

Fluid and Tissue Biomarkers of Lewy Body Dementia: Report of an LBDA Symposium

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Abstract

The Lewy Body Dementia Association (LBDA) held a virtual event, the LBDA Biofluid/Tissue Biomarker Symposium, on January 25, 2021, to present advances in biomarkers for Lewy Body Dementia (LBD), which includes Dementia with Lewy Bodies (DLB) and Parkinson's Disease Dementia (PDD). The meeting featured eight internationally known scientists from Europe and the United States and attracted over 200 scientists and physicians from academic centers, the National Institutes of Health and the pharmaceutical industry. Methods for confirming and quantifying the presence of Lewy body and Alzheimer pathology as well as novel biomarkers were discussed.

Introduction

The Lewy Body Dementia Association (LBDA) Research Centers of Excellence presented a virtual symposium on biomarkers for consideration in clinical trials on January 25, 2021. Goals were to identify biomarkers that may be useful as inclusion criteria and as outcome measures in clinical trials in Lewy Body Dementia (LBD) which is a major opportunity to improve clinical trials for these individuals(1). The discussion was confined to biomarkers in body fluids and tissues and did not extend to other modalities such as neuroimaging. Given that the majority of people with LBD harbor both Lewy body and Alzheimer Disease (AD) pathology (2–5), options for identifying and quantifying the presence of each of these types of pathology were reviewed. Novel biomarkers for related disease processes such as endolysosomal processing were also evaluated. Although the focus of the symposium was to evaluate candidate biomarkers for potential use in clinical trials, the role of biomarkers in disease pathophysiology was also considered.

Biomarkers of Alpha-Synuclein Pathology

Two modalities for detecting pathologic alpha-synuclein were discussed: seeded aggregation assays and immunohistochemical detection of pathologic alpha-synuclein and studies are summarized in Table 1. Seeded aggregation assays, also described as protein misfolding cyclic amplification (PMCA) or real-time quaking-induced conversion (RT-QuIC) assays, amplify small amounts of aggregated protein in body fluids or tissue homogenates in an iterative process of aggregation and partial disaggregation (6). Such assays have become a focus of alpha-synuclein biomarker research since measurement of total, phosphorylated, and oligomeric alpha-synuclein in cerebrospinal fluid (CSF) and serum have to date failed to demonstrate acceptable diagnostic value (7). Seeded aggregation assays are well developed as a clinically useful assay in prion disease, and several labs have now adapted the methods for detecting aggregated alpha synuclein in biofluids (8–11). Dr. Lebovitz described published studies documenting that the specificity and sensitivity of the PMCA assay in CSF samples from clinically diagnosed individuals with Parkinson's Disease (PD) is greater than 90% (6,10,12). Evaluation of diagnostic value in pathologically confirmed individuals is under way, and performance of the assay in plasma samples is also being assessed. In Dementia with Lewy Bodies (DLB), seeding activity is significantly increased over PD so it is perhaps not surprising that test specificity and sensitivity are also greater than 90% for individuals with DLB versus controls and for individuals with DLB versus non-synucleinopathies (9,13,14). Finally, in autopsy-confirmed AD, the seeded aggregation assay correctly detected all negative cases as those with no evidence of Lewy bodies at autopsy versus positives as all cases of diffuse neocortical Lewy bodies (unpublished). Interestingly, people with AD who harbored Lewy bodies mainly in the amygdala at autopsy were detected approximately 50% of the time with the CSF seeded aggregation assay. Quantitative aspects of the assays (e.g., time to amplification, maximal fluorescence) still require further testing. Data

examining these metrics across individuals of advancing disease state (measured by postmortem alpha-synuclein) and within individuals longitudinally are needed to demonstrate clinical utility.

Dr. Beach provided a history of efforts since 2007 to develop immunohistochemical detection of pathologic alpha-synuclein in peripheral tissues as a biomarker of PD. Initial studies in colon biopsies were limited by high false-positive rates and poor inter-rater reliability, but subsequent multicenter studies concluded that these problems could be addressed by screening of multiple candidate methods and training of raters. Following such optimization, sensitivity and specificity of colon biopsy in autopsy tissue was excellent (100% accuracy for one method albeit in limited numbers of individuals) and could be useful diagnostically (15–17). However, the question of whether colon was the best site for biopsy remained, due to an insufficient amount of submucosa obtained with current biopsy methods, so subsequent studies compared colon, submandibular gland, and skin biopsy. The Systemic Synuclein Sampling Study (“S4 Study”) employed consensus slide-reading by a panel of 5 specially trained neuropathologists, all blinded to diagnosis (15). The S4 study found >90% specificity but disappointingly low sensitivity (56% in submandibular gland, 24% in skin, and very low sensitivity in colon). This may in part be attributable to the overrepresentation of early PD cases in S4: one-third of cases had a median disease duration of only 8 months and the median disease duration for the entire PD group was 42 months (15). Sensitivity was greater in participants with more advanced PD, particularly in submandibular gland biopsies (76%). Dr. Beach concluded that improvement in the sensitivity of immunohistochemical methods was needed, or alternatively, seeding assays such as RT-QuIC may soon supersede them and he hypothesized that while RT-QuIC is already being done on CSF with very promising potential, peripheral tissue deposits may be a better model of brain tissue deposits and also perhaps a better measure of target engagement for monoclonal antibodies and other new therapeutic approaches.

Skin biopsy in the S4 study consisted of two 3-mm skin punch biopsies obtained from the paravertebral posterior–inferior cervical area and mid-thigh; immunohistochemical labeling was performed with an alpha-synuclein antibody after protease pretreatment. Dr. Gibbons presented a distinct approach to skin biopsy involving three punch biopsies from distal leg, distal thigh, and posterior cervical region in people with PD and DLB, using a double-label immunofluorescence method to detect and colocalize both alpha-synuclein and neuronal markers (PGP 9.5) in thick sections (18–20). His team found higher sensitivity than the S4 study for a single biopsy (74%) and increasing sensitivity when considering two (90%) or three (96%) biopsies (unpublished data presented at American Academy of Neurology Conference, 2020). They reported overall outstanding sensitivity and specificity for skin biopsy in this study and subsequent validation (accuracy 99.1%) as a lab-developed test, the “SYN-ONE” test (CND Life Sciences™), for discriminating peripheral synucleinopathies (PD, DLB, Multiple System Atrophy, Pure Autonomic Failure) from controls (unpublished). In discussion, it was noted that Dr. Gibbons’ unpublished data correlated with clinical scores including the Unified Parkinson’s Disease Rating Scale (UPDRS), Orientation and Mobility Severity Rating Scale (OMSR), and DLB Cognitive Scale (unpublished). Biopsy acquisition was performed as part of a multicenter study supported by the NIH Office of Rare Diseases (Autonomic Disorders Consortium). An additional multi-site blinded study funded by NIH is currently under way to evaluate how this approach fares in the environment of multiple academic and private practice sites with a core reference lab-developed test. The skin biopsies prepared in this manner can also be quantified, and the first quantitative studies show significant autonomic and sensory nerve fiber density differences between groups, individuals with DLB having the most severe autonomic and sensory neuropathies, followed by idiopathic PD, and finally Multiple System Atrophy (MSA), without evidence of peripheral nerve degeneration. Dr. Gibbons’ group also recently reported that cutaneous phospho-alpha-synuclein is moderately correlated ($r = 0.6$) with both sympathetic and total autonomic impairment in individuals with isolated REM sleep behavior disorder (iRBD) and is more common in iRBD with hyposmia (21).

The final speaker in this session, Dr. Kanthasamy, linked the seeded aggregation assay and tissue biopsy approaches by presenting results from skin biopsy samples that were analyzed not with immunohistochemistry but instead homogenized and processed with a seeded aggregation assay, RT-QuIC. After establishing the method with autopsied brain tissue and submandibular gland (8,22), his group compared skin samples from individuals with PD and controls, and found that the seeded aggregation assay performed on skin homogenates yielded specificity of 96% and sensitivity of 96% (23). The maximal fluorescence metric from the seeding assay also correlated with disease severity (UPDRS $p < 0.0001$; Mini Mental Status Examination (MMSE) $p = 0.0035$), suggesting that quantitative aspects of this assay might be useful as a marker of disease state and potentially as an outcome measure in clinical trials targeting alpha synuclein. An advantage of seeding assays over immunohistochemistry is that they do not require extended review by specially trained neuropathologists.

Biomarkers for Alzheimer's Disease Pathology

Dr. Lemstra provided a summary of CSF biomarkers for AD pathology in LBD and studies are also summarized in Table 2. Studies in neuropathologically-confirmed cases have shown that mixed pathology can be detected antemortem with CSF biomarkers using similar cutoffs employed for AD. Larger *in vivo* cohorts including the European DLB Consortium (E-DLB), Mayo Clinic Cohort and Amsterdam Dementia Cohort, have shown that AD biomarkers, either CSF (most commonly CSF tau/A β 42 ratio) or PET markers, are increased in DLB over PD and PDD and correlate with DLB dementia, progression and survival. Evidence suggests that these Alzheimer markers likely reflect concomitant Alzheimer pathological process along with the Lewy body disease. Specifically, AD biomarkers in DLB (DLB-AD) are associated with increased age, female sex, increased *APOE* ϵ 4 genotype, decreased memory, increased delusions and hallucinations, less REM-behavior sleep disorder and parkinsonism, worse language performance, faster progression, increased temporal thinning and tau pathology, and greater risk of institutionalization and mortality (2–4,24–28). These findings are corroborated by post-mortem clinicopathologic studies showing that AD pathologic features (neuritic plaques and also tangles) in cases clinically defined as “probable DLB” are associated with an atypical “Alzheimerized” clinical presentation (e.g. worse performed on orientation and memory testing) (29,30). Dr. Lemstra also presented studies showing CSF AD biomarkers do not appear to influence positivity rates of DaTscan (31) or electroencephalography (32) but emphasized that more studies are needed. Different amyloid-beta species were also discussed as possible biomarkers for discriminating DLB from AD. Unlike AD, in which A β 42 is more selectively decreased, multiple studies have shown that DLB exhibits a broader decrease of multiple amyloid-beta species (A β 38, A β 40, A β 42). Furthermore, these studies show that some species of amyloid-beta (A β 38, A β 40) decrease independently of AD biomarkers (CSF tau/A β 42) and *APOE* genotype, and some species (A β 38) correlate with disease duration. Ratios (A β 42/A β 38) can discriminate clinical DLB from AD with moderate accuracy (sensitivity 78%, specificity 67%) (33,34). Limited data suggests a negative association between symptomatic treatment with acetylcholinesterase inhibitors in DLB and longitudinal changes in AD biomarkers (35). It was proposed that DLB with AD biomarkers (DLB-AD) could be considered for recruitment into clinical trials for amyloid-modifying therapy in the research setting, but future work is needed to clarify the relationship between amyloid deposition and clinical symptoms in AD and DLB-AD. DLB-AD (discussed below) is defined by the presence of AD biomarkers unlike DLB-pure (DLB-p) and is a common subtype (at least 50%) of DLB characterized by more rapid clinical deterioration and mortality (24).

The final speaker, Dr. Shaw, summarized progress in blood biomarkers for AD, mainly focusing on targets whose studies are well underway (A β 42/40, pTau181 and 217, neurofilament light chain [NfL]) with brief mention of earlier-stage targets (alpha-synuclein, TDP-43, GFAP, NPTX2). Plasma A β 42/40 as a biomarker shows small but reproducible absolute differences in amyloid-positive (by PET) versus amyloid-negative patients and shows early promise as a screening test alongside

covariates, *APOE* $\epsilon 4$ genotype and age. In general, area under the receiver operating characteristic curve (AUCs) has been better in mass spectrometry studies (AUC 0.82 – 0.89) (36,37) than immunoassays (AUC 0.65 – 0.77, up to 0.80 when A β 42 and A β 40 were used in a logistic regression model instead of the A β 42/A β 40 ratio) (38). A large head-to-head round robin study (Foundation for the National Institutes of Health Biomarkers Consortium (FNIH BC) / Alzheimer's Disease Neuroimaging Initiative (ADNI)) nearing completion will compare 3 mass spectrometry and 3 immunoassays for plasma A β 42/40 ratio concordance with amyloid-PET. pTau181 and pTau217, tested by immunoassay or by mass spectrometry, can reliably detect tau pathology and levels correlate with amyloid PET, cerebral atrophy, and cognitive decline (39,40). Some reports suggest pTau217 has somewhat superior sensitivity and specificity versus pTau181 for discriminating AD from other disorders and healthy controls (41), but more head-to-head studies are needed to definitively address this question. Pre-analytical, analytical, and clinical replication studies are underway in international groups and there are multiple companies developing diagnostic tests for these targets. Dr. Shaw also highlighted the importance of detailed pre-analytical studies to test variables such as delayed centrifugation at room temperature and freeze-thaw cycles that can affect these measurements. The Alzheimer's Association Global Biomarker Standardization Consortium recently reported these findings at the 2021 Alzheimer's Association International Conference, underscoring the need to resolve inter-lab differences before widespread clinical applications are implemented. Neurofilament light chain (NfL) was also briefly mentioned as a third well-studied biomarker of non-specific neurodegeneration that needs larger head-to-head confirmatory studies. During the discussion, other promising pTau targets were mentioned including pTau231 and di-phosphorylated peptides. Low DLB enrollment in these studies was noted and ongoing consortia were highlighted that are measuring pTau181, pTau231 as well as A β 42/40, NfL and glial fibrillary acidic protein (GFAP). Newer CSF and plasma biomarkers for AD, Lewy body, and non-AD neurodegenerative disorders are also reviewed elsewhere (42).

Biomarkers of Other Disease Mechanisms

Two modalities for discovery of novel CSF biomarkers were discussed: mass spectrometry and antibody array proteomics and these are also summarized in Table 3. Mass spectrometry is an unbiased approach with longstanding precedent in laboratory testing and biomarker research (e.g., plasma AD markers discussed above) whereas newer antibody arrays, such as O-link® Proximity Extension Assay (PEA) discussed below, offer more targeted, higher throughput, and potentially more sensitive multiplexed immunoassays. Interestingly, these technologies have been shown to cover different fractions of the proteome, leading to partly complementary results (43).

Dr. Zetterberg presented CSF mass spectrometry data of a panel of proteins involved in endolysosomal and autophagosome processing. These fundamental intracellular sorting organelle systems have been implicated by genetic and histologic studies in DLB (reviewed in (44)) but have rarely been studied in CSF. Dr. Zetterberg presented data from the measurement of these proteins quantitatively in AD and PD and achieved simultaneous measurement of 18 related proteins in approximately 0.2ml of CSF. The team's results show a pattern of increase in AD vs. controls for many lysosomal markers (e.g., cathepsin B, LAMP2) as well as endocytosis (AP2) and ubiquitin whereas there was a decrease in PD for the same group of proteins. The causes of increased protein release in AD and decreased release in PD are unknown but results argue against non-specific neurodegeneration since results for AD and PD are in the opposite direction. Discussants noted similar decreases across multiple proteins (synuclein, tau, A β) in the Parkinson's Progression Markers Initiative (PPMI) cohort and one speculated unifying explanation was retromer dysfunction (45). Studies in DLB patients have not yet been performed using this approach.

The second speaker, Dr. Teunissen, first discussed biomarker discovery in DLB using mass spectrometry and ELISA validation. Six potential targets were identified in DLB versus control

including: down-regulation of neurosecretory protein VGF, neuronal pentraxin-2 (NPTX2), neuroendocrine convertase 2 (PCSK2), and neuronal Pentraxin receptor (NPTXR) and up-regulation of ubiquitin carboxyl-terminal hydrolase (USP14) and proteasome subunit beta type-7 (PSMB7) (46). The extent of downregulation of NPTX2 and VGF in DLB was greater than but overlapped with the extent of downregulation in AD and PD. It is unclear if DLB patients with lower NPTX2 have more amyloid pathology, a potentially important distinction given that AD studies (e.g., ADNI) also find NPTX2 dysregulation (47). In the second half of his presentation, Dr. Teunissen presented early results of a broad search for distinguishing a DLB subgroup with AD CSF biomarkers (DLB-AD) using multiplexed immunoassay arrays, Olink®, discussed briefly above. Interestingly, Dr. Teunissen's early data disagree with the hypothesis that biomarkers for DLB-AD represent a simple combination of biomarkers for Lewy body disease plus AD. The team found that DLB-AD had unique features compared to DLB-p and AD, including lower CSF protein levels and specific protein differences. In contrast, few differences in proteins were observed between DLB-p and AD in these data. The identity of these proteins enriched in DLB-AD included cell adhesion, cytokine-cytokine interactions, axon guidance, and neurogenesis. Preliminary validation studies with a more quantitative immunoassay, the Ella® system, show replication of Olink® hits and further discriminatory power of candidate proteins in addition to pTau and tTau in distinguishing DLB from AD.

Conclusion and Future Directions

Alpha-synuclein biomarkers

There was agreement by the presenters regarding the urgent need for a sensitive and specific *in vivo* biomarkers to detect alpha-synuclein pathology, which has the potential to improve therapeutic targeting by inclusion of patients after verifying the presence of target neuropathology and also to inform disease pathogenesis. Panel discussants provided insights into the logistics and needs for further validation of emerging peripheral tissue biopsies and seeding aggregation assays for these approaches to reach utility in therapeutic trials and eventually clinical use. Key gaps include the need for further autopsy-validation, longitudinal analyses, and standardization of assay methods. Indeed, there are animal and cell-model data suggesting alpha-synuclein pathology can spread throughout the nervous system (48,49), but it remains unclear whether and how this occurs in humans. The Braak staging system for PD suggests that pathology begins in the peripheral autonomic nervous system or olfactory bulb and then migrates proximally to the amygdala and brainstem and in the more severe cases, to the cerebral cortex (50). This model is supported by some human studies, such as those of multiple groups finding high levels of alpha-synuclein pathology in skin biopsies in early PD and in REM-sleep behavior disorder (RBD) without other clinical evidence of synucleinopathy (20,21,51–54). However, the model is not supported by other large autopsy-based studies that find no evidence of peripheral-first synucleinopathy (e.g. stomach versus brain) and that peripheral synucleinopathy is more common and severe in later stages (55,56). Overall these observations, combined with the fact that limbic Lewy body pathology may occur prior to brainstem pathology in the course of DLB, indicate that the initiation and propagation of Lewy body pathology is variable among the synucleinopathies (57), may vary by predictors such as genotype, and that future work is needed to resolve these findings.

AD biomarkers in LBD

In contrast to biomarkers of alpha-synuclein, sensitive AD biomarkers are established and provide a link between clinical and pathologic aspects of DLB with and without AD pathology. Panelists agreed with the potential importance of stratifying therapeutic trial inclusions and/or outcomes based on AD biomarker profiles in LBD due to the strong association of these biomarkers

with clinical outcomes, exemplified by the prospective data in the European DLB (E-DLB) consortium. Frequency of AD pathology in DLB (DLB-AD) varies between 25-89% depending on biomarker cutoffs and diagnostic criteria (2–5). Distinct lines of evidence have shown that DLB-AD may represent a biological interaction of these mixed amyloid-beta, tau and alpha-synuclein pathologies. In clinically defined DLB, unpublished data suggest that DLB-AD appears to exhibit a distinct CSF immunophenotypic pattern, raising the importance of exploratory biomarker discovery work to further refine biological subgroups in DLB. Moreover, in autopsy-defined DLB there is lower overall tau compared to AD and higher temporal lobe enrichment of tau that is associated with both cortical thinning and cognitive impairment (28,58). Although the longitudinal assessment of AD biomarker progression in DLB is understudied, there are conflicting results in PD (59,60) that appear to be explained by variable disease stages and methods of measurement in individual studies, but also intrinsic biological variability between patients. Thus, future prospective longitudinal DLB-specific studies with autopsy-confirmation are needed to quantitatively compare the time course of amyloid and tau biomarkers and to consider potential DLB-specific cutoffs. By enabling the study of homogenous patient populations with similar underlying biology, these efforts will increase the capacity to assess treatment outcomes in DLB-focused clinical trials.

Overall, rapid progress has been made in the development of fluid and tissue-based biomarkers for Lewy body dementia and they show promise as useful tools. Further external validation and translational research is needed specifically in individuals with Lewy body dementia in order to accurately determine biomarker test characteristics and overall determine how these individuals may benefit from such a biomarker test.

Conflict of Interest

TGB has conducted consultation for a peripheral synuclein assay and speakers honorarium from Roche Diagnostics. RML is employed full time by Amprion, serves on Amprions Board, and is a shareholder. CET research is supported by the European Commission (Marie Curie International Training Network, grant agreement No 860197 (MIRIADE), and JPND), Health Holland, the Dutch Research Council (ZonMW), Alzheimer Drug Discovery Foundation, The Selfridges Group Foundation, Alzheimer Netherlands, Alzheimer Association. CET is recipient of ABOARD, which is a public private partnership receiving funding from ZonMW (#73305095007) and Health Holland, Topsector Life Sciences & Health (PPP allowance; #LSHM20106). More than 30 partners participate in ABOARD. ABOARD also receives funding from Edwin Bouw Fonds and Gieskes Strijbisfonds. IV is appointed on a research grant by Alzheimer Nederland (NL 17004). CET has a collaboration contract with ADx Neurosciences, Quanterix and Eli Lilly, performed contract research or received grants from AC Immune, Axon Neurosciences, Biogen, Brainstorm Therapeutics, Celgene, EIP Pharma, Eisai, PeopleBio, Roche, Toyama, Vivoryon. CET serves on editorial boards of *Medidact Neurologie/Springer*, *Alzheimer Research and Therapy*, *Neurology: Neuroimmunology & Neuroinflammation*, and is editor of a *Neuromethods* book by Springer. HZ has served at scientific advisory boards and/or as a consultant for Abbvie, Alector, Eisai, Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics, Nervgen, AZTherapies, CogRx, and Red Abbey Labs, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure and Biogen, and is a co founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (all outside submitted work). CM an inventor on several US and international Georgetown University patents to use tyrosine kinase inhibitors (TKis) for the treatment of neurodegenerative diseases. CM is cofounder and shareholder and receives consulting fees from KeifeRx LLC. CM receives consulting fees from Neumentum LLC, SUN Pharmaceuticals Research

Industry and SkyBio. CM received NIH NIA funding, Alzheimers Association and Sun Pharmaceuticals Research Industry Funding to study TKis in neurodegeneration, including LBD. KLP has received consulting fees from Curasen and is funded by grants from the NIH , Michael J Fox Foundation for Parkinsons Research, Lewy Body Dementia Association, and Alzheimers Drug Discovery Foundation, and has received funding from Sanofi US Services, Inc. to perform clinical trials. LSR declares ownership interest (Stock or stock options): Brain Health Inc, NeuroTau, Optimal Cognitive Health Company, uMethod Health, Versanum, Athira, Cognoptix and consulting work for: Alzheon, Biogen, Cortexyme, Roche Genentech, Stage 2 Innovations/Renew Research, Acadia, T3D, Eisai, KeifeRx. LSR also declares royalties: HarperCollins, Humanix and speakers bureau: Health and Wellness Partners. MJA receives research support from the NIA (R01AG068128, P30AG047266), the Florida Department of Health (grant 20A08), and as the local PI of a Lewy Body Dementia Association Research Center of Excellence. MJA serves on the data safety monitoring boards (DSMBs) for ACTC/ATRI and ADCS. MJA receives royalties from the publication of the book Parkinsons Disease: Improving Patient Care. MJA serves on the level of evidence editorial board for Neurology and related publications (uncompensated). JGG declares Grants/research - Acadia, Michael J. Fox Foundation, Parkinsons Foundation, Consultant - Worldwide Med, Honoraria - American Academy of Neurology, Davis Phinney Foundation, International Parkinson and Movement Disorders Society, Medscape, Parkinsons Foundation, Other: Lewy Body Dementia Association Research Center of Excellence.

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Author Contributions

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Table 1: Fluid and tissue biomarkers of alpha-synuclein pathology

Biomarker	Utility for inclusion/exclusion criteria	Utility as an outcome measure	Procedure	Comments
CSF, seeded aggregation assay for alpha synuclein	PD vs Ctrl: good SN, SP, AUC (0.89-0.95) (6) PD/DLB/PAF: good SN, SP (61) RBD vs Ctrl: good SN, SP (62)	Changes over time not established. PD: MIF correlates with UPDRS score RBD: PD/DLB risk (62).	Lumbar puncture	Commercial test to be released in 2021
Skin biopsy, seeded aggregation assay for alpha synuclein	PD: good SN/SP (23,63) PD/DLB/MSA vs other: good SN/SP (63)	Change over time not established PD: MIF correlates with UPDRS & MMSE (23)	Punch biopsy, frozen	Promising results, numbers of patients still modest, lower SN/SP for FFPE
Skin biopsy(-ies), IF synuclein & PGP 9.5	PD: good SN/SP when optimized (19) PD/DLB/MSA/PAF vs other: good SN/SP, better for IF vs. CSF or skin RT-QuIC in one study (54)	One study shows 7% increase per year (unpublished)	1-3 punch biopsies, frozen or ZF	Promising results; needs multi-site validation; commercial test offered since 2020
Skin biopsy-single biopsy (scalp), single-label IHC for alpha synuclein	PD: good SP with optimized methods; SN limited (24%) especially in early PD. Better SN than colon (15)	Changes over time not established	Punch biopsy, FFPE	Not likely to be developed further.
Submandibular gland biopsy	PD: good SP with optimized methods; limited SN (56%) especially in early PD; better SN than colon and skin (15)	Changes over time not established	Submandibular biopsy by ENT	Not likely to be developed further
Colon biopsy	PD: good SP with optimized methods; low SN (<15%) especially in early PD (15)	Changes over time not established	Colonoscopy with biopsy	Not likely to be developed further
Designation of “good” means greater than 90%. Abbreviations: PD = Parkinson’s disease, DLB = dementia with Lewy bodies, PAF = pure autonomic failure, RBD = REM-behavior sleep disorder, UPDRS = Unified Parkinson’s Disease Rating Score, MMSE = Mini Mental State Exam, SN = sensitivity, SP = specificity, AUC = area under the curve, MIF = maximum intensity fluorescence, FFPE = formalin-fixed paraffin embedded tissues, IF = immunofluorescence, ZF = Zamboni’s fixative				

Table 2: Fluid and tissue biomarkers of AD pathology

Biomarker	Utility for inclusion/exclusion criteria	Utility as an outcome measure	Procedure	Comments
CSF A β , tau, pTau	DLB-AD > DLB-p: tTau/A β ₁₋₄₂ good sensitivity and specificity (2) DLB vs. AD: A β ₄₂ /A β ₃₈ modest sensitivity and specificity (AUC 0.76) (33,34) AD vs MCI/Ctrl: core biomarkers, improve detection of early stages (64,65)	PD: Progression (59) DLB: Changes over time are not established DLB-AD vs. DLB-p tTau/A β ₁₋₄₂ : worse progression (2–4,24–28) AD: Prodromal / baseline A β & tau related to later atrophy, amyloid, decline (66–69)	Lumbar puncture	AD: soon universal A β cutoffs. Timecourse data marks AD subtypes (67,70); indicates drug-target engagement (71,72)
CSF NfL	DLB: Sensitive and early marker, non-specific (73). Elevations in DLB > Ctrl (AUC 0.94), proDLB > Ctrl (AUC 0.87), DLB > proDLB (AUC 0.6), DLB-AD > DLB-p (lower AUC vs. tau/A β , not shown) (74) MSA, PSP, CBS > other parkinsonian dz (73,75) AD: sensitive, nonspecific (65)	DLB: Changes over time are not established AD: Correlates with degeneration (76); increase with dementia, decline, atrophy (74,77).	Lumbar puncture	
Plasma A β _{42/40}	DLB: A β ₄₂ unchanged in small study (78); ratio not studied AD: Small effect but good AUC (~0.85), complements covariates (<i>APOE</i> ϵ 4, age)	Changes over time are not established	Blood draw	AUC better for mass spec vs. IA; however stability unproven
Plasma tTau/pTau	DLB: Non-specific for DLB, small study, ratio untested (78); predicts abnormal tau-PET and CSF A β ₄₂ /A β ₄₀ (79) AD vs Ctrl, NDD: pTau181 & pTau217 accurate (AUCs (0.87 – 0.98); DLB part of NDD controls (80)	DLB: Changes over time are not established AD: pTau181 & pTau217 predict and correlate with ongoing progression (39,80,81); more amyloid-specific than plasma NfL	Blood draw	Head-to-head comparison needed
Plasma NfL	Non-specific marker of damage (81,82) DLB & proDLB vs Ctrl: Elevated but unclear test performance (pre-print) (83)	DLB: Predicts cognitive progression (pre-print) (83); changes over time not established AD: Progression independent of tau but not AD-specific (81)	Blood draw	
Designation of “good” means greater than 90%. Abbreviations: AD = Alzheimer’s disease, PD = Parkinson’s disease, DLB = dementia with Lewy bodies, DLB-AD = dementia with Lewy bodies and AD biomarkers, DLB-p = “DLB-pure” / dementia with Lewy bodies and lacking AD biomarkers, proDLB = prodromal DLB, MSA = multiple system atrophy, PSP = progressive supranuclear palsy, CBS = corticobasal syndrome, tTau = total tau, pTau = phosphorylated tau, NDD = neurodegenerative diseases/dementias, NfL = neurofilament light chain, AUC = area under the curve, Ctrl = controls, dz = diseases, IA = immunoassay				

Table 3: Fluid and tissue biomarkers for other disease mechanisms

Biomarker	Utility for inclusion/exclusion criteria	Utility as an outcome measure	Procedure	Comments
CSF autophagy markers	DLB: Not established AD: Increase PD: Decrease	DLB: Changes over time not established	Lumbar puncture	
Novel CSF biomarkers NPTX2, VGF	DLB vs. Ctrl: • NPTX2 (AUC 0.81) • VGF (AUC 0.81) • NPTX2+VGF+age+ αSyn (AUC 0.94) AD vs. Ctrl/MCI: NPTX2 (47)	DLB: Low VGF associated with cognitive decline at time of presentation, high VGF associated with future cognitive decline (84); changes over time are not established AD: NPTX2 change correlates with cognitive decline (47)	Lumbar puncture	VGF correlated with CSF tau & αSyn

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