

α -Synuclein Seeding Amplification Assay: A Breakthrough in Diagnosing Parkinson Disease?

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Research over the last several decades implicates brain accumulations of misfolded proteins as a primary pathologic feature of neurodegenerative diseases. These brain deposits can now be interrogated *in vivo* in some neurodegenerative diseases using PET imaging or fluid biomarkers (1). In our diagnostic algorithms for clinical research trials, these new biomarkers are generating a nosologic shift that may improve the accuracy and timeliness of diagnosis and provide more consistency for monitoring effects of treatment (2). The conceptual framework for diagnosis and monitoring of neurodegenerative disorders is undergoing a revolutionary paradigm shift—from diagnoses based on clinical signs and symptoms described by eponymic syndromes to a definition of disease guided by a biologic understanding of the primary pathophysiology as demonstrated by highly specific biomarkers.

A recent example of this trend is the integration of imaging and nonimaging biomarkers of Alzheimer disease into clinical treatment trials. PET imaging biomarkers for amyloid β and tau now serve important roles in enrolling cohorts for trials of amyloid-targeting treatments, as well as in detecting changes in amyloid or tau burden in the brain as a measure of drug target engagement. The amyloid/tau/neurodegeneration system (3) is based on the presence and timing of biomarkers over the course of disease rather than strictly the severity of cognitive impairment for diagnosing and staging Alzheimer disease. Most important, a diagnosis can be made very early, when the impact of a disease-modifying treatment may be greatest, even before the manifestation of common clinical symptoms.

Clinical researchers on Parkinson disease (PD) now have access to biomarkers of the primary disease process, α -synuclein (a-syn) brain deposition in the form of Lewy bodies. The systematic spread of Lewy bodies in brain deduced from cross-sectional post-mortem data focuses the field on targeting a-syn for therapies that may potentially slow disease progression. This offers the challenge and the opportunity to make an earlier, more accurate diagnosis for timely management with therapies that target a-syn.

It is now logistically feasible to assay *in vivo* the presence of brain a-syn. Recent work has shown that the method referred to as the a-syn seeding amplification assay (SAA) provides a highly sensitive and accurate test for synucleinopathies (4,5). The test takes advantage of the characteristic property of misfolded a-syn

fibrils—promotion of misfolding of the normal protein. These misfolded a-syn fibrils spread along specific neural networks to other neurons, moving across synapses. This is a process thought to occur *in vivo* as the mechanism for spreading of Lewy bodies over the course of disease progression. When a small sample of cerebrospinal fluid is coincubated with normally folded recombinant a-syn, the latter misfolds, creating more aberrant protein that goes on in serial fashion to amplify the assay to the point that it may be detectable through standard histochemical techniques. SAAs have also been pursued with serum and tissue, most notably skin biopsy, with the presence of small amounts of a-syn being found in PD patients (6). These latter approaches are not as sensitive as the cerebrospinal fluid but are improving and moving toward the goal of having a simple assay that may provide binary information or even semiquantitative data regarding the a-syn status of the patient.

Preliminary data on a large PD patient cohort ($n = 1,123$) with cerebrospinal fluid samples from the Parkinson Disease Progression Marker Initiative evaluated a-syn SAA at baseline presentation of idiopathic PD participants ($n = 545$), healthy controls ($n = 173$), participants with clinical signs or symptoms but without evidence of dopaminergic deficits ($n = 54$), participants with prodromal PD based on either rapid-eye-movement sleep behavior disorder or olfactory impairment ($n = 51$), and nonmanifesting genetic carriers of LRRK2 or GBA ($n = 310$) (7). The sensitivity for detection of clinically well-characterized PD was 88%, whereas specificity was 96% for healthy controls. When evaluated in PD participants with an olfactory deficit, a-syn SAA was positive in 98.6%. In the prodromal cohort, rapid-eye-movement sleep behavior disorder or hyposmic/anosmic participants were positive in 86% of cases. Within the prodromal group, there were subjects who were a-syn SAA-positive and dopamine transporter imaging-negative but not a-syn SAA-negative and dopamine transporter imaging-positive. This finding suggests that the a-syn SAA may be more sensitive than dopaminergic imaging with ^{123}I -ioflupane SPECT and is consistent with the fact that motor symptoms do not appear until the nigrostriatal pathways are affected by Braak stage 3, well after the process of Lewy body formation has begun (8).

The rapid development of a-syn SAAs has motivated groups to develop biologically based diagnostic schemes. Höglinger et al., in preliminary work, propose a system based on defined gene variants, a-syn pathology, and imaging evidence of neurodegeneration, with the associated clinical syndrome defined by ranked, high-specificity features (9).

In fall 2022, a working group was formed to incorporate these advances in a paradigm-shifting, biologically based, and functionally relevant staging of PD. This group includes academic physicians

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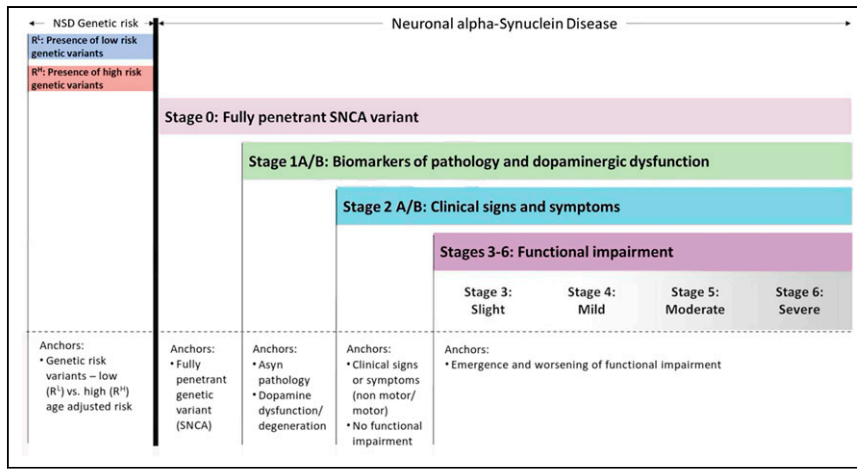


FIGURE 1. Schematic of neuronal NSD-ISS, biologically rooted, evidence-based hybrid staging schema for clinical research on neuronal synucleinopathies, PD, and Lewy body dementia. (Reprinted with permission of (11).)

and movement disorder specialists, basic scientists, pharmaceutical company scientists, regulatory scientists, PD patients, and nonprofits such as the Critical Paths Institute under the organizational umbrella of the Michael J. Fox Foundation. Interest in such a staging system comes from, first, the immediate clinical trial requirement for testing PD as early in the course as possible, even before motor manifestations required of the current clinically based diagnosis; second, a lack of evidence-based measures of progressing functional impairment with more specificity than Hoehn–Yahr staging for a more patient-relevant and standardized way to assess the impact of novel, disease-targeting therapeutics on longitudinal evaluation of clinical trial participants; and third, more accurate prognoses for patients and caretakers, despite phenotypic heterogeneity.

The conceptual framework keys off the premise that neuronal a-syn pathology and dopaminergic dysfunction measured with biomarkers are the sine qua non of PD diagnosis and that the functional impact of symptoms is more important than taxonomic symptom classification per se to patients, their caregivers, and regulatory authorities. These considerations resulted in an initial iteration of the neuronal a-syn disease integrated staging system (NSD-ISS). The NSD-ISS is a hybrid system of biologically based diagnosis and functional staging that is designed to reflect the current state of the art in understanding of the pathophysiology of neuronal a-syn disease, which includes PD and Lewy body dementia. The other disorder associated with a-syn is multiple-system atrophy, which features glial cell involvement as its primary pathology and hence is excluded from this classification and staging (10). This conceptual schema is described in Figure 1.

Briefly, there are 6 stages. Stage 1 is a-syn pathology and dopaminergic dysfunction demonstrated by a-syn SAA and ¹²³I-ioflupane SPECT, respectively, without any clinical signs or symptoms. At stage 2, clinical signs and symptoms are evident but cause no functional impairment. At stage 3, there is some slight functional impairment, which progresses to mild in stage 4, moderate in stage 5, and finally severe in stage 6. Both biologic and functional stages are evidence-based. The particular biomarkers for the targeted pathology—indeed, even the pathologic target—may change as new information becomes available.

Although offering some advantages, the NSD-ISS does represent a paradigm shift that will challenge some individuals. The

diagnosis of PD in the absence of motor symptoms may feel counterintuitive to some clinicians and patients. The bundling of PD and Lewy body dementia under one classification schema will be challenging to others. The fact is that the terms *PD* and *Lewy body dementia* will still be used referring to patients; the NSD-ISS parses along lines of biology, which in the end is critical to drug development.

These are research staging criteria currently, but it is conceivable that biologically based phenotype classification may have wider utility for clinical purposes such as ensuring the appropriateness of a treatment or offering more accurate prognoses that allow care providers to better plan resources and families to better manage expectations. The consequence of these developments for the nuclear medicine community may also be profound, with the high sensitivity of scintigraphy, the information about spatial extent in the brain, and the possibility of quantification being critical aids in the effort to slow progression or even prevent clinical expression of these α-synucleinopathies.

DISCLOSURE

No potential conflict of interest relevant to this article was reported.

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