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REVIEW ARTICLE

In vivo detection of Alzheimer's and Lewy body disease concurrence: Clinical implications and future perspectives

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Abstract

INTRODUCTION: The recent introduction of seed amplification assays (SAAs) detecting misfolded α-synuclein, a pathology-specific marker for Lewy body disease (LBD), has allowed the in vivo identification and phenotypic characterization of patients with co-occurring Alzheimer's disease (AD) and LBD since the early clinical or even preclinical stage.

METHODS: We reviewed studies with an in vivo biomarker-based diagnosis of AD-LBD copathology.

RESULTS: Studies in large cohorts of cognitively impaired individuals have shown that cerebrospinal fluid (CSF) biomarkers detect the coexistence of AD and LB pathology in approximately 20%–25% of them, independently of the primary clinical diagnosis. Compared to those with pure AD, AD-LBD patients showed worse global cognition, especially in attentive/executive and visuospatial functions, and worse motor functions. In cognitively unimpaired individuals, concurrent AD-LBD pathologies predicted longitudinal cognitive progression with faster worsening of global cognition, memory, and attentive/executive functions.

DISCUSSION: Future research studies aiming for a better precision medicine approach should develop SAAs further to reach a quantitative evaluation or staging of each underlying pathology using a single biofluid sample.

KEYWORDS
dementia, Lewy body disease, prions, real-time quaking-induced conversion, RT-QuIC, synuclein

Highlights

- α-Synuclein seed amplification assays (SAAs) provide a specific marker for Lewy body disease (LBD).
- SAAs allow for the in vivo identification of co-occurring LBD in patients with Alzheimer's disease (AD).
- AD-LBD coexist in 20-25% of cognitively impaired elderly individuals, and ∼8% of those asymptomatic.
- Compared to pure AD, AD-LBD causes a faster worsening of cognitive functions.
- AD-LBD is associated with worse attentive/executive, memory, visuospatial and motor functions.

1 | INTRODUCTION

Neurodegenerative diseases represent the primary cause of cognitive decline in the elderly population.[1,2] The accumulation of misfolded proteins is characteristic of these disorders.[3] Amyloid-β (Aβ) and phospho-tau (p-tau) deposits generating amyloid plaques and neurofibrillary tangles in Alzheimer's disease (AD) and α-synuclein (α-syn) forming Lewy bodies and dystrophic neurites in Lewy body disease (LBD) constitute the most prevalent neurodegenerative proteinopathies.[4–6] Identifying the pathological neurodegenerative process causing a cognitive decline in a living patient has relied for many years only on clinical criteria. However, neuropathological studies have highlighted the profound limitations of this approach. These studies not only underscored the wide spectrum of clinical features associated with a single pathology, especially at disease onset but also revealed the concurrence of multiple pathologies in many of these patients, thus making the need for biomarkers that identify the different proteinopathies in vivo pivotal for a precision medicine approach.

The introduction of accurate biofluid and imaging biomarkers of AD pathology, including positron emission tomography (PET) imaging and cerebrospinal fluid (CSF) detection of Aβ- and p-tau-related pathologies, has provided the first significant step in this direction, starting the era of in vivo neuropathologic assessment.[6] Later, accurate tau biomarker assays have been developed to detect tau variants in the blood that reflect Aβ and tau proteinopathies in the brain,[7] and a recent study has shown the same or even better diagnostic performance compared to clinically approved CSF tests.[8] The recent introduction of in vitro seed amplification assays (SAAs) detecting misfolded α-syn in CSF and other tissues contributed a second fundamental advance by providing a pathology-specific biomarker for LBD.[9]

Here, we review the contributions these biomarkers have given to improve clinical characterization, diagnosis, and prognostic evaluation of patients with concurrent AD and LBD pathologies (AD-LBD). After briefly reviewing the molecular pathology and staging systems of AD and LBD, the basic principles behind SAAs and CSF biomarker changes in AD and LBD, and the results of anatomic-clinical correlations in neuropathological series, we will focus on the contributions these biomarkers have given to establishing prevalence, clinical phenotype, and longitudinal trajectories of patients with AD-LBD.

2 | NEUROPATHOLOGICAL STAGING LBD AND AD

Based on the topographic distribution and progressive ascending central nervous system involvement, three stages of LBD were initially recognized: (i) brainstem predominant, (ii) limbic (or transitional), and (iii) diffuse neocortical.[10,11] In 2003, Braak and colleagues refined this staging system by hypothesizing early involvement of the olfactory bulb, the enteric nervous system, and the dorsal motor nucleus of the vagus nerve (in the medulla oblongata) (stage 1), followed by LB pathology spreading through the brainstem to the pontine tegmentum and the pars compacta of the substantia nigra (in the midbrain) (stages 2 and 3).[12] The involvement of the basal forebrain, amygdala, anteromedial temporal mesocortex (transentorhinal region), and allocortex (CA2-plexus) follows (stage 4). Then, LB pathology spreads to the neocortex, reaching the sensory association areas (stage 5) and finally the entire neocortex (stage 6).[12] However, the generalizability of Braak's staging system, initially developed for sporadic Parkinson's disease (PD), to the whole spectrum of LBD, including dementia with Lewy bodies (DLB), has been disputed.[13] Although most studies support the value of this classification, a few have reported that in some cases, the distribution of LB pathology was inconsistent with that of Braak stages mainly because of the absence of α-syn deposits in the lower brainstem despite the involvement of other more rostral areas.[14–17] In 2006, Uchikado et al. described an LBD variant characterized by prominent amygdala involvement in patients with a primary neuropathological diagnosis of AD.[18] Subsequent studies confirmed the almost exclusive association of the amygdala-predominant variant with AD or other neurodegenerative pathologies, with tau or TDP-43 deposition affecting the amygdala.[19–21] Other studies in individuals with mixed AD-LBD documented a significantly lower LB pathology burden in brains with the amygdala-predominant variant than in those following the LBD Braak staging.[18,22] Currently, the amygdala-predominant variant is

considered the main deviation from the stereotypic ascending progression described by Braak.[22–25] Accordingly, the latest proposed staging systems for LBD include the recognition of the amygdala-predominant variant (as well as an olfactory-only stage) in addition to the typical staging systems based on the ascending course from the medulla oblongata to the cerebral neocortex.[26–28]

The neuropathological hallmarks of AD are (i) senile plaques, which are extracellular deposits of Aβ peptides, and (ii) neurofibrillary degeneration related to the intraneuronal accumulation of aggregated p-tau protein.[4] Aβ and p-tau accumulations also follow a stereotyped, hierarchical, topographic progression, although not coincidental, across brain regions. Specifically, Aβ first accumulates in neocortices with a potential preference for starting in the medial frontal and parietal areas,[29] followed by the limbic regions (allocortex, hippocampus, and amygdala), deep gray nuclei, brainstem, and cerebellum.[30] In contrast, p-tau accumulation starts from the transentorhinal region and then involves the entorhinal cortex and Ammon's horn. As the disease progresses, it expands to the neocortical association areas, usually distributing first to the temporal cortex and then virtually to all isocortices,[31,32] even though at least two to four different p-tau spreading trajectories might exist.[33,34] The current National Institute on Aging–Alzheimer's Association guidelines for the neuropathologic diagnosis of AD recommend the use of these staging systems for Aβ and p-tau together with the evaluation of neuritic plaques (i.e., a subgroup of amyloid plaques surrounded by dystrophic p-tau-immunoreactive neurites) according to the Consortium to Establish a Registry for AD protocol.[35] The combined analysis of Aβ, p-tau, and neuritic plaque staging (ABC score) defines three levels of AD neuropathological changes (ADNC): low, intermediate, and high.[4] In patients with cognitive impairment, only intermediate and high ADNC levels are considered an adequate explanation of cognitive symptoms or dementia.[4]

RESEARCH IN CONTEXT

1. **Systematic review**: The co-occurrence of Alzheimer's disease (AD) and Lewy body disease (LBD), the two most common neurodegenerative disorders, is frequent in elderly individuals. Due to the lack of a specific in vivo biomarker, until recently, the identification and phenotypic characterization of patients with AD-LBD copathology has been evaluated retrospectively in *post-mortem* neuropathologic studies.
2. **Interpretation**: Seed amplification assays (SAAs) detecting misfolded α-synuclein allow for the early identification of comorbid AD-LBD patients, even in the preclinical stage. AD-LBD copathology has been associated in vivo with a characteristic neurocognitive profile and faster longitudinal, global cognitive decline.
3. **Future directions** : The assessment of multiple neurodegenerative co-pathologies in vivo will be a fundamental requirement for a precision medicine approach in patients with cognitive decline. Future research studies should develop SAAs further to reach a quantitative evaluation/staging of each underlying pathology using a single biofluid sample.

3 | CO-OCCURRENCE OF AD AND LBD: PREVALENCE AND RISK FACTORS

AD and LB pathologies are highly prevalent in cognitively impaired individuals and frequently coexist[36,37] (Figure 1). Abnormal deposition of α-syn aggregates affects 33%–66% of sporadic AD patient brains evaluated post-mortem, with similar incidences in early-onset (EO) (<65 years) and late-onset (LO) (>65 years) AD.[18,38–42] In addition to sporadic AD, LB copathology involves a noticeable proportion of the brains of individuals with dementia harboring genetic mutations associated with autosomal-dominant AD (ADAD), with significant variability reported among studies (27%–85%).[19–21,43] The small size of the ADAD cohort and individual genetic features likely contributed to this variability, as demonstrated by the greater frequency of LB pathology in *PSEN1* carriers than in *PSEN2* carriers and discordant findings between relatives.[19] Finally, LB pathology also characterizes 14-50% of patients with Down syndrome-associated AD (DSAD).[44–47] Despite the largely overlapping frequency of LB copathology among the sporadic LOAD, sporadic EOAD, ADAD, and DSAD cohorts, increasing evidence indicates that the brain regional distribution and type of LBD significantly differ between groups, mainly depending on the age of disease onset. Indeed, in LOAD brains, concurring LB pathology usually follows the classic Braak's staging system, with less than 20% of cases showing LB pathology confined to the amygdala and limbic areas.[42] In contrast, sporadic EOAD and ADAD brains show a higher proportion (between 20% and 50%) of the amygdala-predominant variant.[20,42] Similarly, LB pathology in DSAD mainly involves the limbic regions (i.e., amygdala and entorhinal cortex), and it is less pronounced or absent in the brainstem and neocortices.[46,47]

On the one hand, these findings indicate that the co-occurrence of "typical" (i.e., following the Braak staging) LBD pathology in AD patients increases with age, which is in line with the increasing prevalence of PD and DLB after 60 years.[48–50] On the other hand, the data support the idea that the ADNC burden, in particular, p-tau pathology,[40,51] which is on average more severe in EOAD and ADAD than in LOAD, is a strong determinant of α-syn accumulation in the amygdala, a vulnerable region in neurodegenerative proteinopathies.[52,53] Evidence corroborating the latter claim includes the following observations: (i) limbic α-syn aggregates can be found in other diseases with tau pathology (e.g., Pick disease, argyrophilic grain disease, amyotrophic lateral sclerosis-parkinsonism/dementia complex of Guam)[54,55]; (ii) misfolded α-syn and p-tau often colocalize in neurons in the amygdala of AD and DSAD brains[46,56,57]; (iii) misfolded p-tau can cross-seed α-syn aggregation and accelerate its propagation in vitro and animal models,[58,59] and (iv) the amygdala of AD-LBD brains shows a distinct molecular signature characterized by a higher α-syn load and

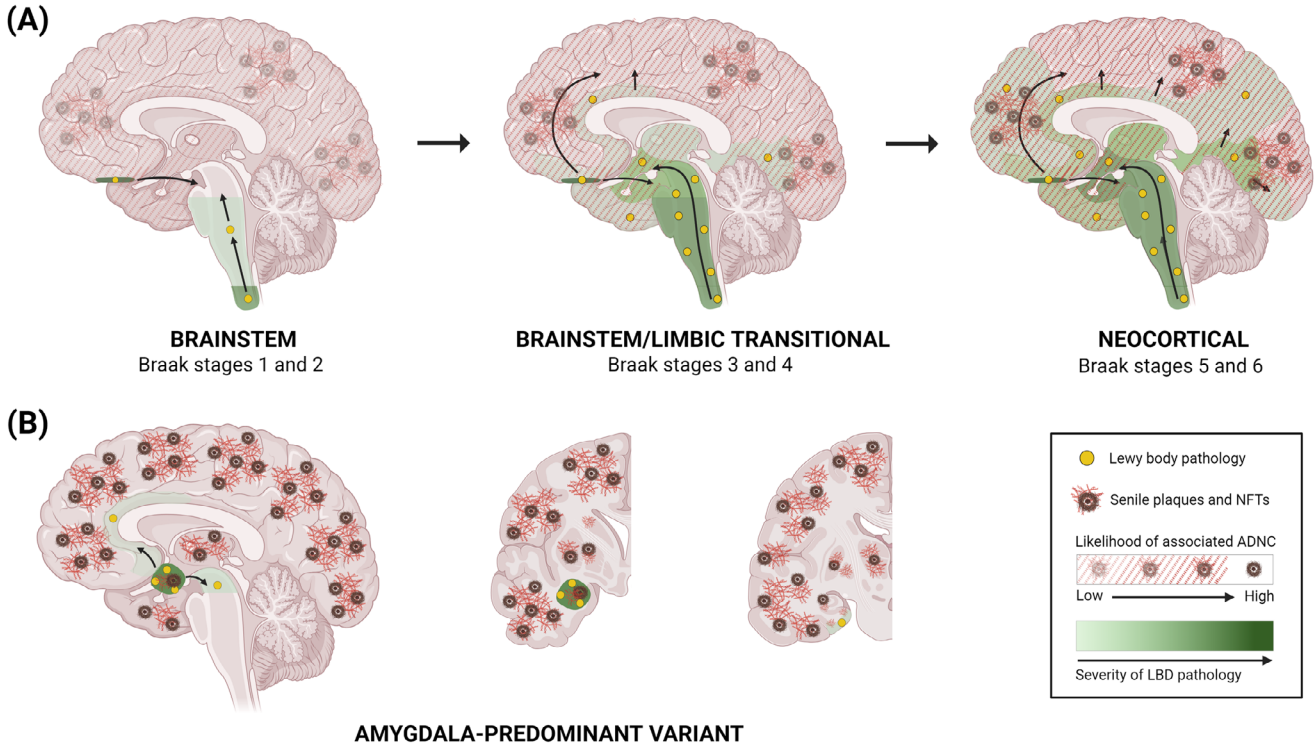

FIGURE 1 Schematic representation of LBD and AD copathology. (A) According to the McKeith/Braak staging, LB pathology initially involves the lower brainstem (left). Then, following an ascending course, it spreads to the limbic regions (middle) and eventually reaches the neocortices (right). As LBD progresses, the likelihood of coexisting AD pathology increases because of the older mean age and a likely synergistic effect between the two pathologies. (B) The amygdala-predominant variant of LBD is almost invariably associated with AD pathology. Besides the amygdala, the area showing the greatest LB pathology load in this variant, LBs can sometimes be detected in the anterior cingulate gyrus, the entorhinal cortex and the upper brainstem (limited to the midbrain). Hemibrain sagittal sections (A, B-left) and coronal sections at the level of the amygdala (B-middle) and hippocampus (B-right). AD , Alzheimer's disease; ADNC , AD neuropathologic change; LB , Lewy bodies; LBD , Lewy body disease; NFTs , neurofibrillary tangles. Figure created with BioRender.com.

increased detection of insoluble phosphorylated α-syn as compared with PD with dementia (PDD) brains lacking AD p-tau pathology.[60]

When looking at the issue of coexisting LB and AD pathology from the LBD side, individuals with a clinical diagnosis of DLB show an even greater frequency (48%–91%) of concurrent ADNC at autopsy.[40,50,61–64] However, when only patients with intermediate or high ADNC are considered, the percentage is approximately 50%.[64] In DLB patients with concurrent ADNC, there is a direct correlation between the extent of ADNC and the α-syn pathological load, with ADNC being greater in the brains of patients with neocortical LBD than in those at the limbic or brainstem stage.[50,62,64] Accordingly, ADNC is more frequent in DLB than in PDD,[64] given the lower cortical α-syn pathology detected in PDD.[65]

Several studies have investigated the factors predisposing patients to AD-LBD comorbidity, but the results are inconclusive. For example, whether the apolipoprotein E (*ApoE*) ε4 status is a specific risk factor for the coexistence of AD and LB pathologies is still debated.[42,51,65–68] Early genome-wide association studies (GWAS) in DLB found an association with *ApoE* ε4,[69,70] but did not assess the effect of concomitant AD pathology. In a subsequent GWAS study, Kaivola et al. stratified the DLB population according to the extent of AD copathology. They confirmed the association with *ApoE* ε4 only in DLB with severe or intermediate ADNC but not in pure DLB.[71] Additionally, extensive GWAS analyses in PD did not detect *ApoE*,[72] supporting further the view that the frequent concomitant AD pathology mainly drives the association between *ApoE* ε4 and DLB.

Regarding the effect of race, a study found a greater incidence of AD-LBD in black patients than in white patients,[73] a finding likely related to a setting (clinical) bias. A recent study combining multiple cohorts from different settings, including community-based cohorts, did not confirm the race difference.[74] Because of the heterogeneity of the cohorts, study settings, and pathological burdens, these results should be interpreted cautiously and need further confirmation. Finally, whether males are more susceptible to AD-LBD comorbidity than females is also uncertain.[42,67,75]

4 | EFFECT OF AD AND LBD COPATHOLOGY ON CLINICAL PHENOTYPE AND DISEASE PROGRESSION: EVIDENCE FROM CROSS-SECTIONAL *POST-MORTEM* STUDIES

Until recently, due to the lack of in vivo pathology-specific markers, especially for LBD, studies on the clinical effect of AD-LBD

copathology were limited to retrospective cross-sectional studies in individuals with a *post-mortem* neuropathologic evaluation. The clinical impact of co-occurring AD-LBD has been significantly associated with the extent and relative burden of the two proteinopathies assessed *post-mortem* (Aβ and p-tau vs. α-syn). Overall, all but one[39] studies reported an additive effect of AD-LBD copathology on the clinical phenotype,[76,77] leading to a faster worsening of cognition.[64,78–81] The effect was most evident in patients with diffuse LBD and neocortical neurofibrillary tangles compared to patients with transitional/limbic LBD (with or without neocortical tangles).[81] Similarly, patients with a primary diagnosis of AD showing a co-occurring LB pathology not fulfilling the distribution and density thresholds as defined by consensus criteria for DLB had a faster cognitive decline than typical AD patients did.[80] Coexisting AD in subjects with a primary neuropathological LBD diagnosis was also associated with reduced survival.[50,64]

Regarding the clinical phenotype, studies have shown a significant overlap in disease presentation between patients with pure and mixed AD-LBD, making it difficult to predict copathology individually.[82] Overall, the occurrence of DLB core features and, consequently, the likelihood of a clinical DLB diagnosis were more evident in pure LBD than in LBD-AD.[80,83] In the latter group, the severity of ADNC was negatively associated with the detection rate of the core features of DLB.[61,81,84] Notably, the diagnostic sensitivity for probable DLB decreased to 46% in individuals with transitional/limbic LBD and neocortical neurofibrillary tangles.[81]

Compared with the cognitive profile of pure DLB patients, characterized by prominent visuospatial, attention, and executive function impairment, patients with AD-LBD presented with more pronounced memory and language deficits, which significantly overlapped with the cognitive profile of pure AD.[79,81,84,85] In another study, the coexistence of LBD in patients with a primary AD diagnosis was associated with worse performance on trial-making test A, which assesses visual attention.[68]

Two studies reported a significant effect of concomitant LBD on motor function in individuals with a primary AD diagnosis, as demonstrated by higher Unified Parkinson's Disease Rating Scale-part III (UPDRS-III) scores.[67,68] In the study in which the LB pathology burden was assessed,[68] it was significantly associated with the UPDRS scores.

Finally, a wealth of evidence suggests that LB copathology also influences neuropsychiatric manifestations in AD.[67,68,86] Chung and colleagues reported higher Neuropsychiatric Inventory Questionnaire scores in AD-LBD patients than in pure AD patients[67]; the subitem scores were associated with a significantly greater incidence of delusions, hallucinations, aberrant motor behavior, and sleep behavior problems. Similarly, Bayram and colleagues reported increased anxiety, irritability, and appetite problems in a comorbid group.[86]

5 | BIOMARKERS TO INVESTIGATE LBD AND AD COPATHOLOGY IN VIVO

For several years, indicative LBD biomarkers were limited to those revealing downstream effects of LB pathology, including dysfunction of nigro-striatal pathway (dopamine transporter [DAT] imaging), brainstem sleep-related nuclei (polysomnography), or the autonomic nervous system (^{123}I-metaiodobenzylguanidine [MIBG] scintigraphy).[87] However, none of these markers is fully specific for LBD since they can be altered under other conditions (e.g., reduced DAT uptake in other parkinsonian syndromes; cardiac MIBG uptake influenced by medications, diabetes, etc.).[88] Additionally, the sensitivity of these biomarkers decreases significantly at the prodromal stage of the disease.[89] The development of SAAs for α-syn represented a breakthrough in the field by providing a highly specific biomarker able to detect misfolded α-syn in vivo, even in prodromal LBD conditions (isolated rapid eye movement [REM] sleep behavior disorder, isolated autonomic failure, and mild cognitive impairment [MCI]).[90] SAAs are novel, in vitro techniques allowing the ultrasensitive detection of prion-like amyloidogenic proteins, including α-syn. These methods exploit the seeded polymerization mechanisms by which these proteins propagate in the nervous system. Briefly, in SAAs, the tested biosamples (containing the misfolded protein of interest) are incubated with an excess of "native" recombinant protein and a fluorescent dye (thioflavin T [ThT]) with a high affinity for the beta-sheet structure. The interaction between the "native" recombinant protein and the misfolded isoform promotes a conformational change and aggregation of the former, ultimately forming amyloid fibrils. Since ThT binds newly formed amyloid aggregates, the reaction can be monitored by assessing ThT fluorescence emission in real-time. Intermittent shaking induces aggregate fragmentation, which promotes the formation of novel, free-reactive seeds and accelerates amyloid formation (Figure 2). α-syn-SAA has been applied successfully to CSF (the most commonly used biofluid), skin punches,[91] nasal swabs,[92] and enteric biopsies,[93] with different performances across biomatrices and the LBD spectrum. Recent studies demonstrated the possible application of SAA technology to blood, which is of great interest. Using neuron-derived extracellular vesicles as a biomatrix enriched for α-syn,[94,95] isolated from plasma, Kluge and colleagues reported a full sensitivity and specificity of the adapted assay in 30 individuals with PD and 50 controls.[96] In another study, Okozumi et al., using serum samples and a modified SAA protocol with a preliminary immunoprecipitation step, reported an over 85% accuracy in distinguishing PD from controls in both an exploratory cohort (n = 221 and n = 128, respectively) and a confirmatory-blinded cohort (n = 20 and n = 26).[97] These studies are promising but await validation by other laboratories and in larger patient cohorts.

Currently, commercial and in-laboratory-made α-syn-SAAs are available, which raises the question of their relative diagnostic value. Despite some differences in methods or data analysis, two inter-laboratory studies demonstrated a high result concordance between the three α-syn SAAs protocols initially proposed, all showing high diagnostic performance in identifying PD patients from controls.[98,99] These findings indicate that α-syn SAAs might represent a robust and reliable technology in expert hands, providing consistent results and allowing comparison between studies. However, more multisite ring trials are needed for a deeper assessment of the different assays' reliability, including patients at various LB pathology stages who are assessed neuropathologically. The most significant difference between

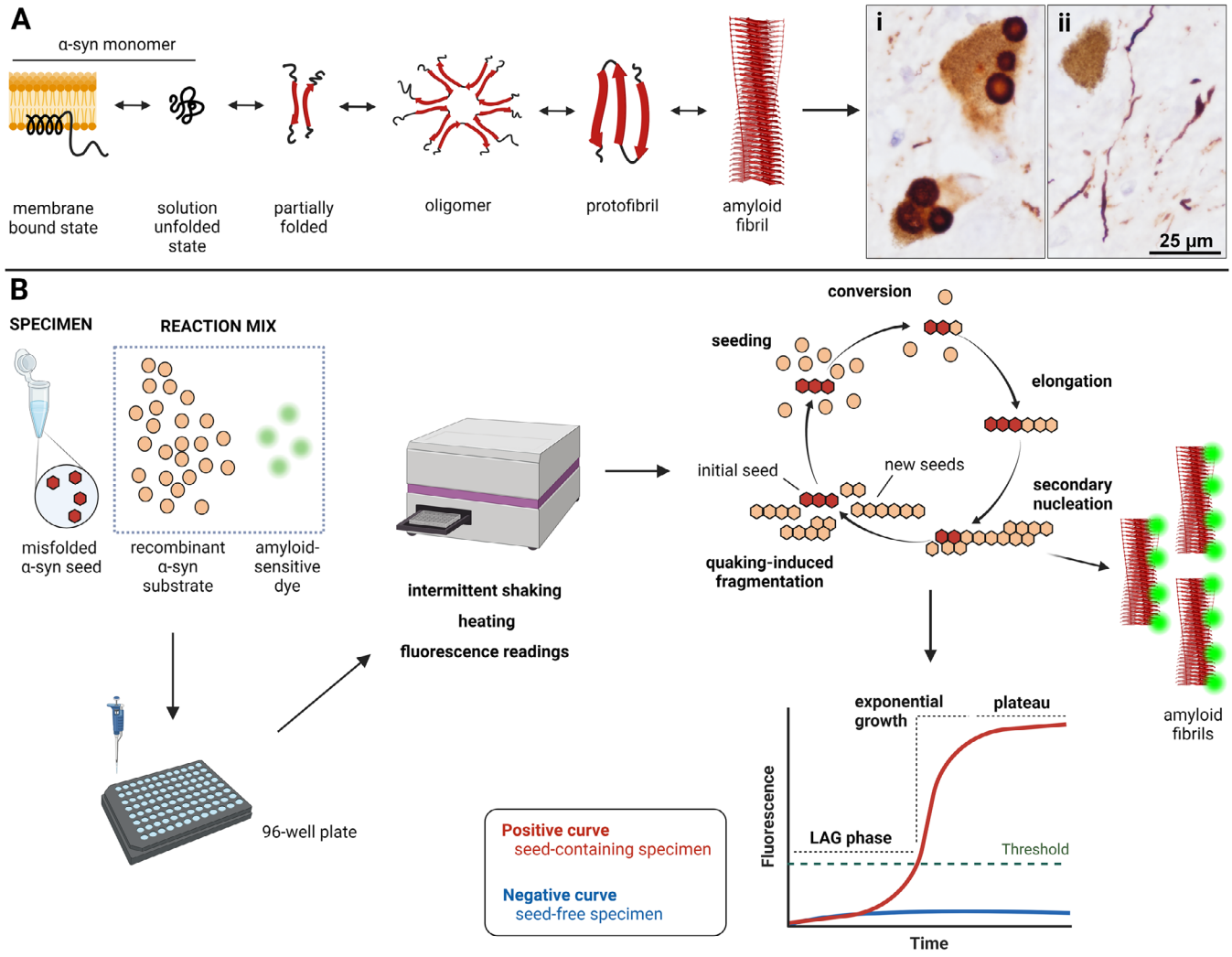

FIGURE 2 Schematic representations of the α-syn misfolding process and working principles of the α-syn SAA. (A) The α-syn monomer exists in two states: membrane-bound (partially helical conformation) and in solution (unfolded); partially folded α-syn states precede the formation of oligomers > protofibrils > fibrils with amyloidogenic properties. In the LBD, α-syn fibril aggregates accumulate as cytoplasmic neuronal inclusions in cell bodies (Lewy bodies = i) and nerve terminals (Lewy neurites = ii). (B) In the SAA reaction, the tested biosample, in which misfolded α-syn is detected, is loaded together with the reaction mixture, which includes a relatively large amount of recombinant α-syn substrate and an amyloid-sensitive dye, in a 96-well plate (usually, each sample is loaded in multiple wells). Then, the plate is incubated at a given temperature, and the samples are subjected to intermittent rest/shaking cycles. During incubation, α-syn seeds progressively elongate through recombinant substrate recruitment and conversion. The newly formed oligomers are partially fragmented by quaking, producing novel seeds, while in part, they continue to elongate to the status of protofibrils and, eventually, fibrils. By acquiring amyloid proprieties, the protofibrils/fibrils bind the amyloid-sensitive dye (thioflavin T), which produces a fluorescent signal. The fluorescent signal intensity from each well is monitored through serial readings at fixed time intervals. In the reaction's readout, fluorescence intensity (y) is plotted as a function of time (x): if the curve crosses a defined threshold (dashed line), the well is deemed "positive"; if not, it is considered "negative." α-syn, α -synuclein; LBD, Lewy body disease; SAA, seed amplification assay. Figure created with BioRender.com.

the available assays concerns their performance in multiple system atrophy (MSA), another neurodegenerative disease characterized by α-syn aggregation and tissue deposition, mainly in oligodendrocytes. Only one of the proposed α-syn SAA distinguishes between LBD and MSA based on the kinetic of fluorescence curves.[100,101] In contrast, the other SAAs show either a negative reaction, also allowing the distinction with LBD,[90] or a low rate of positive responses.[102] However, this issue is not relevant to the topic of the present review since AD and MSA association is unfrequent, and there is a predominant motor and autonomic dysfunction over cognitive impairment in MSA.

In contrast to LBD, biological markers of AD, including those measured in the CSF, have been introduced earlier[6] and already represent a standard for diagnosing AD.[103,104] The leading current approach is based on the A/T/(N) system, which provides a binary classification of biomarker results for the three pathological processes: "A" refers to biomarkers that measure Aβ deposition, "T" to those that assess p-tau accumulation, and "N" to those that assess neurodegeneration

TABLE 1 Performance of the CSF α-syn SAA according to the LBD pathology stage and timing (*ante-mortem* vs. *post-mortem*) of biofluid collection

CSF collection	*Ante-mortem*			*Post-mortem*		
Reference	[114]	[113]	Overall (by CSF collection)	[114]	[112]	Overall (by CSF collection)
N	66	59	125	30	48	78
Neocortical/limbic	44/45 (97.8)	32/32 (100)	76/77 (98.7)	19/20 (95.0)	28/29 (96.6)	47/49 (95.9)
Brainstem	na	14/23 (60.9)	14/23 (60.9)	na	2/5 (40.0)	2/5 (40.0)
Olfactory bulb only	na	na	na	na	1/3 (33.3)	1/3 (33.3)
Amygdala-predominant	3/21 (14.3)	2/4 (50.0)	5/25 (20.0)	6/10 (60.0)	7/11 (63.6)	13/21 (61.9)
Overall sensitivity	47/66 (71.2)	48/59 (81.4)	95/125 (76.0)	25/30 (83.3)	38/48 (79.2)	63/78 (80.8)

Note: The data are expressed as positive/tested (%).
Abbreviations: α-syn, α-synuclein; CSF, cerebrospinal fluid; LBD, Lewy body disease; na, not available; SAA, seed amplification assay.

or neuronal injury.[105] The positivity of the A/T categories is required for AD diagnosis, while A+ alone is considered evidence of AD pathology as part of the AD continuum.[103] The "N" category is often labeled in brackets since it is not specific to AD physiopathology. Focusing on AD core CSF biomarkers, A+ status is defined by decreased levels of Aβ42 peptide or Aβ42/Aβ40, with the ratio better correlating with the amyloid burden measured by amyloid PET or neuropathologic examination,[106,107] while T+ status is defined by increased CSF levels of distinct p-tau isoforms classified in two subgroups, T_1 including p-tau181, p-tau217, and p-tau231, and T_2 including pT205 and MTBR-243 [https://aaic.alz.org/diagnostic-criteria.asp]. Increasing evidence indicates that A/T_1 markers are helpful for diagnostic purposes, while T_2 markers are for staging and prognosis. Besides core biomarkers, the CSF N+ category is defined by abnormally elevated neurofilament light chain protein. Finally, a new category, "I" (= inflammation), characterized by the increase of glial fibrillary acid protein, a marker of astrocytic activation, has also been proposed [https://aaic.alz.org/diagnostic-criteria.asp].

6 | SENSITIVITY OF THE α-SYN SAA FOR IDENTIFYING LBD: RESULTS FROM NEUROPATHOLOGICAL COHORTS

Increasing evidence indicates that α-syn SAA has a high diagnostic value for synucleinopathies, especially for detecting LBD.[108] CSF α-syn SAAs showed 91.6%–100% sensitivity and 94.3%–100% specificity in neuropathologically confirmed DLB and PD patients.[90,109–113] Recent studies have also shown that the sensitivity of CSF α-syn SAAs depends on the LB pathology burden. In patients following classic Braak staging, the test sensitivity decreases from 100% in the limbic and neocortical stages (Braak 4-6) to 35-60% when the LBD is limited to the brainstem (Braak stages 1-3)[112,113] (Table 1). However, the sensitivity increased significantly from Braak stage 1-2 (37%) to Braak stage 3 (73%).[113] Finally, studies have consistently reported relatively low sensitivity of CSF α-syn SAA, ranging between 14.3% and 63.6%, in patients with predominant amygdala involvement (i.e., with concomitant AD in most cases).[112–114] Two studies analyzing CSF samples obtained via lumbar puncture (LP) reported 14.3% (3 positives of 21) and 50% (2 of 4) positivity rates, respectively.[113,114] Two additional studies obtained a slightly greater sensitivity when CSF was obtained *post-mortem* from the brain ventricle at autopsy (7 positives of 11, 63.6%[112]; 6 of 10, 60%[114]) (Table 1). The low percentage of α-syn SAA positivity in patients with focal or amygdala-predominant LB pathology was confirmed in a recent study evaluating CSF from 10 patients in the Dominantly Inherited Alzheimer Network (DIAN)—ADAD cohort with neuropathologic confirmation and evidence of α-syn immunoreactivity in the brain.[115] Indeed, the only positive CSF (10%) was from the individual with the highest LB pathology burden with a diffuse involvement reaching the neocortical areas. In patients tested negative by SAA, α-syn immunoreactivity was limited to the olfactory cortex or showed a limbic/amygdala-predominant distribution. Given the long mean time from CSF collection to autopsy (32.4 ± 18.4 months), the possibility that the LB pathology was even sparser at the time of LP provides an additional explanation for the high rate of negative SAA results in these patients.[115]

Although all studies published to date demonstrated an association between LBD stage and α-syn SAA sensitivity, further work is needed to overcome some limitations associated with these studies. First, the number of samples analyzed is still relatively low, especially for the cases with focal LB pathology, which are the most important for studies comparing different SAAs' performance. Second, LB pathology staging mainly derives from the analysis of the topographic distribution of α-syn aggregates. Still, the overall α-syn load may vary significantly among brains at the same LB pathology stage. Moreover, because of the multiple staging systems proposed for LBD, the comparisons between studies might not be straightforward. Future research studies should harmonize and standardize the quantification of α-syn burden in the brain and more systematically assess the SAAs' performance in cases with a relatively low LB pathology load.

Finally, initial evidence indicates that some kinetic parameters of α-syn SAA reaction are associated with the α-syn burden,[113] paving the way for staging LBD in vivo. Developing an assay able to predict the spread of LB pathology in vivo would have relevant implications for

stratifying patients enrolled in clinical trials for both AD and LBD and defining disease trajectories.

7 | INVESTIGATING THE PREVALENCE AND CLINICAL EFFECT OF MIXED AD-LBD USING BIOMARKERS IN VIVO

Early studies investigating the diagnostic value of CSF α-syn SAA across disease groups showed that the rate of positive results in AD patients was greater than that in individuals with other (non-LBD) neurodegenerative conditions or age-matched non-neurological controls.[90,100] The first significant study of this kind involved a clinical cohort of MCI patients with CSF biomarker evidence of AD pathology and no clinical LBD features at baseline.[116] The results showed that 13% of the MCI-AD patients tested positive for CSF α-syn SAA. Among them, 6 out of 16 patients developed at least one clinical core feature of DLB at follow-up, and one developed orthostatic hypotension (i.e., a supportive clinical criterion for DLB). Hence, this study demonstrated that (1) the CSF α-syn SAA can identify LBD in patients clinically diagnosed with AD at the prodromal stage and (2) the clinical phenotype of AD-LBD patients can be assessed (and eventually predicted) in vivo. This study paved the way for subsequent investigations on the frequency and clinical effect of LBD in individuals with MCI/dementia due to AD in vivo by using CSF α-syn SAA. Regarding the frequency, the largest of these studies reported an AD-LBD incidence of 10-20%, with lower values in preclinical AD, intermediate values in MCI and higher values at the dementia stage.[37,117] Two additional recent studies, however, reported a higher incidence of AD-LBD respectively of 45% (in 80 AD patients at the dementia stage) and 30% (in 240 patients, ranging from 26% in the preclinical/MCI group to 36% in the dementia group).[118,119] The prevalence of AD-LBD detected in these studies is almost comparable to that observed in neuropathological cohorts at the terminal stage (including the amygdala-predominant variant), which is intriguing because it would indicate that there is no further significant increase of AD-LBD comorbidity during disease progression which may last several years. Given that these divergent results have been obtained in different laboratories, it will be important to address the possible effect of the SAA protocols applied, including their accuracy for detecting LB pathology. Future studies are required to harmonize α-syn SAA protocols, standardize raw data analysis, and assess the assay's reliability in multisite ring trials.

Two recent studies evaluated the incidence of LB copathology by CSF α-syn SAA in patients with ADAD.[115,120] In 27 symptomatic ADAD patients from the DIAN cohort, α-syn SAA was positive in 3 of them (11.1%), a prevalence lower than that in sporadic AD cohorts. The second study analyzed 18 ADAD patients who had CSF available and *post-mortem* neuropathology. Concomitant LB pathology was present in 44% ADAD brains; still only 5.6% of CSF samples were positive for misfolded α-syn.[120] The lower sensitivity of α-syn SAA in patients with focal and/or amygdala-predominant LBD, which is relatively more common in ADAD,[19,20] and EOAD[42] than in LOAD, and likely occurs later in the disease course, secondarily to AD pathology, might explain these findings.

Regarding the effect on the clinical phenotype, cross-sectional analyses in the large Swedish BIOFINDER 1 and BIOFINDER 2 cohorts revealed that in cognitively impaired individuals (MCI or dementia), the AD-LBD and pure AD groups had worse overall cognition and memory functions than the pure LBD group, while LBD pathology mainly influenced visuospatial performance.[37]

Regarding the prevalence of DLB clinical features in AD-LBD patients, the results are contradictory: one study reported a slight increase in symptomatic orthostatic hypotension and parkinsonism (but no difference in motor scores according to the Movement Disorder Society UPDRS-III),[118] whereas another found no difference between AD-LBD patients and pure AD patients.[37] Interestingly, in the latter study, there was a greater frequency of visual hallucination and worse motor performance in the pure LBD group than in the AD-LBD group.[37] According to the analysis of the effect of each contributing pathology, LBD was an independent predictor of worse attention/executive and visuospatial functions, more significant motor impairment, and a greater incidence of visual hallucinations. The greater visuo-constructional impairment (i.e., worse performance in copying drawings) in AD-LBD compared to pure AD has been confirmed in a recent study.[119] In contrast, Aβ and tau were associated with worse global cognition and memory performance.[37] Finally, another study failed to detect clinical differences in AD patients with or without LBD comorbidity.[121] The limited size of the cohort investigated likely accounts for the divergent findings (Table 2).[37,90,100,115–122]

Quadalti et al. also evaluated the longitudinal associations of AD-LBD copathology with cognitive outcomes: the comorbid AD-LBD group had a greater rate of worsening of all cognitive outcomes, namely, global cognition, attention/executive, memory, and visuospatial functions, over time than did the pure AD or LBD groups.[37] LBD was an independent predictor of longitudinal worsening of visuospatial skills (alone), memory, attention/executive functions, and global cognition. A faster longitudinal decline in Mini-Mental State Examination (MMSE) scores in AD-LBD compared to pure AD individuals has been confirmed in a recent study.[119]

In individuals who were unimpaired at baseline cognition and neurological testing, the effect of AD-LBD pathology was even more striking, particularly in predicting longitudinal cognitive progression. Indeed, AD-LBD patients had a faster worsening of global cognition, memory, and attentive/executive functions, making LB pathology an independent predictor.[117] Moreover, according to the cross-sectional analysis, LBD was associated with smell reduction in both the pure LBD and AD-LBD groups.

Taken together, these data identify the LBD as an independent factor in addition to AD in determining the clinical presentation and disease trajectory in both cognitively impaired and unimpaired patients.

Mirroring the use of α-syn SAA in patients with AD, other studies have characterized the A/T/(N) profile in patients with a clinical diagnosis of DLB to investigate the presence and effect of concurrent AD pathology. The first study in a sizable cohort showed

TABLE 2 Sensitivity of the CSF α-syn SAA in AD clinical cohorts

Reference	Diagnosis/cohort	*N*	α-syn +/-	Sensitivity (%)
[100]	AD[a]	14	5/9	35.7
[122]	probable AD (NIA-AA 2011)	11[b]	0/11	0.0
[90]	MCI/dementia due to AD (IWG-2)	43	7/36	16.3
[116]	MCI due to AD (IWG-2)	120	16/104	13.3
[121]	MCI/dementia due to AD (IWG-2)	97	15/82	15.5
[118]	MCI/dementia due to AD (NIA-AA 2018)	80	36/44	45.0
[37]	MCI/dementia due to AD (DSM-5)	425	73/352	17.2
[117]	Preclinical AD (NIA-AA 2018)	167	20/147	11.9
[119]	Preclinical/MCI/dementia due to AD (NIAA-2018)	240	72/160[c]	30.0
[120]	ADAD	18	1/17	5.6
[115]	ADAD – DIAN:			
	• Symptomatic	27	3/24	11.1
	• Asymptomatic mutation carriers	26	0/26	0.0

Note: Only studies with $N > 10$ were included.
Abbreviations: α-syn, α-synuclein; AD, Alzheimer's disease; ADAD, autosomal-dominant Alzheimer's disease; CSF, cerebrospinal fluid; DIAN, Dominantly Inherited Alzheimer Network, MCI, mild cognitive impairment; SAA, seed amplification assay; +/-, positive/negative SAA results.
[a]Unspecified selection criteria.
[b]The authors stated that because of the recurrence of visual hallucinations at follow-up, two patients initially classified as having AD were shifted to DLB group.
[c]Results were inconclusive in eight cases.

that a CSF AD profile, defined as abnormal (low) Aβ42 combined with abnormal (high) t-tau and/or p-tau, characterizes 25% of DLB patients, compared with only 9% of PDD patients and 3% of PD patients without dementia.[123] In a group of 417 patients with DLB stratified according to the A/T profile determined by CSF Aβ42 and p-tau in the European cohort and by Pittsburgh compound B and AV-1451 PET in the Mayo Clinic cohort, Ferreira et al.[124] found that 32% of patients were A+/T− and 15% were A+/T+. The two most recent studies produced similar results, showing that approximately 50% of DLB patients harbor CSF abnormalities within the AD continuum (A+/T+ or A+/T-).[125,126] However, approximately half of the patients did not have significant p-tau pathology (A+/T-), indicating a low ADNC burden. Although a few patients even changed from A+ to A- during a 12-month follow-up, there was a good relationship between the A+ state in vivo and amyloid deposition at neuropathology as determined by the Thal stage. In contrast, the A+/T+ positivity biomarker state did not change during the 12-month follow-up and correlated with a high Braak stage of neurofibrillary degeneration in the few patients evaluated neuropathologically.[125] Thus, DLB patients with an A+/T- profile may represent a subgroup with dominant LBD pathology and mild ADNC.

Regarding the clinical effect of concomitant AD pathology, a first study by the European DLB consortium revealed that reduced CSF Aβ42 levels were associated with more rapid cognitive decline in DLB patients, as measured by the MMSE.[127] Similarly, using a CSF t-tau/Aβ42 ratio > 0.52 as a criterion for an AD-positive profile, Lemstra et al.[128] documented worse memory performance, a greater frequency of hallucinations and a greater mortality risk in DLB-AD patients than in pure DLB patients. However, there was no significant difference in the rate of cognitive decline between the two patient groups. According to the study by Ferreira et al.,[124] A+ was the main predictor of worse cognitive performance when considered together with T+ (determined by CSF p-tau or AV-1451 PET), and T+ was associated with a lower frequency of clinical features of DLB, such as parkinsonism and probable REM sleep behavior disorder. In another study,[129] 43 patients with LBD (DLB/PDD) and AD, determined by a t-tau/Aβ42 ratio > 0.3, performed worse on confrontation naming tasks (30-item Boston Naming Test) than patients with pure LBD but similarly on executive abilities (letter fluency, reverse digit span), and global cognition (MMSE). The latest of these studies also documented a steeper cognitive decline and increased mortality risk in DLB patients within the AD continuum.[126]

8 | CONCLUSION

The introduction of biomarkers has led to significant advances in the field of neurodegenerative diseases by improving diagnostic accuracy in vivo and prognostic estimates, recruiting subjects in clinical trials and monitoring the response to disease-modifying agents.[6] In particular, the availability of pathology-specific markers for both AD and LBD, the two most prevalent neurodegenerative disorders, has allowed for the first time early in vivo identification and characterization of patients with concurrent AD-LBD. Recent studies in large cohorts have shown that in approximately 20%–25% of patients with sporadic late onset disease (either AD or LBD), the two disorders coexist (at the clinical stage) when identified using CSF-based biomarkers. However, in EOAD and ADAD, the percentage of patients with concurrent biomarker-defined AD-LBD drops to 10%, in line with "typical" LBD

being an age-related disorder whose incidence increases significantly only after 55-60 years.[48]

Regarding the "clinical" impact, evidence indicates that the coexistence of "typical" LBD and AD pathologies significantly affects the phenotype and course (including response to therapy) by worsening global cognition and especially attentive/executive, memory, and visuospatial functions. However, this will likely not be the case for those with only focal LBD, including the amygdala-predominant form, also considering that it develops late in the course of the disease, likely secondarily to AD pathology.

One significant current drawback of in vivo neuropathologic assessment concerns the limitations of current biofluid markers in providing a quantitative evaluation or staging of the underlying pathology. Although this will be the main research challenge in the "neurodegenerative" biomarker field, recent data raise the hope that this gap will be filled soon. On the AD side, it has been shown that distinct p-tau isoforms primarily correlate with Aβ pathology (i.e., p-tau181 and p-tau217),[130,131] but other tau variants (e.g., MTBR-tau243) are more strongly associated with tau proteinopathy.[132] Thus, measuring multiple tau isoforms in a single sample may provide a tool for staging AD pathology in vivo.[133] However, staging LBD in vivo is more challenging. Preliminary data indicate that consistently measuring the number of positive replicates in serial dilution experiments associated with a greater number of replicate analyses increases the "quantitative" capacity of the α-syn SAA and the ability to predict the LB pathology burden. Should this goal be accomplished, biomarker analysis of CSF will become an essential (and virtually the only one in the preclinical stage) precision medicine tool for estimating the relative burden of pathology in patients with AD-LBD.

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CONFLICT OF INTEREST STATEMENT

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