in the DeNovo Parkinson (DeNoPa) Cohort, Mov Disord. 38 (2023) 567-578. doi: 10.1002/mds.29329.

39. A. Siderowf, L. Concha-Marambio, D.E. Lafontant, C.M. Farris, Y. Ma, P.A. Urenia, H. Nguyen, R.N. Alcalay, L.M. Chahine, T. Foroud, D. Galasko, K. Kieburtz, K. Merchant, B. Mollenhauer, K.L. Poston, J. Seibyl, T. Simuni, C.M. Tanner, D. Weintraub, A. Videnovic, S.H. Choi, R. Kurth, C.S. Caspell-Garcia, C.S. Coffey, M. Frasier, L.M.A. Oliveira, S.J. Hutten, T. Sherer, K. Marek, C. Soto, B, Parkinson's Progression Markers Initiative, Assessment of heterogeneity among participants in the Parkinson's Progression Markers Initiative cohort using α-synuclein seed amplification: a cross-sectional study, Lancet Neurol. 22 (2023) 407-417. doi: 10.1016/S1474-4422(23)00109-6.

40. G. Bellomo, S. Paciotti, L. Concha-Marambio, D. Rizzo, A.L. Wojdała, D. Chiasserini, L. Gatticchi, L. Cerofolini, S. Giuntini, C.M.G. De Luca, G. Pieraccini, S. Bologna, M. Filidei, E. Ravera, M. Lelli, F. Moda, M. Fragai, L. Parnetti, C. Luchinat, Cerebrospinal fluid lipoproteins inhibit α-synuclein aggregation by interacting with oligomeric species, Commun Biol. 6 (2023) 105. doi: 10.1038/s42003-022-03041-y.

41. I. Martinez-Valbuena, S. Lee, E. Santamaria, J. Fernandez-Irigoyen, B. Couto, N. Reyes, H. Tanaka, S. Forrest, A. Kim, H. Qamar, J. Li, A. Karakani, K. Senkevich, E. Rogaeva, N. Visanji, A. Lang, G. Kovacs, Introducing the molecular subtyping of Progressive Supranuclear Palsy: a proof of concept [abstract], Mov Disord. 38 (2023) (suppl 1).

42. W. Paslawski, S. Khosousi, E. Hertz, I. Markaki, A. Boxer, P. Svenningsson, Large-scale proximity extension assay reveals CSF midkine and DOPA decarboxylase as supportive diagnostic biomarkers for Parkinson's disease, Transl Neurodegener. 12(2023) 42. doi: 10.1186/s40035-023-00374-w.

43. M. Del Campo, L. Vermunt, C. F. W. Peeters, A. Sieben, Y. S. Hok-A-Hin, A. Lleó, D. Alcolea, M. van Nee, S. Engelborghs, J. L. van Alphen, S. Arezoumandan, A. Chen-Plotkin, D. J. Irwin, W. M. van der Flier, A. W. Lemstra, C. E. Teunissen, CSF proteome profiling reveals biomarkers to discriminate dementia with Lewy bodies from Alzheimer´s disease, Nat Commun, 14 (2023) 5635. doi: 10.1038/s41467-023-41122-y.

44. J.B. Pereira, A. Kumar, S. Hall, S. Palmqvist, E. Stomrud, D. Bali, P. Parchi, N. Mattsson-Carlgren, S. Janelidze, O. Hansson, DOPA decarboxylase is an emerging biomarker for Parkinsonian disorders including preclinical Lewy body disease, Nat Aging. 3 (2023) 1201-1209. doi: 10.1038/s43587-023-00478-y.

45. A. Okuzumi, T. Hatano, G. Matsumoto, S. Nojiri, S. I. Ueno, Y. Imamichi-Tatano, H. Kimura, S. Kakuta, A. Kondo, T. Fukuhara, Y. Li, M. Funayama, S. Saiki, D. Taniguchi, T. Tsunemi, D. McIntyre, J.J. Gérardy, M. Mittelbronn, R. Kruger, Y. Uchiyama, N. Nukina, N. Hattori,

Propagative α-synuclein seeds as serum biomarkers for synucleinopathies, Nat Med. 29 (2023) 1448-1455. doi: 10.1038/s41591-023-02358-9.

46. A. Kluge, J. Bunk, E. Schaeffer, A. Drobny, W. Xiang, H. Knacke, S. Bub, W. Lückstädt, P. Arnold, R. Lucius, D. Berg, F. Zunke, Detection of neuron-derived pathological α-synuclein in blood, Brain. 145 (2022) 3058-3071. doi: 10.1093/brain/awac115.

47. A. Stuendl, T. Kraus, M. Chatterjee, B. Zapke, B. Sadowski, W. Moebius, M.A. Hobert, C. Deuschle, K. Brockmann, W. Maetzler, B. Mollenhauer, A. Schneider, α-Synuclein in Plasma-Derived Extracellular Vesicles Is a Potential Biomarker of Parkinson's Disease, Mov Disord. 36 (2021) 2508-2518. doi: 10.1002/mds.28639.

48. S. Dutta, S. Hornung, A. Kruayatidee, K.N. Maina, I. Del Rosario, K.C. Paul, D.Y. Wong, A. Duarte Folle, D. Markovic, J.A. Palma, G.E. Serrano, C.H. Adler, S.L. Perlman, W.W. Poon, U.J. Kang, R.N. Alcalay, M. Sklerov, K.H. Gylys, H. Kaufmann, B.L. Fogel, J.M. Bronstein, B. Ritz, G. Bitan, α-Synuclein in blood exosomes immunoprecipitated using neuronal and oligodendroglial markers distinguishes Parkinson's disease from multiple system atrophy, Acta Neuropathol. 142 (2021) 495-511. doi: 10.1007/s00401-021-02324-0.

49. S. Petrillo, T. Schirinzi, G. Di Lazzaro, J. D'Amico, V. L. Colona, E. Bertini, M. Pierantozzi, L. Mari, N.B. Mercuri, F. Piemonte, A. Pisani, Systemic activation of Nrf2 pathway in Parkinson's disease, Mov Disord. 35 (2020) 180-184. doi: 10.1002/mds.27878.

50. M. Avenali, S. Cerri, G. Ongari, C. Ghezzi, C. Pacchetti, C. Tassorelli, E.M. Valente, F. Blandini, Profiling the Biochemical Signature of GBA-Related Parkinson's Disease in Peripheral Blood Mononuclear Cells, Mov Disord. 36 (2021) 1267-1272. doi: 10.1002/mds.28496.

51. T. Schirinzi, I. Salvatori, H. Zenuni, P. Grillo, C. Valle, G. Martella, N.B. Mercuri, A. Ferri, Pattern of Mitochondrial Respiration in Peripheral Blood Cells of Patients with Parkinson's Disease, Int J Mol Sci. 23 (2022) 10863. doi: 10.3390/ijms231810863.

52. O. Hansson, S. Janelidze, S. Hall, N. Magdalinou, A.J. Lees, U. Andreasson, N. Norgren, J. Linder, L. Forsgren, R. Constantinescu, H. Zetterberg, K. Blennow; Swedish BioFINDER study, Blood-based NfL: A biomarker for differential diagnosis of parkinsonian disorder, Neurology. 88 (2017) 930-937. doi: 10.1212/WNL.0000000000003680.

53. N.C. Chen, H.L. Chen, S.H. Li, Y.H. Chang, M.H. Chen, N.W. Tsai, C.C. Yu, S.Y. Yang, C.H. Lu, W.C. Lin, Plasma Levels of α-Synuclein, Aβ-40 and T-tau as Biomarkers to Predict Cognitive Impairment in Parkinson's Disease, Front Aging Neurosci. 12 (2020) 112. doi: 10.3389/fnagi.2020.00112.

54. T. Botta-Orfila, X. Morató, Y. Compta, J.J. Lozano, N. Falgàs, F. Valldeoriola, C. Pont-Sunyer, D. Vilas, L. Mengual, M. Fernández, J.L. Molinuevo, A. Antonell, M.J. Martí, R. Fernández-Santiago, M. Ezquerra, Identification of blood serum micro-RNAs associated with idiopathic and LRRK2 Parkinson's disease, J Neurosci Res. 92 (2014) 1071-7. doi: 10.1002/jnr.23377.

55. M.C.T. Dos Santos, M.A. Barreto-Sanz, B.R.S. Correia, R. Bell, C. Widnall, L.T. Perez, C. Berteau, C. Schulte, D. Scheller, D. Berg, W. Maetzler, P.A.F. Galante, A. Nogueira da Costa, miRNA-based signatures in cerebrospinal fluid as potential diagnostic tools for early stage Parkinson's disease, Oncotarget. 9 (2018) 17455-17465. doi: 10.18632/oncotarget.24736. 56. T.M. Marques, B. Kuiperij, I.B. Bruinsma, A. van Rumund, M.B. Aerts, R.A.J. Esselink, B.R. Bloem, M.M. Verbeek, MicroRNAs in Cerebrospinal Fluid as Potential Biomarkers for Parkinson’s Disease and Multiple System Atrophy, Mol. Neurobiol. 2016;54:7736–7745. doi: 10.1007/s12035-016-0253-0.

57. A. Pérez-Soriano, P. Bravo, M. Soto, J. Infante, M. Fernández, F. Valldeoriola, E. Muñoz, Y. Compta, E. Tolosa, A. Garrido, M. Ezquerra, R. Fernández-Santiago, M.J. Martí; Catalan MSA registry (CMSAR), MicroRNA Deregulation in Blood Serum Identifies Multiple System Atrophy Altered Pathways, Mov Disord. 35 (2020) 1873-1879. doi: 10.1002/mds.28143.

58. Y. Miki, M. Tomiyama, T. Ueno, R. Haga, H. Nishijima, C. Suzuki, F. Mori, M. Kaimori, M. Baba, K. Wakabayashi, Clinical availability of skin biopsy in the diagnosis of Parkinson’s disease, Neurosci. Lett. 469 (2010) 357–359.

59. R. Haga, K. Sugimoto, H. Nishijima, Y. Miki, C. Suzuki, K. Wakabayashi, M. Baba, S. Yagihashi, M. Tomiyama, Clinical Utility of Skin Biopsy in Differentiating between Parkinson’s Disease and Multiple System Atrophy, Park. Dis. (2015) 167038.

60. V. Donadio, A. Incensi, C. Piccinini, P. Cortelli, M.P. Giannoccaro, A. Baruzzi, R. Liguori. Skin nerve misfolded -synuclein in pure autonomic failure and Parkinson disease, Ann. Neurol, 79 (2016) 306–316.

61. N. Wang, J. Garcia, R. Freeman, C.H. Gibbons. Phosphorylated Alpha-Synuclein within Cutaneous Autonomic Nerves of Patients with Parkinson’s Disease, The Implications of Sample Thickness on Results, J. Histochem. Cytochem. 68 (2020) 669–678.

62. I. Rodríguez-Leyva, E.G. Chi-Ahumada, J. Carrizales, et al. Parkinson disease and progressive supranuclear palsy: protein expression in skin, Ann Clin Transl Neurol,3 (2016) 191-199. doi:10.1002/acn3.285.

63. D. Yoo, J.I. Bang, C. Ahn, V.N. Nyaga, Y.E. Kim, M.J. Kang, T.B. Ahn. Diagnostic value of α-synuclein seeding amplification assays in α-synucleinopathies: A systematic review and meta-analysis. Parkinsonism Relat Disord. 104 (2022) 99-109. doi: 10.1016/j.parkreldis.2022.10.007.

64. V. Donadio, Z. Wang, A. Incensi, G. Rizzo, E. Fileccia, V. Vacchiano, S. Capellari, M. Magnani, C. Scaglione, M. Stanzani Maserati, P. Avoni, R. Liguori, W. Zou, In Vivo Diagnosis of Synucleinopathies: A Comparative Study of Skin Biopsy and RT-QuIC. Neurology, 96 (2021) e2513-e2524. doi: 10.1212/WNL.0000000000011935.

65. A. Iranzo, A. Mammana, A. Muñoz-Lopetegi, S. Dellavalle, G. Mayà, M. Rossi, M. Serradell, S. Baiardi, A. Arqueros, C. Quadalti, A. Perissinotti, E. Ruggeri, J.S. Cano, C. Gaig, P. Parchi. Misfolded α-Synuclein Assessment in the Skin and CSF by RT-QuIC in Isolated REM Sleep Behavior Disorder. Neurology. 100 (2023) e1944-e1954. doi: 10.1212/WNL.0000000000207147.

66. R. Liguori, V. Donadio, Z. Wang, A. Incensi, G. Rizzo, E. Antelmi, F. Biscarini, F. Pizza, W. Zou, G. Plazzi, A comparative blind study between skin biopsy and seed amplification assay to disclose pathological α-synuclein in RBD, NPJ Parkinsons Dis. 9 (2023) 34. doi: 10.1038/s41531-023-00473-5.

67. A. Kuzkina, C. Panzer, A. Seger, D. Schmitt, J. Rößle, S.R. Schreglmann, H. Knacke, E. Salabasidou, A. Kohl, E. Sittig, M. Barbe, D. Berg, J. Volkmann, C. Sommer, W.H. Oertel, E. Schaeffer, M. Sommerauer, A. Janzen, K. Doppler. Dermal Real-Time Quaking-Induced Conversion Is a Sensitive Marker to Confirm Isolated Rapid Eye Movement Sleep Behavior Disorder as an Early α-Synucleinopathy. Mov Disord. 38 (2023) 1077-1082. doi: 10.1002/mds.29340.

68. L.M. Chahine, T.G. Beach, C.H. Adler, M. Hepker, A. Kanthasamy, S. Appel, S. Pritzkow, M. Pinho, S. Mosovsky, G.E. Serrano, C. Coffey, M.C. Brumm, L.M.A. Oliveira, J. Eberling, B. Mollenhauer; Systemic Synuclein Sampling Study, Central and peripheral α-synuclein in Parkinson disease detected by seed amplification assay, Ann Clin Transl Neurol. 10 (2023) 696-705. doi: 10.1002/acn3.

69. F.J. Jiménez-Jiménez, H. Alonso-Navarro, E. García-Martín, D. Santos-García, I. Martínez-Valbuena, J.A.G. Agúndez, Alpha-Synuclein in Peripheral Tissues as a Possible Marker for Neurological Diseases and Other Medical Conditions, Biomolecules. 13 (2023) 1263. doi: 10.3390/biom13081263.70. T. Schirinzi, D. Maftei, F.M. Passali, P. Grillo, H. Zenuni, D. Mascioli, R. Maurizi, L. Loccisano, M. Vincenzi, A.M. Rinaldi, M. Ralli, S. Di Girolamo, A. Stefani, R. Lattanzi, C. Severini, N.B. Mercuri, Olfactory Neuron Prokineticin-2 as a Potential Target in

Parkinson's Disease, Ann Neurol. 93 (2023) 196-204. doi: 10.1002/ana.26526.71. A. Stefani, A. Iranzo, E. Holzknecht, D. Perra, M. Bongianni, C. Gaig, B. Heim, M. Serradell, L. Sacchetto, A. Garrido, S. Capaldi, A. Sánchez-Gómez, M.P. Cecchini, S. Mariotto, S. Ferrari, M. Fiorini, J. Schmutzhard, P. Cocchiara, I. Vilaseca, L. Brozzetti, S. Monaco, M. Jose Marti, K. Seppi, E. Tolosa, J. Santamaria, B. Högl, W. Poewe, G. Zanusso; SINBAR (Sleep Innsbruck Barcelona) group, Alpha-synuclein seeds in olfactory mucosa of patients with isolated REM sleep behaviour disorder, Brain. 144 (2021) 1118-1126. doi: 10.1093/brain/awab005.72. M. Boerger, S. Funke, A. Leha, A.E. Roser, A.K. Wuestemann, F. Maass, M. Bähr, F. Grus, P. Lingor, Proteomic analysis of tear fluid reveals disease-specific patterns in patients with Parkinson's disease - A pilot study, Parkinsonism Relat Disord. 63 (2019) 3-9. doi: 10.1016/j.parkreldis.2019.03.001.

73. M. Figura, E. Sitkiewicz, B. Świderska, Ł. Milanowski, S. Szlufik, D. Koziorowski, A. Friedman, Proteomic Profile of Saliva in Parkinson's Disease Patients: A Proof of Concept Study, Brain Sci. 11 (2021) 661. doi: 10.3390/brainsci11050661.

Figure 1. Representative curves of CSF α-SYN RT-QuIC from three patients studied in our laboratory (equipment: FLUOSTAR OMEGA). A = a PD case with a typical positive curve; B = an MSA patient with a positive curve too but with a larger lag phase and a lower maximum fluorescence compared to PD; C = negative seeding rection in a control subject case.

Table 1. Overview of the diagnostic value of PD biomarkers.

Biomarker	Tissue	Technique	Comments and challenges
α-synuclein species	CSF	ELISA	**t-α-syn** Reduced levels in PD compared with HC and neurological controls. Overlap of results, resulting in modest discriminant ability [**6-8**]. Other studies did not find significant differences between groups [**9,10**]. This biomarker alone should not be considered for the diagnosis of PD. **o-α-syn** and **p-α-syn** Increased levels in PD compared with HC and neurological controls. Remarkable discriminant ability, yet some overlap [**13**]. Increased levels were not found among people with iRBD [**10**]. Few studies, still lack of validation in independent laboratories [**16**].
	Blood and serum	ELISA Immunoprecipitation + immunodetection or SAA	Important content of α-synuclein in red blood cells with risk for erythrocyte contamination. Recently, studies using immunoprecipitation have shown promise of the study of extracellular vesicles[~~**41,42**~~][46,47]. Its value must be confirmed in larger studies.
	Skin	Immunohistochemistry	~~Lower diagnostic accuracy compared to SAAs.~~ High sensitivity and specificity [**64, 66**]. A study comparing diagnostic accuracy with SAAs was limited by a suboptimal accuracy of CSF-SAAs in sharp contrast to other studies with high accuracy of CSF-SAA in iRBD [**38,65**] ~~or have~~and analyzed CSF-SAAs in a reduced sample size compared to the sample used for immunohistochemistry analyses[**64**]. More studies are needed to elucidate which technique is better.
Pro-aggregating forms of α-syn	CSF	RT-QuIC PMCA	Almost perfect sensitivity and specificity, even in iRBD cases [**29,30**]. Lesser positive results in LRRK2 monogenic variant of PD. Analytical issues (i.e. reproducibility).

	Skin	RT-QuIC PMCA	~~Lower diagnostic accuracy compared to SAA in CSF.~~Less invasive procedure compared to lumbar puncture. Standardization of site and number of biopsies is lacking.
	Other tissues (saliva, colon, etc.)	RT-QuIC PMCA	Methods still need further development, whereas for the submandibular gland despite moderate accuracy the performance of biopsy in terms of valid amount of tissue has been often suboptimal while carrying remarkable side effects[5]
NFL	CSF	ELISA	Useful to differentiate PD vs. atypical parkinsonism. [52] Difficult to establish standard cut-off.
	Blood	SIMOA	Correlations are moderate or even modest with CSF NFL. [48]
miRNAs	Blood (cell-free or within extracellular vesicles)	Transcriptomic studies	Distinct miRNA signatures have been found in the CSF of early [55] as well as more advanced PD stages, differentiating them from HC and atypical parkinsonism [56,57]. Still need for standardized methodological approaches. Inconsistent results between miRNA studies.
β–glucocerebrosidase	CSF	Artificial fluorescent substrates combined with in vitro systems. In situ GCase activity (cell culture, PBMC) Dry blood spot assay.	Reduced activity in PD vs. HC.

CSF = cerebrospinal fluid; ELISA = enzyme-linked immunosorbent assay; HC = healthy controls; iRBD = idiopathic REM sleep behaviour disorder; o-α-syn = oligomeric α-synuclein; p-α-syn = phosphorylated α-synuclein; PD = Parkinson's disease; PMCA = protein misfolding cyclic amplification; RT-QuIC = real-time quaking-induced conversion; SAAs = seed~~ing~~ amplification assays; SIMOA = single molecule array; t-α-syn = total α-synuclein.

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Figure(s)

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