Personal View:

Limitations of the alpha-synuclein seed amplification assay in clinical practice - understanding the pathological diversity of Parkinson's syndrome

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Recent publications have reported that alpha-synuclein seed amplification assays (α-syn-SAA) offer potential as a biomarker test for Parkinson's disease (PD) related to alpha-synuclein pathology. This has led to recommendations to integrate α-syn-SAA into new classification schemes to facilitate an earlier diagnosis of the underlying disease process, assessing the presence of alpha-synuclein pathology in prodromal (pre-diagnostic) PD [1–3]. This has led to a research based definition of Neuronal Alpha-Synucleinopathy to encompass a pathological process with clinical and preclinical phases [1–3]. A suggestion to use α-syn-SAA to guide clinical trial enrollment, particularly for trials targeted against alpha-synuclein, has also been made. However, although the α-syn-SAA is an important research tool, caution is needed in deploying this as a clinical "test" in general neurology practice.

α-syn-SAAs rely on the seeding properties of abnormal alpha-synuclein in cerebrospinal fluid or other bio-samples, including skin biopsies, and measure the kinetics of propagating alpha-synuclein aggregation. They have been developed using post-mortem brain tissue and a recent pooled meta-analysis has shown that around 88% of clinically diagnosed patients with Parkinson's disease using accepted clinical criteria have a positive SAA [4].

Evaluating the usefulness of this assay in clinical practice involves an acknowledgment of the current limitations of the published literature and the recognised uncertainty in the early diagnosis of PD. The Queen Square Brain Bank (QSBB) clinical diagnostic criteria have been widely used as operational criteria for the definition of PD in clinical research studies and trials, and have influenced general neurological practice. They were established by careful documentation of the clinical features of patients followed through their disease course, who at autopsy had pathologically defined PD with Lewy bodies and neurites [5]. Although this predated the description of alpha-synuclein as the major protein in Lewy bodies it provided a firm clinical basis for alpha-synuclein related Parkinson's disease. In sharp contrast, most of the recent papers on SAA have relied on a cross-sectional diagnosis of PD.

Carefully conducted longitudinal clinical studies have shown that around 10% of patients diagnosed with PD at their initial neurological assessment have an alternative disorder, including conditions that are more benign (essential tremor, dystonic tremor, normal ageing) and more malignant (Parkinson's Plus Syndromes - Progressive Supranuclear Palsy - PDP, Multiple System Atrophy - MSA, Corticobasal syndrome - CBS). Because a clinical diagnosis of PD can be error prone after examining a patient at a single timepoint, the QSBB criteria require following particular symptoms over time in order to make a diagnosis (e.g. persistent asymmetry, levodopa response, levodopa induced dyskinesias, prolonged disease course). Similar prospective criteria are included in the MDS diagnostic criteria for PD [6]. These clinical criteria emphasise the need to observe patients over time to improve diagnostic certainty The true diagnostic value of SAA cannot be established,

therefore,without longitudinal follow-up of patients with positive and negative SAA tests and post-mortem examination where possible.

Many SAA studies have used healthy controls unaffected by neurodegenerative disease. However, the main clinical challenge is not in distinguishing Parkinson's disease from healthy controls, but in differentiating it from MSA, PSP, CBS, normal pressure hydrocephalus and cerebrovascular disease. The potential cross-reactivity of the SAA across different neurodegenerative disease proteins needs to be clearly established in patient CSF samples and correlated with post-mortem follow-up before it can be safely used in clinical practice. The specificity of α-syn-SAA is much lower in distinguishing PD from MSA, when compared to distinguishing PD from healthy controls [4]. There will also be a significant false positive rate for α-syn-SAA (in diagnosing PD), in patients who may have alpha-synuclein co-pathology in addition to their primary diagnosis, for example the well documented occurrence at autopsy of Lewy body pathology in primary Alzheimer's disease (AD). The α-syn-SAA will enable definition of the effects of co-pathology in large patient series. With respect to asymptomatic individuals incidental Lewy body pathology occurs in 1 in 6 of the elderly population, who die without any signs of Parkinson's disease [5]. The high rates of α-syn-SAA positivity in patients with clinically diagnosed PSP, Alzheimer's disease, Guam amyotrophic lateral sclerosis and normal pressure hydrocephalus may relate to background co-pathology. In addition although reference laboratories have shown a high degree of consistency in reporting test results, as has been reported with dopamine transporter SPECT imaging in the investigation of Parkinsonism, there can be variation in reporting from different laboratories [7]. The work of Russo and colleagues, reporting the results of α-syn-SAA assays in a common set of samples in three laboratories, showed that 5/30 (17%) of the baseline assay results in clinically diagnosed PD patients, and 4/30 (13%) of the year 3 clinically defined healthy controls were inconsistent. There are therefore three possible interpretations of a reported positive α-syn-SAA assay in patients being assessed for PD: true positive with PD - the patients has Lewy body PD and a positive α-syn-SAA assay; true positive with co-pathology - the patient has a positive α-syn-SAA assay but does not currently have a clinically syndrome primarily attributable to Lewy body pathology; and false positives - the patient does not have underlying Lewy body pathology.

Why do around 10% of PD patients meeting accepted clinical criteria have a negative α-syn-SAA test? The answers may already be evident in the neuropathological and clinical literature. The 1992 study by Hughes and colleagues showed that 6% of patients diagnosed with PD in life had pathological findings compatible with PSP at post-mortem, and a recent study from the Banner Brain Bank using a community case ascertainment approach showed that about 10% of patients with a movement disorder had underlying PSP [8,9]. The 1992 QSBB work on the accuracy of diagnosis of PD was interpreted by some as indicating that British neurologists were failing to identify features of atypical Parkinsonism such as supranuclear gaze palsy, falls and early severe autonomic failure, and so were misdiagnosing

PSP and MSA as PD. An alternative explanation, however, is that some patients with PSP and MSA pathology are clinically indistinguishable from PD in the first few years after diagnosis. This possibility was supported by the subsequent recognition of PSP-parkinsonism. There are other non-alpha synuclein based causes of Parkinson's syndrome. For example, in the 1930s another tau related disorder, post-encephalitic parkinsonism (PEP) was as common as paralysis agitans / PD. Neurofibrillary tangle pathology has also been recognised as a common cause of Parkinson's syndrome in the elderly in a now frequently overlooked paper [10]. Genetic forms of Parkinson's syndrome are a further potential confounder. As highlighted in the study from Siderowf and colleagues, patients with pathogenic *LRRK2* and *PRKN* mutations appear to have a lower rate of alpha-synuclein pathology defined either by post-mortem or α-syn-SAA and this would also be anticipated for patients with *MAPT* or SCA gene mutations.

Clarifying whether PSP-P and other tauopathies explain why patients who are clinically diagnosed with PD have negative α-syn-SAA testing will require careful clinical follow-up and re-analysis of α-syn-SAA negative PD cases The recent PPMI study of α-syn-SAA showed that patients with negative α-syn-SAA testing and mutations in LRRK2 had increased unadjusted neurofilament light chain (NF-L) levels a known (although non-specific) biomarker for PSP, suggesting that some SAA negative PD patients have PSP type tau pathology.

The development of four repeat tau amplification assays and of tau and alpha-synuclein protein-based imaging, together with rapid genetic analysis of newly diagnosed cases and the use of a range of SAAs may ensure a more accurate early pathological diagnosis of Parkinson's syndrome in the future. This will encompass a recognition of the pathological diversity of Parkinson's syndrome and detailed evaluation of clinical features will be combined with multi-modal evaluation of the underlying pathology using *in vivo* biochemical assays.

However, currently it is premature to base the definition of Parkinson's disease solely on the use of α-syn–SAA. More work is needed in evaluating co-pathology, establishing the use of α-syn-SAA in diagnosing different Parkinsonian syndromes with longitudinal follow-up and in resolving the pathogenesis of α-syn–SAA negative cases. Although α-syn–SAA may provide new insights into the biology of Parkinson's syndrome and improve early patient selection for future clinical trials, we would recommend maintaining the concept of Parkinson's syndrome as the initial clinical diagnosis in patients presenting with an asymmetric akinetic-rigid syndrome with or without tremor, with definition of clinically probable Parkinson's disease depending on the follow-up features as defined in the QSBB and MDS diagnosed criteria.

Disclosure

Dr Morris is employed by UCL. In the last 24 months he reports paid consultancy from Roche, Aprinoia, AI Therapeutics and Amylyx ; lecture fees/honoraria - BMJ, Kyowa Kirin, Movement Disorders Society. Research Grants from Parkinson's UK, Cure Parkinson's Trust, PSP Association, Medical Research Council, Michael J Fox Foundation. Dr Morris is a co-applicant on a patent application related to C9ORF72 - Method for diagnosing a neurodegenerative disease (PCT/GB2012/052140)

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Search strategy and selection criteria

References for this Review were identified by searches of PubMed between February 2019 and February 2024 using the search terms "alpha-synuclein", "SNCA", "synuclein" AND "amplification", "SAA", "re-QUIC", "PMCA" and "Protein misfolding cyclic amplification". Articles were restricted to humans and English language publications.