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VIEWPOINT

Biomarker-Based Approach to α-Synucleinopathies: Lessons from Neuropathology

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Recently, proposals have attempted to reclassify Lewy body diseases in vivo by merging the long-established clinicopathological entities of Parkinson's disease (PD), Parkinson's disease dementia (PDD), and dementia with Lewy bodies (DLB), and some also to include rapid eye movement (REM)-sleep behavior disorder (RBD). A position paper[1] and a personal view paper[2] proposed biomarker-based staging and classification of these conditions. As both papers emphasize,[1,2] clinical diagnosis has challenges and limitations including early diagnosis that reflects the pathogenesis and clinical staging hindered by disease heterogeneity even within the same proteinopathy types. The suggested staging system is founded on the idea that diagnoses should rely on biomarkers, independent of the clinical syndrome, and the term neuronal α-synuclein disease (NSD) is proposed to redefine these conditions.[1] Together with the paper on biological classification[2] it was proposed that the detection of α-synuclein in cerebrospinal fluid (CSF) or skin and dopaminergic dysfunction assessed via positron emission tomography (PET) or single photon emission computed tomography (SPECT) possess the necessary sensitivity and specificity to identify the gold standard neuropathological alterations associated with Lewy body diseases.[1,2] We welcome these initial attempts to redefine these diseases incorporating biological constructs, particularly for the early and in vivo diagnosis of these disorders. Imaging and biofluid biomarkers for neurodegeneration have potential advantages in assessing disease presence and progression during life and have made major contributions in the research setting, most notably in enriching clinical trials for Alzheimer's disease (AD). Biology-based disease definition and classification has always been a central focus of neuropathology. As noted in the original publications,[1,2] this initial research framework will require much work to fill gaps in technology and knowledge, validate, and improve as we attempt transition from research biomarkers to disease surrogates. For example, currently α-synuclein seeding amplification assays (SAA) lack sensitivity and specificity for brain region and cell type, features known from neuropathology to be critically important to clinical outcomes. Our goal is to share the collective experience of our international group of neuropathology experts by suggesting future research priorities to further improve the proposed research frameworks.

The first description of Lewy bodies detectable on hematoxylin and eosin staining[3] and glial cytoplasmic inclusions in multiple system atrophy (MSA), observed first using Gallyas silver staining[4] was followed by the discovery of the central role of α-synuclein, which linked these diseases together as α-synucleinopathies.[5] Application of various anti-α-synuclein antibodies used in immunohistochemistry[6-8] revealed a wide range of cytopathologies beyond the classical Lewy bodies, diffusely distributed in neuronal processes and the perikarya, and beyond that, in astrocytes[9-12] and oligodendroglia.[11,13] In MSA, the pathognomonic glial cytoplasmic inclusions ("Papp-Lantos bodies")[4] are accompanied by neuronal cytoplasmic and nuclear inclusions.[14] New subtypes of MSA, where neuronal α-synuclein pathology in the limbic system is a predominant feature, have also been recognized.[15,16]

Several genes associated with the clinical features of PD are unaccompanied by Lewy bodies on neuropathological examination. Other, not PD-related mutations and genetic conditions, including those in *PRNP* (ie, genetic prion disease),[17] *APP*,[18] *PSEN* 1, *PSEN* 2,[19] and trisomy 21[20] (ie, AD-related neuropathology), infantile neurodegenerative disorders,[21] or neurodegeneration with brain iron accumulation,[22] can also show Lewy body or other types of α-synuclein pathology.

From a neuropathological viewpoint, two major categories of conditions with α-synuclein pathology can be distinguished: those where α-synuclein pathology is consistently versus inconsistently detected (Table 1). One of the latter conditions is AD. The combination of tau and α-synuclein pathology can be a foundation for disease diversity, for example, in DLB,[23] but also in AD, and that along with other factors (genetic, environmental, etc) might reflect many distinct "biological" associations, warranting unique approaches to clinical diagnosis, prevention, and therapy to aid in precision medicine. Indeed, individuals with AD and α-synuclein pathology might not necessarily have dopaminergic alterations. Even within parkinsonian disorders, many concomitant clinicopathological diagnoses exist.[24]

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TABLE 1 *Conditions with α-synuclein pathology with or without SAA examinations of CSF or skin*

	Seeding assay	
	CSF	**Skin**
α-Synuclein pathology consistently detected		
Cellular distribution: neuronal >> astroglial >> oligodendroglial		
I) PD or PDD		
• Sporadic PD	+	+
• Genetic PD (*SNCA* mutations)	+	n/a
• Genetic PD (certain genes)	+	n/a
• Incidental/preclinical/subclinical/premotor PD	−+	n/a
II) DLB		
• Sporadic DLB	+	+
III) Other LBD		
• "Amygdala-only" α-synuclein pathology	−+	n/a
• "Olfactory-only" α-synuclein pathology	−+	n/a
• "Periphery-only" α-synuclein pathology*	n/a	n/a
• Lewy body dysphagia	n/a	n/a
• REM sleep behavior disorder	+*	+*
• Some cases with pure autonomic failure	+	n/a
• Neuropathology confirmed brainstem only LBD	−+	n/a
Cellular distribution: oligodendroglial >> neuronal		
MSA		
I) Striatonigral degeneration (MSA-P)	+	+
II) Olivopontocerebellar atrophy (MSA-C)	+	+
III) aMSA (FTLD-synuclein)**	n/a	n/a
α-Synuclein pathology not consistently detected		
Cellular distribution: mostly neuronal		
I) Brain ageing	n/a	n/a
II) Alzheimer's disease	−+	n/a
III) NBIA and neurometabolic disorders	n/a	n/a
III) Certain gene mutations (including *LRRK2*)	+ (*LRRK2*)	n/a

Note that results for MSA-P and MSA-C are not reported separately in the literature. Of note, skin has been used in immunofluorescence studies aimed at identifying α-synuclein pathology. However, we would like to emphasize the importance of expanding SAA studies using skin, which is already underway, to broaden the scope and reach of SAA testing.

SAA, seeding amplification assay; CSF, cerebrospinal fluid; PD, Parkinson's disease; PDD, Parkinson's disease with dementia; +, examination performed and positive results; n/a, data not available; −+, examination performed but low sensitivity; DLB, dementia with Lewy bodies; LBD, Lewy body disorders; REM, rapid eye movement; MSA, multiple system atrophy; MSA-P, multiple system atrophy with parkinsonism; MSA-C, multiple system atrophy with cerebellar; aMSA, atypical MSA; FTLD, frontotemporal lobar degeneration; NBIA, neurodegeneration with brain iron accumulation.

*Need to be confirmed in autopsy-based studies.

**Neuronal α-synuclein pathology predominates in the limbic system.

Novel approaches to diagnosis of α-synucleinopathy using peripheral tissues have emerged, but important caveats remain. α-Synuclein pathology is found in peripheral organs, which have been implicated as a site of initiation.[25] Because some studies did not find α-synuclein pathology in the periphery without brain pathology,[26-28] in contrast to another study,[29] current autopsy studies do not consistently support this position. Studies on peripheral organs may show variable results depending on the sampling, the processing methods, antibodies used, and

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type of cohorts evaluated, including whether autopsy confirmation was included. Furthermore, the clinical implications of α-synuclein detected in nasal swab[30] or postmortem in the retina[31] need to be explored.

α-Synuclein SAA has been established not only in CSF, but also in peripheral tissues such as skin. Meta-analysis on studies, most of which did not include autopsy confirmation, have shown that the pooled sensitivity and specificity to differentiate α-synucleinopathies from controls using CSF samples and SAA is high, and that overall the biological samples tested to date, the CSF and skin α-synuclein SAA have demonstrated the best diagnostic performance.[32-34] Current CSF SAA show that α-synuclein aggregates are detectable only after α-synuclein pathology reaches a threshold in the brain. In particular, SAA CSF is less sensitive to detect α-synuclein pathology restricted to the brainstem or amygdala.[35-38] Furthermore, several conditions with α-synuclein pathology defined by neuropathology are yet to be examined using in vivo α-synuclein SAA biomarkers and the most reliable method needs to be validated (Table 1).

Here, we highlight several open tasks where involvement of neuropathology can facilitate understanding the reliability of biomarkers:

- Caution is needed to consider mere detection of seeding of misfolded α-synuclein in bodily fluids or peripheral tissue as α-synucleinopathy reflecting a brain disease. Therefore, a descriptive (presence or absence of α-synuclein seeding) approach is recommended because it is not necessarily associated with neuronal degeneration and disease. For example, misfolded prion protein was reported only in the appendix in individuals who then did not develop lethal brain disease.[39] Longitudinal autopsy studies are necessary to determine whether stage 1 of NSD (ie, "S(+)D(−)")[1] or Parkinson's type synucleinopathy (ie, "G(−)S(+)N(−)")[2] represent the universal first stage of brain disease. It remains unclear whether the sensitivity of the assay for low amounts of seeds might reveal a transient event, which is corrected by a functional protein clearance system hindering the pathology to progress in the brain. Furthermore, it cannot be excluded that the proteins present in the CSF (ie, inflammatory or other neurodegenerative-related proteins) can alter the outcome of the SAA, in particular that other proteinopathies co-exist frequently in LBD.[40] The reporting of seeding of misfolded α-synuclein without immunohistochemically detectable α-synuclein pathology[41] warrants further investigation as well. A solution to validate the state represented by a given biomarker would be the use of additional biomarkers,[42] ideally also confirmed by autopsy studies.
- Despite identical protofilament folds of α-synuclein,[43] lumping clinicopathological entities based on the presence of α-synuclein seeding alone would not decrease heterogeneity, but rather would cause misinterpretations. Neuropathology experts prioritize building classification schemes based on well-defined subtypes reflecting not only the pathoanatomical pattern, but also the co-pathologies that all might have an effect on seeding capacities.[44,45] We recommend focusing on how biomarkers might distinguish clinicopathological conditions, including AD with α-synuclein pathology, showing different disease course and prognosis. Autopsy materials are ideally suited to facilitate developing of SAA protocols to distinguish different "strains" of α-synuclein.[46]
- Indeed, some specialists advocate that Lewy body disease should be viewed as a single encompassing disease entity where phenotypic variance is caused by the presence of individual risk factors, disease mechanisms, and co-pathologies.[47] Further levels of classification will need to integrate the strain concept supported by distinct α-synuclein folds in Lewy body disease versus MSA.[48] These have relevance for SAAs based on reports claiming its ability to discriminate between samples of CSF from patients diagnosed with PD versus MSA.[49] Indeed, studies using human brain samples have demonstrated the physicochemical factors governing the in vitro amplification of α-synuclein to generate strain-specific reaction buffers.[46]
- Differences in seeding from the same diseases associated with distinct proteomic profiles suggest that distinct molecular populations of α-synuclein may contribute to heterogeneity in phenotypes and progression rates.[44] This may reflect the molecular behavior of α-synuclein associated with differing prognoses (ie, duration of illness)[44] and not necessarily the pathological stage (ie, Braak stage[50] or other systems[51]) of protein deposition in the brain, warranting novel approaches to standardized quantification and interpretation.[52] These concepts will need to be integrated into the interpretation of SAA results supporting better stratification of patients.
- Most of the PET or SPECT imaging meta-analyses focused on the distinction between PD and atypical parkinsonisms.[53,54] DLB and AD with Lewy body co-pathology and cognitive predominant clinical symptoms may be associated with positive α-synuclein SAA without dopamine transporter imaging suggesting dopaminergic neurodegeneration. In combined disease forms (eg, AD with LBD) the disease pathogenesis and therapy approach might be very different.[55,56] Indeed, recent SAA studies address this issue by combining AD biomarkers for better stratification.[57-60] The addition of molecular imaging would also help with more

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quantitative approaches of assessing α-synuclein pathology burden.

- SAA meta-analyses focused on examining the sensitivity and the specificity of SAA in differentiating PD from controls.[32-34] Recent studies evaluated other conditions and show that α-synuclein assay can be useful in the diagnosis of parkinsonian disorders.[61] Although this is helpful in most of the cases, in addition to AD, many atypical parkinsonism forms harbor LBD as co-pathology raising caution to attribute the clinical syndrome purely to α-synuclein disease. This well-known neuropathology observation is less integrated into the clinical practice, although it is promising that α-synuclein SAA positivity is increasingly reported in various non-PD clinical cohorts[62,63] interpreted as concomitant proteinopathy. The presence of α-synuclein SAA positivity in the CSF in MSA[61] could also lead to false interpretation as NSD[1] or Parkinson's type α-synucleinopathy[2] unless the SAA specifically detects MSA-associated α-synuclein, or the SAA is complemented by other tests such as neurofilament light chain.[2,64,65]
- Additional effort is needed to incorporate pathways driving the disease in inherited parkinsonism lacking α-synuclein inclusions. Encouraging neuropathological examination for these conditions can aid in translating pathogenic markers into in vivo biomarkers.
- Most reported cohorts used as basis for the proposals do not contain the full spectrum and severity of disease/s; therefore, expanding the cohorts to additional community-based studies reflecting diversity (ie, epidemiologic neuropathology), and prioritizing full body autopsy, would be of high value. Caution is needed before concluding that CSF SAA positivity is the first detectable clinical stage of Lewy body diseases, because an insufficient number of SAA studies have been completed on skin or several gastrointestinal tract sites. Therefore, further studies[35,36,66] are needed to correlate CSF/blood/skin α-synuclein SAA positivity with neuropathological deposition patterns in the brain and periphery.

The integration of blood-based molecular findings[42] complemented by tissue-based bulk, single cell,[67] spatial transcriptomics,[68] proteomics,[44] imaging to detect α-synuclein in vivo,[69] genetic studies[70] reflecting pathogenic scenarios of disease, observations on the spectrum of biochemical modifications[71] of the protein used as the marker of pathology (ie, α-synuclein), and anatomical overlap with other proteins[40] with potential effect on seeding[45] will pave the path for stratified medicine. Artificial intelligence-based methods for the image analysis[72] or for the detection of novel histological subtypes of disease[73] with clinical relevance are also expected to expand.

We believe that these tasks need to be addressed with harmonized SAA methods and human tissue-based studies with expanded expertise, for example, as performed for the validation and the Food and Drug Administration approval of the amyloid PET tracers.[74] Although biomarkers have already contributed to clinical trial successes, there is much research to be done to ensure their appropriate and effective use in clinical settings. The neuropathology community is eager to work with our clinical and neuroimaging colleagues to achieve this important goal. ●

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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