



We cannot exclude the possibility that some of the patients in our cohort may have had MSA or, conversely, that some PD patients had been erroneously misdiagnosed as MSA and therefore excluded from this cohort as a result of early autonomic dysfunction. However, a review of our data showed that 37/104 (35.6%) of the patients included in our cohort had early autonomic impairment, suggesting that the latter possibility, if not fully ruled out, is improbable.

We do agree that autonomic dysfunction represents one of “the hidden game changers in PD” because it is an underrecognized and, therefore, undertreated source of PD disability, particularly orthostatic hypotension (OH). For instance, a phase III, randomized, placebo-controlled, double-blind clinical trial of 224 nondemented PD patients with OH showed a reduction in the rate of falls by 295% during an 8-week observational period in those receiving droxidopa compared to placebo.² In a chart review of 316 PD patients, we found that OH increased health care utilization independently from age, disease duration, motor severity, dopaminergic treatment, and cognitive function.³ Importantly, even “asymptomatic” OH (ie, not endorsing postural lightheadedness) yields similar impairment in quality of life and rate of falls than symptomatic OH,⁴ suggesting that traditional screening questionnaires may not be sufficient to uncover this treatable complication.

Multiple studies, including the recent publication from De Pablo-Fernandez and colleagues titled “Association of Autonomic Dysfunction With Disease Progression and Survival in Parkinson Disease,” have shown that autonomic dysfunction may affect patients with PD from the very early stages or even in premotor stages.⁵ In that publication, the authors further noted that the earlier appearance of autonomic dysfunction increased the risk of reaching milestones of PD disability and shortened survival despite the lack of correlation with α -synuclein pathologic staging. Although confirming the key role of dysautonomia as a determinant of functional disability in PD, these data suggest that our current pathological model fails to recapitulate the complexity of autonomic dysfunction involved in the dysautonomia of MSA and PD.

In conclusion, we agree with the central premise from De Pablo-Fernandez and Warner that “the possibility of either clinical misdiagnosis or inappropriate exclusion of patients must be considered in design and data interpretation, as it may lead to potential bias affecting the conclusions of the study.” We also advocate for the institution of a cohort employing comprehensive autonomic assessments as well as skin biopsies, peripheral biomarkers, and autopsy material to assist the next generation of biomarker development efforts and, ultimately, ushering the era of precision medicine in PD.

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Data Access and Responsibility Statement

A. Merola and A. Romagnolo had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. ■

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References

1. Merola A, Romagnolo A, Rosso M, et al. Autonomic dysfunction in Parkinson’s disease: a prospective cohort study. *Mov Disord* 2018;33:391-397.
2. Hauser RA, Heritier S, Rowse GJ, Hewitt LA, Isaacson SH. Droxidopa and reduced falls in a trial of Parkinson disease patients with neurogenic orthostatic hypotension. *Clin Neuropharmacol* 2016;39:220-226.
3. Merola A, Sawyer RP, Artusi CA, et al. Orthostatic hypotension in Parkinson disease: impact on health care utilization. *Parkinsonism Relat Disord* 2018;47:45-49.
4. Merola A, Romagnolo A, Rosso M, et al. Orthostatic hypotension in Parkinson’s disease: does it matter if asymptomatic? *Parkinsonism Relat Disord* 2016;33:65-71.
5. De Pablo-Fernandez E, Tur C, Revesz T, Lees AJ, Holton JL, Warner TT. Association of autonomic dysfunction with disease progression and survival in Parkinson disease. *JAMA Neurol* 2017; 74:970-976.

Detection of α -Synuclein in Saliva: The Importance of Preanalytical Assessment

We read with great interest the paper by Goldman and colleagues¹ regarding the detection of α -synuclein (α -syn), tau, and A_{beta}-42 in different biological fluids from the Bio-Find cohort. To improve early diagnosis and monitor future disease-modifying therapies, a reliable and easily accessible biomarker for Parkinson’s disease (PD) is an important challenge. To compare different biological fluids in the same patient could also bring new knowledge on neurodegenerative mechanisms underlying PD.

Saliva is a promising biomarker’s source for its easy and noninvasive collection method. Despite previous studies showing reduced total α -syn concentration in the saliva of

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PD patients in comparison to healthy controls,²⁻⁴ Goldman and colleagues¹ found no significant differences. It is important to note that the authors analyzed the saliva of a small cohort of PD patients and healthy controls. Moreover, some important methodological issues could explain the conflicting results or at least should be considered when these results are compared with those of previous studies. In this respect, we address 3 preanalytical aspects that are worth considering in future analysis of salivary α -syn.

- 1. Collection and storage.** Protocols for collection and storage of salivary samples are not described by the authors.¹ Saliva contains different proteins and electrolytes, including enzymes that could affect the detection of α -syn. In previous studies,²⁻⁴ protease inhibitors have been added to each salivary sample after collection and before assay procedures. Saliva could also contain cells' membrane fragments and desquaming epithelial cells. It is well known that α -syn has a high propensity to bind lipid membranes.⁵ Therefore, the presence of epithelial cells could affect the concentration of free α -syn detectable by enzyme linked immunosorbent assay (ELISA). To avoid these difficulties, in previous studies²⁻⁴ saliva has been centrifugated after collection and only the supernatant has been stored and submitted to analytical assays. The storage temperature and pH are other crucial issues to take into account because in vitro α -syn aggregation or disaggregation could undergo in samples stored for long time at +4 °C or -20 °C.⁶ In vitro modifications, in turn, could affect the ELISA detection of α -syn, interfering with the free protein available to antibody binding.
- 2. Total protein concentration in saliva.** Protein concentration in saliva may vary during different times of the day, according to the salivary secretion rate. Furthermore, in PD patients, salivary secretion varies in the different stages of the disease, perhaps affecting biochemical composition of saliva⁷ and producing a bias in α -syn quantification, especially when the size of the samples analyzed is small.¹ For this reason, preassay measurement of total salivary protein concentration should be provided. Concentration of α -syn should be normalized in each sample with total protein concentration,^{2,3} or each sample should be prediluted before assay procedures in accordance to its total protein concentration.⁴
- 3. Blood contamination.** Hong and colleagues⁸ previously reported that α -syn in blood resided predominantly in red blood cells (>95%) followed by platelets (1%-4%), indicating that variations in blood contamination could have a significant

effect on α -syn levels. In CSF, α -syn levels increased substantially when the hemoglobin level was >200 ng/ml, whereas when it was <200 ng/ml, the effects on α -syn levels were negligible.⁸ In accordance, Goldman and colleagues¹ found a positive correlation between levels of hemoglobin and α -syn in saliva, but they excluded from the analysis only samples with a concentration of hemoglobin higher than 1200 ng/ml. The contribution of blood-derived α -syn should always be addressed in all biological fluids analyzed and more restrictive inclusion criteria for blood-contaminated samples should be recommended⁸ to prevent the possible overestimation of salivary α -syn concentration.

Besides these crucial preanalytical assessments, as a result of the heterogeneity of α -syn aggregation, an intercohort variability is expected. ELISA, indeed, as an immunoenzymatic assay, could be affected by the epitope masking as a result of the formation of α -syn aggregates. Further studies are needed to design new methods to detect α -syn aggregates in biological fluids. ■

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References

- Goldman JG, Andrews H, Amara A, et al. Cerebrospinal fluid, plasma, and saliva in the biofind study: relationships among biomarkers and Parkinson's disease features. *Mov Disord* 2018;33:282-288.
- Devic I, Hwang H, Edgar JS, et al. Salivary alpha-synuclein and DJ1: potential biomarkers for Parkinson's disease. *Brain* 2011;134:e178. <https://doi.org/10.1093/brain/awr015>
- Al-Nimer MS, Mshat SF, Abdulla HI. Saliva a-synuclein and high extinction coefficient protein: a novel approach in assessment biomarkers of Parkinson's disease. *N Am J Med Sci* 2014;6(12):633-637.
- Vivacqua G, Latorre A, Suppa A, et al. Salivary total and oligomeric alpha-synuclein in Parkinson's disease. *PLoS ONE* 2016;11:e0151156.
- Davidson WS, Jonas A, Clayton DF, et al. Stabilization of alpha-synuclein secondary structure upon binding to synthetic membranes. *J Biol Chem* 1998;273:9443-9449.
- Volpicelli-Daley LA, Luk KC, Lee VM. Addition of exogenous α -synuclein preformed fibrils to primary neuronal cultures to seed recruitment of endogenous α -synuclein to Lewy body and Lewy neurite-like aggregates. *Nat Protoc* 2014;9:2135-2146.
- Tumilasci OR, Cersósimo MG, Belforte JE, et al. Quantitative study of salivary secretion in Parkinson's disease. *Mov Disord* 2006;21:660-667.
- Hong Z, Shi M, Chung KA, et al. DJ-1 and alpha-synuclein in human cerebrospinal fluid as biomarkers of Parkinson's disease. *Brain* 2010;133:713-726.