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Detection of Alzheimer's Disease Pathology in patients using biochemical biomarkers: prospects and challenges for use in clinical practice.

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Abstract

Background: Amyloid- β 1-42 peptide was identified 34 years ago in amyloid plaques obtained from Alzheimer's disease (AD) and Down's Syndrome patients' autopsied brain tissue. This led to development of immunoassay methods for measuring this marker of amyloid plaque burden in cerebrospinal fluid (CSF) 10 years later. Following this were development of research use only immunoassays for tau proteins, one for total tau the other for tau phosphorylated in the threonine 181 position. These early studies were followed by extensive studies that documented the clinical utilities of these biomarkers that reflect either amyloid plaque burden or tau tangle pathology in living patients in different patient cohorts.

Content: Here we describe: (1) published experience on clinical utilities of AD biomarkers; (2) challenges for robust and reliable measurement including recent development by diagnostic companies of fully automated immunoassays and development of mass spectrometry-based reference methods; (3) development of "Appropriate Use Criteria" (AUC) guidelines for safe and appropriate use of CSF testing for AD diagnosis developed by a workgroup of neurologists, a neuroethicist and laboratory scientists; (4) a framework for defining AD based on CSF and imaging methods for amyloid plaque burden detection, tau tangle pathology and neurodegeneration. This framework, sponsored by the National Institute of Aging together with the Alzheimer's Association, was designed for research studies but with important implications for future clinical practice; (5) growing recognition of additional co-pathologies in AD patients and challenges involved in development of methods to detect these in patients.

Summary: Based on experience over the years we expect soon the availability of validated research tools for AD pathology detection that can support clinical treatment trials of disease-

modifying agents, and ultimately use in clinical practice. Although validated methods are becoming available for CSF testing we can expect in the next few years validated methods for AD biomarkers in plasma to emerge.

The development, analytical validation and assessments of the diagnostic performance of the biomarkers for the two hallmarks of Alzheimer's disease (AD) neuropathology amyloid plaques and neurofibrillary tangles--A β 42, A β 40, t-tau and p-tau phosphorylated in the 181 threonine position (p-tau181), measured in cerebrospinal fluid (CSF) by immunoassay or liquid chromatography-tandem mass spectrometry (LC/MSMS) in various research settings--is at a mature stage of development (1,2). Amongst the major signposts of this progress are: detection and quantitative measurements in CSF of A β 42, A β 40 and the tau proteins (3–8); and establishing the diagnostic utilities of these measurements over the years despite the fact that the early immunoassays were "Research Use Only" (RUO). These RUO immunoassays had significant limitations, including: sub-par lot-to-lot performance, poor center to center performance even using replicate sample aliquots, and the same lot of reagents, and most of the early studies used clinical diagnosis as the standard of truth for presence of AD neuropathology (9). Despite these limitations the diagnostic utilities of these CSF AD biomarkers were repeatedly confirmed in many studies (9,10; see Tables 1 and 2 for summary). Nevertheless, with observed inter-laboratory precision values (%CV) of 20-29%, 14-26% and 14-27% for A β 42, t-tau, and p-tau181, respectively (11,12), making establishment of universal cut-points a nearly impossible task, a growing impetus for improved performance led to Important advances in the standardization of analytical methods including:

- The launch in 2004 of the Alzheimer's Disease Neuroimaging Initiative (ADNI) (13,14). A core value of this multicenter study of the longitudinal brain changes in elderly cognitively normal, mild cognitive impairment (MCI) and early AD dementia participants is the use of highly standardized neuroimaging, biochemical biomarker and genetic testing methods. The value of this study is heightened by a second core value, namely upload, and availability to the scientific community for interrogation, of quality-controlled clinical, ADNI participant biochemical, neuroimaging and genetic biomarker data prior to publication. This study has had a significant impact, which led to development of ADNI-like organization of studies world-wide, and provided sustained strong support for standardization of biofluid biomarker and neuroimaging methods (13–18) in AD and Parkinson's disease (PD) research. An important feature of this study is that overall funding is via public (National Institute of Aging [NIA] plus private (industrial, private foundations) support and there has been ongoing scientific input from each of these stakeholders under the overall guidance under the leadership of Michael Weiner, PI, and direction of the NIA;
- The establishment in 2009 of an international quality control program for CSF AD biomarkers, sponsored by the Alzheimer's Association, that provides valuable feedback regarding precision performance over time and comparison across methods to participating laboratories including diagnostic companies (19). The QC program will soon include blood based AD biomarkers;

- The development and validation of reference method mass spectrometry-based measurements of CSF A β 42 (20,21) and considerable progress toward that goal for A β 40 and tau proteins (22,23);
- The development and validation of fully automated immunoassays for A β 42, A β 40 and the tau proteins (24–26);
- The development and release in December 2017 of neat CSF-based Certified Reference Materials (CRMs) at low, mid- and high A β 42 concentrations based on reference mass spectrometry methods (27).
- Repeated observations across different study populations of high concordance between CSF AD biomarkers and amyloid PET detection of amyloid plaque burden (Table 2) and earlier observations of a high degree of correlation of CSF AD biomarkers and autopsy-based diagnosis of AD (9, 25, 28–30). The gold standard for detection of AD neuropathological following following brain autopsy (see review in reference 9) and, in the living patient, 3 amyloid PET imaging tracers, Florbetapir, Flutemetamol and Florbetaben, have been recognized by the US FDA as a surrogate for autopsy detection of amyloid neuritic plaque burden (31–34) and recently shown to detect cerebrovascular amyloid deposits as well (Charidimou 2017).

Thus, we can look forward to the realistic prospect of harmonized CSF A β 42 concentration measurement across different analytical platforms and, it is hoped, based on work at an earlier stage, a similar outcome for t-tau (37). Several diagnostic companies are well along the pathway in the development of IVD-level immunoassays thus paving the way toward the introduction of these tests of AD pathology in clinical practice. In a number of European countries, tests for CSF A β 42, t-tau, and p-tau181 have achieved CE Marking (abbreviation of French phrase “Conformite Europeene” which means “European Conformity”) status and are used in routine clinical practice (9). In the USA these tests have primarily been used in research studies. With the prospect of the availability of CSF AD biomarker tests for routine testing in the not too distant future, the Alzheimer Association convened a multidisciplinary team to develop appropriate use criteria (AUC) to provide guidance for safe and optimal use of lumbar puncture (LP) and CSF testing for detection of AD pathology in clinical practice. This effort was modeled after the earlier AUC for amyloid PET testing (34). Here, we discuss two of the primary outcomes of the CSF AUC effort, the safety of LP and recommendations for best practice performance of the procedure and ratings, appropriate or inappropriate, for 14 clinical indications for LP and CSF testing in AD diagnosis.

Safety of the lumbar puncture procedure.

The CSF AUC reviewed studies that evaluated the safety of obtaining CSF by LP in patients with suspected AD involving more than 7,000 individuals (9). Furthermore, the safety record for LP had been documented in more than 30,000 patients with a broad range of neurological diseases (35,36) including the differential diagnosis of infectious diseases of the central nervous system (CNS), neuro-inflammatory conditions such as multiple sclerosis and autoimmune mediated encephalitis, certain central nervous system (CNS) cancers, for measurement of CSF pressure, and is widely used in the research setting for detection of AD

pathology. A lumbar puncture involves withdrawal of CSF via a needle introduced into the subarachnoid space of the lumbar sac, at a level that is safely below the spinal cord (35,36).

Recommendations for safe LP performance.

Accumulated experience and based on careful, thoughtful critical reviews by clinicians who perform the LP procedure routinely has demonstrated that CSF can be collected safely and reliably by LP (36). Recognition of patient- and LP-procedure-related risk factors are key to maximizing patient safety. Evaluation of the patient for potential contraindications including use of anti-coagulant medications, recent seizures, some disorders of blood clotting, intracranial lesions, impaired consciousness, papilledema is important for best assurance of least risk. Absence of these can provide for reassurance to the patient in preparation for the LP procedure. In addition, taking into account known procedural risk factors such as needle size by including use of an atraumatic narrow bore needle, using the lateral recumbent position, associated with less risk for post-LP headache (PLPH), avoidance of more than 3 attempts in difficult LP cases since performing 4 or more attempts are associated with increased incidence of back pain, avoidance of collection of more than 30 mL of CSF as it is well documented that up to that volume does not increase risk for PLPH. A final key point that was emphasized in the CSF AUC, to help minimize fear of the procedure, is the importance of the attitude of the clinical staff and the importance of the provision of sensitive, matter-of-fact, verbal communication about the procedure to the patient by clinicians familiar and comfortable with LP (9).

Indications for LP and CSF AD biomarker testing.

The CSF AUC workgroup, following systematic literature reviews, discussions including a one-day face to face meeting, and a formal review process and voting procedure, assembled a listing of 14 possible clinical indications for LP and CSF AD biomarker A β 42, (sometimes normalized to A β 40), t-tau and p-tau181) testing. Based on the formal review process and voting, each of the 14 clinical indications was rated as either inappropriate (n=8) or appropriate (n=6) (Table 3). When describing the appropriate use criteria, the workgroup was fully aware and acknowledged in the report the limitations of currently available medical interventions for AD at a time when researchers pursue the development of disease-modifying treatments. The workgroup stated that CSF testing for AD biomarkers can help to establish an early and accurate confirmation, or rule out, AD pathology thereby helping to provide a foundation for best practice medical care including the following benefits: (1) education, advanced care planning and clinical care early in the disease process, including treatment with AD medications to treat symptoms; (2) necessary time to prepare for adjustments to work responsibilities, safe driving, and financial planning; (3) opportunities to enroll as participants in clinical trials aimed at delaying disease and with hope of providing benefits to other patients and families if successful.

As is the case with any disease process the decision to test, or not, ultimately rests with the patient's physician. The recommendations for testing are made in the context of the many other diagnostic tools often used in patients, usually together with a spouse or close family member, who seek diagnostic evaluation of their cognitive complaints. Thus, in the instance

of a patient with a diagnosis of MCI as described in a case report in this issue (38) following a detailed family history, head MRI to rule out space-occupying lesions or vascular disease, cognitive and memory tests, and a battery of routine laboratory tests for possible metabolic contributors are performed. Pending results of these evaluations if a diagnosis of MCI is made this is an indication that benefits from further testing CSF in order to determine whether or not the MCI is due to AD pathology or not and this is one of the 6 indications the CSF AUC workgroup concluded was appropriate for LP and CSF testing (Table 3). The finding “appropriate” was also a conclusion of the AUC for amyloid PET for this clinical scenario (34). An increasingly recognized clinical abnormality is subjective cognitive decline (SCD), considered in the CSF AUC but not yet recognized in 2013 when the AUC for amyloid PET was published, so not considered in the latter AUC. Patients who are placed in this pre-clinical category are cognitively unimpaired based on objective testing but who are deemed to be at increased risk for AD as compared to an earlier time-point by the patient, a family member and/or the patient’s physician (9). In the workgroup’s view CSF testing in this patient category is appropriate when the patient, a family member and physician all are concerned that the patient has experienced cognitive decline (9). On the other hand, if the SCD patient is found by the physician and family member, after thorough evaluation, to be at low risk for AD, CSF testing was judged to be inappropriate in this group of patients. Among some factors that influence this determination is *APOE ε4* status. Thus if *APOE ε4* is positive this will add weight to the clinician’s judgement about likelihood of presence of AD pathology and need for CSF testing. In the final determination of ordering, or not, CSF AD biomarker testing finally rests with the physician caring for the patient. This indication and judgement as appropriate or not appropriate as well as the others included in Table 3 are fully discussed in the CSF AUC report (9).

Another critical feature of AD pathology that is now well-recognized is heterogeneity. Many individuals with AD pathology at autopsy (amyloid plaques and neurofibrillary tangles) have additional pathology including the presence of Lewy-related pathology, vascular brain injury (VBI), TDP-43 deposits and hippocampal sclerosis, among other conditions (39,40). In moving from clinical diagnosis to biomarker-based detection of AD pathology in the living patient many biomarker studies are evaluating new candidate AD biomarkers to determine added value to the diagnostic utilities of CSF Aβ42, t-tau, and p-tau181. This is especially important in developing more refined prediction of disease progression and in deepening our understanding of the intricacies of AD disease progression throughout the several decades-long continuum that involves amyloidosis, tauopathy, and neurodegeneration (39,40). New biomarkers are under investigation, thought to reflect at least one neuropathologic pathway involved in AD progression, including synapse degeneration and loss, synucleinopathy, TDP-43 pathology or glial activity and inflammation (9).

National Institute on Aging-Alzheimer’s Association Research Framework: Toward a biological definition of Alzheimer’s disease.

One of the underlying principles of the CSF AUC is that AD disease pathology can be detected in living patients using validated surrogates for autopsy pathology such as CSF Aβ42, Aβ40, t-tau and p-tau181 measured by validated immunoassays or by validated mass

spectrometry methodology. The National Institute on Aging-Alzheimer Association (NIA-AA) framework emphasizes that these objective surrogate measurements of AD pathology, or their neuroimaging counterparts, overcome the limitations of clinical diagnosis alone for detection of AD pathology (41): 10-30% patients clinically diagnosed as AD do not have AD pathology at autopsy and similar percentages are devoid of amyloid pathology based on amyloid PET or CSF A β 42 analyses (42). Furthermore, since 30-40% of cognitively normal elderly individuals have abnormal amyloid based on either amyloid PET or CSF testing, reliable detection of AD pathology at its earliest stages is only achievable by biomarker testing (42). The presence of additional pathologies and the prospect of adding validated biomarkers that detect them is further testimony to the importance of moving toward biomarker-based disease detection (9). Table 4 summarizes the 8 possible combinations of A +/-, T +/- and (N) +/- profiles described by the A|T|(N) framework for defining AD based on biomarkers. A report recently described the application of the A|T|(N) framework to ADNI study participants (43). Baseline A|T|(N) combinations were used to predict MCI to AD dementia progression over four years by Cox Proportional Hazards modeling and, declines in memory (Mini-Mental Status Examination, MMSE), cognition (Clinical Dementia Rating sum of boxes, CDR-SOB) and function (Functional Activities Questionnaire, FAQ) were assessed, in 505 ADNI MCI participants who provided CSF and underwent FDG PET (43). In this analysis of ADNI MCI participants those who were A-T-N- at baseline visit had, respectively, the lowest (8.1%), and A+T+N+ the highest (80.9%) progression rates from MCI to a clinical diagnosis of early AD. For A+T-N- and A-T+N- the respective rates were 11.6% and 40.0%. The overall trends in clinical measures (memory-MMSE, cognition-CDR-SOB and function-FAQ) slope declines matched up well with rates of dementia progression across the ATN profiles in these MCI participants (43). Several studies that have utilized the A|T|(N) framework have confirmed the potential utility for this approach to refining the AD status and progression timeline of study participants based on biomarkers (43–45). As clearly described in the A|T|(N) framework this scheme is intended for research purposes but we believe it does provide a potentially valuable approach in future clinical practice especially since both fluid biomarkers as well as imaging biomarker data can be combined for the individual under study and importantly additional markers of disease pathology can be added as they become established (41–45).

The presence of mixed pathology in sporadic AD patients at autopsy is substantial as noted above and new biomarker tests for detection of concomitant pathologies such as Lewy Body Related pathology (LRP), Vascular Brain Injury (VBI) or TDP-43 deposits are needed but challenging to develop so far. The prevalence of co-pathology has been documented in a number of studies. For example, in a study of 1,440 autopsied participants across US Alzheimer's Disease Centers who had neuropathologic (NP) evidence of AD, 42% had ADNP only, 28.3% AD + Lewy Body Pathology (LBD), 20.4% AD + VBI and 9.3% AD + LBD + VBI (46). Thus two major sources of ADNP co-pathology are LRP and VBI and in this study the presence of co-pathology on clinical progression was greater dependent on the level of ADNP, greater in individuals with an intermediate level of ADNP vs those with high levels of ADNP at autopsy (46). Indeed, both in population-based and research autopsy cohorts, "pure" AD neuropathology represents the minority of patients (~35-45%), with most having varying contributions of alpha-synuclein Lewy bodies, TDP-43 pathology

and/or vascular brain injury (39). Indeed, at autopsy >50% of AD patients diagnosed clinically, have alpha-synuclein Lewy pathology together with AD neuropathology. There are currently no CSF or PET imaging biomarkers that can reliably detect alpha-synuclein Lewy pathology in vivo and it is unclear how LBD co-pathology may affect AD CSF biomarkers in clinical AD. Initial studies suggest that assays for CSF total and phosphorylated alpha-synuclein help differentiate pure AD from AD with mixed LBD pathology (47), but further replication is needed. CSF total alpha-synuclein assays have shown limited diagnostic accuracy in clinical LBD (i.e. DLB, Parkinson's disease-PD, PD with dementia-PDD), where in early drug naïve PD CSF alpha-synuclein concentrations are lower in group-wise comparison with healthy controls, but significant overlap between the two groups was observed (48,49). CSF A β 42 levels correlated with postmortem alpha-synuclein pathology, independent of plaque burden, suggesting CSF A β 42 levels in LBD may reflect, in part, alpha-synuclein-dependent mechanisms (50) as well but these results need further replication. TDP-43 pathology is also commonly detected in patients with a clinical diagnosis of AD and aging and has been linked to cognitive impairment (51). There are currently no CSF or PET imaging markers for TDP-43 and it is unclear how this mixed pathology may influence the interpretation of AD CSF biomarkers in the aging population.

Vascular cognitive impairment has a high prevalence which increases with aging and is characterized by a significant topographical and physio-pathological heterogeneity (53). It has been noted that the presence in the brain of VBI increases in prevalence with age (39,54). In addition, vascular risk factors have been associated with hallmark lesions of AD (55). Although currently not formalized within the NIA-AA Research Framework, several biomarkers (including advanced structural and functional MRI protocols, FDG-PET and CSF biomarkers of blood-brain barrier dysfunction and neurovascular dysfunction, like fibrinogen, PDGFR- β and albumin CSF/plasma ratio).have been proposed to characterize the multifaceted vascular lesions involving blood vessels and brain parenchyma *in vivo* (56).

Blood-based biomarkers.

There is an upsurge of interest in defining the clinical utilities of blood based biomarkers as screening tests for the detection of AD pathology. Whereas both imaging and CSF biochemical biomarkers have proven effective in detection of amyloid, tau, and neurodegeneration pathologies, imaging tests are limited by their high cost, and CSF testing is limited by the invasive nature of the test procedure despite its documented safety. On the other hand, blood- based biomarkers have the advantage of the relatively easier procedure that also supports longitudinal collection and lower cost. Thus, there is keen interest in assessments of their usefulness in treatment trials that enroll participants who have evidence of AD pathology. Amongst the most promising biomarkers to date for clinical utility are neurofilament light chain (NfL), p-tau181 and A β 42/A β 40 ratio as discussed in Budelier and Bateman in this issue of JALM (52,57–60). Rigorous analytical and clinical validation studies have been initiated and are required for these biomarkers and if achieved these three are candidates for A, T and (N) for the A|T|(N) framework and their availability would make possible cost-effective longitudinal sampling (e.g., annually or every six months).

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Table 1.

Sensitivity and specificity of CSF biomarkers or combinations of them based on a systematic review of the literature of studies with an N of >100 study subjects. In each of these studies AD diagnosis was determined clinically.

	Sensitivity	Specificity	N*
AD vs HC			
A β 1-42	81.6	82.9	19
t-tau	82.5	86.2	26
p-tau	78.8	79.1	16
A β 1-42 + t-tau	88.7	88.8	16
AD vs non-AD			
A β 1-42	75.4	70.8	16
t-tau	75.4	77.6	18
p-tau	75.2	77.4	15
A β 1-42 + t-tau	86.5	83.7	10
A β 1-42 + p-tau	95.7	89.5	5

* Number of studies with N>100; data in this table adapted from reference #10.

Table 2.

Sensitivity and specificity of CSF biomarkers or ratios based on a systematic review of the literature. In each Of these studies either amyloid PET or autopsy was the basis for detection of AD pathology.

	Sensitivity	Specificity	AUROC*	N
4 consecutive memory clinic studies; amyloid PET				
A β 1-42	87.6	85.2	0.90	748 participants
A β 1-42/A β 1-40	96.0	91.3	0.96	315 participants
13 case control, multicenter & cohort studies; amyloid PET				
A β 1-42	93.2	84.5	0.93	2,346 participants
A β 1-42/A β 1-40	96.0	88.0	0.94	200 participants
5 case control or cohort studies; autopsy diagnosis of AD				
A β 1-42	90.0	84.0	0.92	764 participants
A β 1-42/t-tau	88.7	88.3	0.93	
A β 1-42/p-tau181	92.3	82.3	0.91	

* Area Under the Receiver Operating Characteristic curve; data from reference #9 Appendices.

Table 3.

Clinical indications for appropriate use of LP and CSF testing in the diagnosis of AD.

No.	Indication	Rating
1	Cognitively unimpaired and within normal range functioning for age by objective testing; no conditions suggesting high risk and no SCD or expressed concern about developing AD.	Inappropriate
2	Cognitively unimpaired patient based on objective testing, but considered by patient, family informant, and/or clinician to be at risk for AD based on family history.	Inappropriate
3	Patients with SCD who are considered to be at increased risk for AD	Appropriate
4	Patients with SCD who are not considered to be at increased risk for AD	Inappropriate
5	MCI that is persistent, progressing, and unexplained	Appropriate
6	Patients with symptoms that suggest possible AD	Appropriate
7	MCI or dementia with an onset at an early age (<65)	Appropriate
8	Meeting core clinical criteria for probable AD with typical age of onset	Appropriate
9	Symptoms of REM sleep behavior disorder	Inappropriate
10	Patients whose dominant symptom is a change in behavior (e.g. Capgras Syndrome, paranoid delusions, unexplained delirium, combative symptoms, and depression) and where AD diagnosis is being considered	Appropriate
11	Use to determine disease severity in patients having already received a diagnosis of AD	Inappropriate
12	Individuals who are apolipoprotein <i>E</i> (<i>APOE</i>) ϵ 4 carriers with no cognitive impairment	Inappropriate
13	Use of LP in lieu of genotyping for suspected ADAD mutation carriers	Inappropriate
14	ADAD mutation carriers, with or without symptoms	Inappropriate

Abbreviations: AD, Alzheimer's disease; LP, lumbar puncture; REM, rapid eye movement; SCD, subjective cognitive decline; ADAD, autosomal dominant Alzheimer's disease; MCI, mild cognitive impairment.

*
Table adapted from reference #9.

Table 4.

Biomarker profiles and categories for the NIA_AA Framework (41).

A T (N) profiles	Biomarker category	
A- T- (N)-	Normal AD biomarkers	
A+ T- (N)-	Alzheimer's pathologic change	Alzheimer's continuum
A+ T+ (N)-	Alzheimer's disease	
A+ T+ (N)+	Alzheimer's disease	
A+ T- (N)+	Alzheimer's & concomitant suspected non-alzheimer's pathologic change	
A- T+ (N)-	Non-AD pathologic change	
A- T- (N)+	Non-AD pathologic change	
A- T+ (N)+	Non-AD pathologic change	

A=amyloid plaque burden measured by CSF A β 42 or A β 42/A β 40 or by amyloid PET; T=tau pathology measured by CSF p-tau181 or tau PET; (N) neurodegeneration, measureable using FDG-PET or MRI. When blood based biomarkers become validated they may substitute for CSF biomarkers.