Lights and Shadows of Cerebrospinal Fluid Biomarkers in the Current Alzheimer's Disease Framework

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Abstract.
Background: The most significant biomarkers that are included in the Alzheimer's disease (AD) research framework are amyloid-β plaques deposition, p-tau, t-tau, and neurodegeneration.

Although cerebrospinal fluid (CSF) biomarkers are included in the most recent AD research criteria, their use is increasing in the routine clinical practice and is applied also to the preclinical phases of AD, including mild cognitive impairment. The role of these biomarkers is still unclear concerning the preclinical stage of AD diagnosis, the CSF methodology, and the costs-benefits of the biomarkers' tests. The controversies regarding the use of biomarkers in the clinical practice are related to the concepts of analytical validity, clinical validity, and clinical utility and to the question of whether they are able to diagnose AD without the support of AD clinical phenotypes.
Objective: The objective of the present work is to expose the strengths and weaknesses of the use of CSF biomarkers in the diagnosis of AD in a clinical context.
Methods: We used PubMed as main source for articles published and the final reference list was generated on the basis of relevance to the topics covered in this work.
Results: The use of CSF biomarkers for AD diagnosis is certainly important but its indication in routine clinical practice, especially for prodromal conditions, needs to be regulated and also contextualized considering the variety of possible clinical AD phenotypes.
Conclusion: We suggest that the diagnosis of AD should be understood both as clinical and pathological.

Keywords: Alzheimer's disease, analytical validity, ATN model, clinical utility, clinical validity, CSF biomarkers, diagnosis, mild cognitive impairment, probabilistic model

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disease that provokes neuronal loss and dysfunction primarily causing memory loss and cognitive decline. At the pathological level, AD is characterized by the accumulation of amyloid-β (Aβ) plaques in the extracellular space of the brain and by

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hyperphosphorylated tau deposition intracellularly [1]. During the last thirty years, its diagnostic criteria have been subjected to significant changes. Initially, the diagnosis was only clinical, based on probability and could not be certain until the postmortem examination. Advanced techniques have made possible recently to improve diagnostic accuracy. The diagnosis of AD is more likely when Aβ levels are low in the cerebrospinal fluid (CSF), while total-tau (t-tau) and phosphorylated-tau (p-tau) proteins present high concentrations.

At present, the most significant biomarkers that are included in the AD research framework are Aβ plaques deposition, p-tau, t-tau, and neurodegeneration.

There is growing awareness, in the daily evaluation of patients, that the clinical criteria for the diagnosis of dementia are insufficient [2–4] and that the clinical diagnosis will increasingly require biomarker confirmation.

Indeed, although CSF biomarkers are included in the most recent AD research criteria, their use is increasing in the routine clinical practice, and is applied also to the preclinical phases of AD, including mild cognitive impairment (MCI).

During the last twenty years it has been shown that the combination of CSF biomarkers, which measure the concentration and degradation of proteins at any time point along the course of the disease, and neuroimaging biomarkers, which correlate to the pathological load overtime, allows the highest accuracy for AD diagnosis [5].

In 2007, the International Working Group (IWG) for New Research Criteria for the Diagnosis of Alzheimer's Disease provided a new conceptual framework that proposed to anchor the diagnosis of AD on the presence of biomarkers [6] (see Table 1, adapted from Dubois and colleagues [7]).

The first important refinements to the 2007 criteria were made by the IWG in 2010 [8]. A lexicon was proposed that clearly drew a distinction between the presence of clinical disease (AD) and neuropathology (AD pathology).

In 2014 new IWG research diagnostic criteria for AD were published to provide a simplified algorithm based on specific AD clinical phenotypes with *in vivo* evidence of AD pathology through either the presence of Aβ and tau in CSF or positive amyloid positron emission tomography (PET) scan [9].

This diagnostic algorithm reinforced the notion of AD as a clinic biological entity with cognitive/behavioral and biological components. The authors underlined in the conclusions that the proposed refinements concern the diagnosis of AD in the research setting.

In 2011, the National Institute on Aging and Alzheimer's Association (NIA-AA) created a set of diagnostic guidelines for the symptomatic or "clinical" stages of AD, that is, MCI and dementia [10, 11]. Recommendations were also presented for a stage of AD in individuals without overt symptoms, called "preclinical AD" [12] (Table 1).

In 2018 a better understanding of biomarkers induced the NIA-AA to update and unify the 2011 guidelines [13]. The research AD diagnostic criteria were no more based on the clinical symptoms, but rather shifted towards a biological construct. The research criteria for AD were based on three markers of disease: Aβ deposition (A), pathologic tau (T), and neurodegeneration (N) (commonly referred to as the ATN system), in order to place a subject on the AD spectrum [5, 13].

This unifying update was labelled a "research framework" because its intended use was for observational and interventional research, not routine clinical care, as stressed by NIA-AA [13].

It was suggested that researchers should apply the criteria of the framework to their individual studies and adjust the template in relation to the specific goals of their clinical trials. Further research of alternative testing was also encouraged, as biomarkers might not always be available or appropriate for each specific research goal [13].

In April 2021, the IWG published a Personal View in which it recommends that AD diagnosis should be restricted to people who have positive biomarkers together with specific AD phenotypes, whereas biomarker-positive but cognitively unimpaired individuals should be considered only at-risk for progression to AD [7]. In other words, IWG suggests that the diagnosis of AD should be clinical–biological requiring the presence of both a specific clinical phenotype of AD (phenotype positive) and biomarker evidence of AD pathology (amyloid-positive and tau positive) [7] (Table 1).

In July 2021, according to the NIA-AA research framework, Hampel proposed that validated biomarkers reflecting additional important AD-related pathophysiological processes could enrich and expand the AT (N) system towards an ATX (N) system. Particularly vascular, inflammatory or synaptic biomarkers could be integrated to create ATV (N), ATI (N), or ATS (N) systems, respectively [14]. The authors points out that the AT (N) system remains a

Table 1
Details of successive proposed criteria for Alzheimer's disease diagnosis

	NINCDS-ADRDA 1984	IWG 2007	IWG 2010	NIA-AA 2011	IWG 2014	IWG/AA 2016	NIA-AA 2018	IWG 2021	NIA-AA 2021
Applicable settings	Research and clinical	Research	Research	Research and clinical	Research	Research	Research	Research and Clinical	Research
Clinical Requirements	Dementia, (memory changes, and another cognitive impairment)	Amnestic syndrome of a hippocampal type	Amnestic syndrome of a hippocampal type, posterior cortical variant, logopenic variant, or behavioral-frontal variant	MCI (amnestic or non-amnestic) or dementia	Amnestic syndrome of a hippocampal type, posterior cortical variant, logopenic variant, or behavioral-frontal variant	NONE	NONE	Amnestic variant, posterior cortical atrophy, logopenic variant primary progressive aphasia, behavioral or dysexecutive frontal variant, corticobasal syndrome, semantic and nonfluent variants of primary progressive aphasias*	NONE
Biological requirements	NONE	CSF biomarkers MRI atrophy, ^{18}F-fluorodeoxyglucose PET hypometabolism, amyloid PET positive, or AD autosomal dominant mutation	Pathophysiological markers: CSF changes (low CSF Aβ_{42}, high phosphorylated tau, or high total tau) or amyloid PET positive	Aβ marker (CSF or PET) or marker of degeneration (CSF tau, phosphorylated tau, ^{18}F-fluorodeoxyglucose-PET, and T1-weighted MRI)	CSF Aβ and tau or amyloid PET positive	Aβ marker (CSF or PET) and tau marker (CSF or PET)	Aβ marker (CSF or PET) and tau marker (CSF or PET)	Aβ marker (CSF or PET) and tau marker (CSF or PET)	Aβ marker (CSF or PET) and tau marker (CSF or PET) and neuronal injury and neurodegeneration (FDG PET, MRI, NFL) + possible vascular, inflammatory or synaptic biomarkers to be integrated in the future

Reprinted/adapted from *Lancet Neurology*, Vol. 20, Dubois et al., Clinical diagnosis of Alzheimer's disease: recommendations of the International Working Group, pp. 484–496, 2021 [7], with permission from Elsevier. AD, Alzheimer's disease; ADRDA, Alzheimer's Disease and Related Disorders Association (now the Alzheimer's Association) Work Group; IWG, International Working Group criteria; IWG–AA, International Working Group and Alzheimer's Association joint criteria; NIA–AA, US National Institute on Aging and Alzheimer's Association joint criteria; NINCDS, US National Institute of Neurological and Communicative Disorders and Stroke criteria. *Cognitively unimpaired individuals are considered at-risk for AD.

research framework and much more work is required before it can be introduced into clinical practice [14].

More recently, Frisoni et al. proposed a probabilistic model of AD diagnosis [15], providing arguments in favor of the recommendations of the IWG.

The objective of the present work is to expose the strengths and weaknesses of the use of CSF biomarkers in the diagnosis of AD in a clinical context, proposing some recommendations on their more correct use in daily clinical practice.

METHODS

We used PubMed as main source for articles published in English between January 1, 2007, and December 1, 2021, using the search terms "biomarker" OR "amyloid" OR "tau" OR "neurodegeneration" OR "preclinical" OR "CSF" OR "PET" OR "mild cognitive impairment" AND "Alzheimer's disease" OR "ATN classification". We also searched the references of relevant articles. The final reference list was generated on the basis of relevance to the topics covered in this work.

RESULTS

Biomarkers CSF analysis requires a lumbar puncture (LP) procedure. The procedure is invasive for the patient and sometimes has side effects such as back pain and headache [16]. The fluid is analyzed through enzyme-linked immunosorbent assays (ELISA) and the levels of relevant enzymes and proteins, such as biomarkers, provide insight into the diagnosis of AD [17].

Public healthcare costs for AD diagnosis, increase when clinicians decide to add tests that are not always routinely used in the common clinically based framework yet, as it is for CSF tests. Handels et al. [18] estimated a mean additional cost of €432 per patient when adding the lumbar puncture to the usual-care diagnostic workup for the prognosis of progression to dementia in MCI. The authors created a mathematical model to estimate the costs and quality-adjusted life years (QALYs) based on the effects of reducing worry and stigmatization, the impact of a false-positive/negative prognosis and the side effects of the LP. In addition, they highlighted that this simulation is not yet transferable to clinical practice and must remain in the research stages for further development.

Furthermore, Valcárcel-Nazco et al. [19] highlighted that patients with MCI who have no symptoms

of dementia obtain a cost-effectiveness benefit of the combined use of Aβ and tau proteins against the application of the standard clinical diagnostic criteria of AD alone. This benefit decreases significantly for patients with clear symptoms of dementia. This means that introducing the CSF test for all patients with dementia does not imply a better cost-effectiveness prognostic outcome [20].

An objective constraint on the diffusion of the LP procedure is the high number of cases of MCI and dementia compared to the availability of Memory Units with trained specialized personnel who could perform LP.

It has been suggested that less invasive procedures than CSF biomarkers levels, could include analysis of proteins from swabs of saliva or blood. In 2019, recognizing the major unmet need for blood-based diagnostic tests to identify AD earlier than current practice allows, the U.S. Food and Drug Administration granted a "Breakthrough Device" designation for a blood test through a federal program designed to accelerate the path to approval. As a result, in 49 US States a test is now available, which quantifies $A\beta_{42/40}$ ratio and detects Apolipoprotein E proteotype in blood samples, using a mass spectrometry platform [21, 22]. On the other hand, there is still ongoing debate about which specific proteins and enzymes to consider in the blood analysis of patients with cognitive decline, alongside the limitation of not having standardized values and methodology.

Some promising biomarkers with potential to be included in the assay profile are CSF neurogranin, which measures synaptic degeneration and loss of the neuropil, or the neurofilament light chain (NFL) which measures axonal injury [23]. Neurogranin is concentrated at the dendritic spines mostly in the cortex, hippocampus, and amygdala. Levels of this protein are elevated in AD and MCI patients, and some studies suggest that it is specific to AD [23]. High levels of NFL in the plasma, along with a positive amyloid PET for patients with MCI, have been shown to predict faster cognitive decline, future brain atrophy, and brain hypometabolism. Concerning plasma NFL, Hampel et al. [24] stated that high plasma (or CSF) NFL shows a neurodegenerative ongoing process in the preclinical stage, however it is not specific for AD and it can be found in several other neurodegenerative disorders such as frontotemporal dementia, progressive supranuclear palsy, corticobasal syndrome, and dementia with Lewy bodies [25–27]. Therefore, plasma NFL might be used as a tool in the initial primary care for detec-

tion of neurodegeneration in patients with cognitive deficits [24].

In addition, the Beta-site Amyloid precursor protein Cleaving Enzyme 1 (BACE1) is known to be the first step in the generation of Aβ peptides and is present in MCI and AD with higher levels than healthy elderly individuals. It is thought to predict the progression of MCI to AD, due to the observation that the activity of the enzyme was higher in MCI patients who, within three years, developed AD, versus those who remained cognitively stable within the same period [24].

Introducing blood tests as part of patients screening would allow early detection, reduction of costs and would provide a less invasive alternative to CSF procedure. Access to a blood test would facilitate the subjects' inclusion in trials for disease modifying-therapies as well as continuous monitoring of the progression of the disease at the individual level [24].

The blood test-based biomarkers will be much less invasive and more widely available than current CSF procedure and will dramatically change the management of AD.

The NIA-AA has grouped "distinct profiles and categories" of each biomarker [13]. Profiles refer to the presence or absence of a biomarker, and categories refer to where on the AD continuum the patient falls based on the biomarkers. These profiles facilitate our understanding of the patients' disease and whether they are considered to be or not in the AD disease continuum [13]. However, more research is needed to understand the biomarker signature of different types of dementia.

A limitation of the 2011 NIA-AA recommendations was that biomarkers were grouped into just two categories, amyloid and tau-related neurodegeneration. AT(N) classification provides a solution to this problem, which is to separate biomarkers that are specific for tau pathology from those that are nonspecific measures of neurodegeneration/neuronal injury [13]. These possibly coexisting pathological characteristics have their own effects on the proteins in plasma, further complicating the profile analysis and process to isolate biomarkers relevant to AD [24].

To summarize, there is room for improvement for the biomarkers' framework. For example, including a profile for vascular comorbidities might have a significant impact on the time of onset of dementia, and on the interpretation of CSF neurogranin and the NFL dosages. Understanding the biology of specific biomarkers may lead to improvement in pharmaceutical development, advances in clinical tri-

als designs, contributing to precision medicine and personalized therapies. Biomarker-guided medicine considers methodological advancements and discoveries of the comprehensive pathophysiological profiles of complex multi-factorial neurodegenerative diseases, such as AD [28]. Phenotypic, lifestyle, and psychosocial characteristics, together with genetic data, and biomarkers would make it possible to create a specific "fingerprint" for each patient, directing towards the creation of personalized interventions and therapies [29, 30]. In this approach, also the molecular profiles obtained from the multiple omics (genomics, transcriptomics, proteomics, and metabolomics) perspectives are crucial to create a personalized biomarker-targeted treatment [31].

New and more refined computational analysis techniques, such as machine learning, have been identified to manage the enormous amount of data deriving from the study of omics also in AD. Indeed, machine learning integrates and interprets complex data in scenarios where traditional statistical methods may not be satisfactory [31]. Recently, Clark et al. [32] performed multi-level CSF omics in a cohort of older adults with normal cognition, MCI, and mild dementia. Analyzing proteomics, metabolomics, lipidomics, one carbon metabolism, and neuroinflammation related molecules, they reported novel molecular and pathways alterations associated with AD pathology. These findings are relevant for the development of personalized diagnosis and treatment approaches in AD. In clinical practice, biomarker tests are usually a third option, after psychological assessments and neuroimaging exams, mainly due to the high cost of the procedure, lack of standardization of analytical procedures, and the LP procedure being invasive [33]. Furthermore, patients generally enter the National Health Care system in more advanced stage of dementia. Often patients are referred to the Memory Units with advanced dementia when CSF biomarkers may not be of additional diagnostic value.

The role of available CSF biomarkers is still unclear concerning the preclinical stage of AD diagnosis, the CSF methodology, and the costs-benefits of the biomarkers' tests.

The controversies regarding the use of biomarkers in the clinical practice are related to the concepts of analytical validity, clinical validity and clinical utility [34]. Analytical validity defines how well the test measures what it claims to measure (accuracy, precision, and reproducibility). Clinical validity refers to how well the test measures clinical features of the

disease or treatment outcomes (the relevance of the test as a guide to clinical decision-making). Clinical utility determines how a test can improve patient's outcomes, confirm or change the diagnosis, identify at-risk individuals, and influence therapeutic choices (the balance between benefits and harms in patient's management).

According to Canevelli and colleagues [35], CSF AD biomarkers research lacks of proven methodology: primarily lack of reference values, standards, and statistical models.

The validation of a biomarker used to identify pathology requires knowledge of its distribution in healthy individuals and therefore of a reference group. Individual characteristics that should be considered include ethnicity, sex, clinical history, and frailty, as these could have significant impact on the expression of pathology [36].

In 2017, the Geneva AD Strategic Biomarker Roadmap (SBR) Initiative adapted a framework for the systematic validation of biomarkers to CSF AD biomarkers, encompassing the 42 amino-acid isoform of $A\beta_{42}$, p-tau, and t-tau, with the aim to accelerate their development and clinical implementation [37].

The SBR structures and the validation of AD diagnostic biomarkers into a systematic sequence of five phases each encompassed primary and secondary aims. Phases 1–2 entail the assessment of analytical validity, Phases 3–4 clinical validity, and Phase 5 clinical utility [37].

In 2017, SBR assessed the validation status of the neuropsychological assessment, and of most consolidated AD biomarkers at that time, i.e., amyloid imaging [38], CSF [39], hippocampal atrophy [40], FDG-PET [41], and biomarkers for dementia with Lewy bodies [42], based on evidence published until 2015.

On March 5, 2021, an update of the current validation status of CSF AD biomarkers based on the Biomarker Roadmap methodology was published: Leuzy et al. [43] showed advances such as a unified protocol for the sampling, handling and storage of liquor, the introduction of certified reference methods and materials for $A\beta_{42}$, and the introduction of fully automated assays. Further progress was obtained in defining thresholds for biomarker positivity and assessing the impact of interfering factors. In his work, he showed that the analytical validity was satisfied, while the clinical validity was satisfied only for the early disease stage but not for real world performances. Unfortunately, the clinical utility remained

an unmet goal since disease-modifying treatments for AD are still lacking [43].

On March 10, 2021, a further update on the validation of AD diagnostic biomarkers was published [44]: Boccardi et al. revised the SBR to update it to the current A/T/N framework for research on AD and related disorders [13] and to enable proper assessment of biomarkers of tau pathology [38, 43, 45–47]. With the A/T/N framework, biomarkers were examined and assessed for their individual contribution to an AD or non-AD profiles, allowing more precise diagnosis.

The A/T/N criteria define a clear role of tau biomarkers in the diagnostic procedure of patients. In particular, their positivity is required to define clinical AD, and their positivity in $A\beta$-negative patients denotes the presence of a neurodegenerative disorders belonging to a non-AD continuum.

DISCUSSION

There is large variability between neuropathology and phenotypic clinical expression; some subjects with no clinical evidence of AD have had biomarkers suggesting the presence of the disease. The opposite has also been found in people with AD clinical symptoms but inconclusive biomarker profile [13]. If the presentations of AD are regarded as a continuum, the same should be said for the diagnosis. The dichotomous labelling system of outcomes (abnormal versus normal), should be replaced by a scale (improved, stable, worsened) due to the variable trajectory of the disease. Individuals with an MCI diagnosis might even revert to normal cognition [35]. Currently, there are no biomarkers linked to clinical trajectory or definitive progression of AD [35]. Therefore, validating a biomarker against clinical criteria alone would not result in a reliable prognosis.

What is needed for a more accurate diagnosis with biomarkers are stable reference values that predict when the biomarkers might have a significant effect on cognition. Toward this goal, extensive information needs to be collected for a comparative analysis on the beta amyloid and tau normal range levels in healthy individuals. However, different cut-offs might be needed to answer different research questions based on their aim, which further complicates finding a standard value. For example, cut-offs might be more lenient considering a study on the early advancement of the disease versus stricter cut-offs in a study considering more specific research questions that need higher diagnostic certainty [13]. The Global

Biomarkers Standardization Consortium (GBSC) is working to create a quality control (QC) system to test CSF analysis proficiency across laboratories and maintain protocols for standardization, such as standard methods of analysis, equipment, and proteins to test [48].

Mattsson [17] suggests the method of Selected Reaction Monitoring (SRM) to facilitate the definition of reference points for the $A\beta_{42}$ proteins and peptides analyzed in CSF. This process filters and quantifies small molecules but is a complicated procedure because of the many variables and possible post-translational modifications of $A\beta$. Currently, the measurements of proteins are mostly immunoaffinity-based, but many aspects are hard to control and calibrate to reach standard values. SRM has the benefit that the quantification of the CSF proteins values is unaffected by sample preparation and storage method, unlike the immunoaffinity methods [17].

Importantly, tau is sought to be a dynamic biomarker, not specific to AD, that could facilitate the discovery of therapy efficacious on axonal degeneration [17]. Researchers are working to find a way to connect the different methods of quantification and extract the most important variables for reference standards of tau CSF.

To consider the validation of the biomarkers, Sackett and Haynes [49] proposed to answer five questions in order to evaluate the algorithm of the diagnostic process (Table 2 adapted from Canevelli and colleagues [35]). Very few studies have managed to advance through Phase III, assessing pragmatics and clinical utility, and none have completed Phase V, considering conclusive cost-effectiveness. No biomarker so far has yet been identified to predict clinical benefit endpoints [49].

Each phase requires different statistical methods to progress to the subsequent question, such as null hypothesis testing, prediction models, inferential testing, and cost-effectiveness analysis (Table 2). The main concern with this aspect is that conclusive and communicative models are needed to provide a complete profile on an individual's clinical history.

In addition, the use of pharmacological interventions further challenges the use of AD biomarkers. Acetylcholine (ACh) plays an important role within the cholinergic system of the brain consolidating memory and learning processes. Inhibition of acetylcholinesterase (AChE), an enzyme which hydrolyses ACh, has been linked to a decrease in $A\beta$ aggregates [50]. Anti $A\beta$ drugs like Aducanumab, a human monoclonal antibody, target and reduce $A\beta$ aggregates by binding to both soluble and insoluble aggregated forms of $A\beta$ including oligomers, protofibrils and fibrils [51, 52].

The approval of Aducanumab on June 7, 2021, by the Food and Drugs Administration (FDA) will offer valuable insight into the trajectory of drug development for monoclonal antibodies in AD and other neurodegenerative diseases and will make the use of biomarkers of $A\beta$ even more important [53].

The ATN model expresses a deterministic pathophysiological model in which the amyloid cascade is a necessary and sufficient condition for the consequent phenotypic expression of AD. This construct is sustainable for autosomal dominant cases and for those with *APOE ε*4 but does not find great confirmation for sporadic cases.

In fact, in the latter, the phenotypic manifestation of the disease is probably also conditioned by stochastic contextual factors such as lifestyles and environmental factors. The Lancet Commission on Dementia Prevention, Intervention, and Care estimated that 40% of all cases of dementia are due to 12 modifiable risk factors: hypertension and obesity in midlife, and smoking, physical inactivity and diabetes in later life, lower education level in early life, hearing loss, traumatic brain injury and alcohol abuse in midlife, and depression, social isolation, and air pollution in later life [54].

The concept of cognitive reserve also introduces a component of variability in the possible clinical manifestation of the disease: a greater cognitive reserve

Table 2
Diagnostic research questions

Phase I	Do the test results in patients with the target disorder differ from those in normal people?
Phase II	Are patients with certain test results more likely to have the target disorder than patients with other test results?
Phase III	Does the test result distinguish patients with and without the target disorder among patients in whom it is clinically reasonable to suspect that the disease is present?
Phase IV	Do patients who undergo this diagnostic test have better health outcomes than similar patients who are not tested?
Phase V	Does the use of the diagnostic test lead to better health outcomes at acceptable costs?

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can prevent or slow down the phenotypic presentation of the AD [55].

Preliminary evidence suggests that the different composition of the intestinal microbiota could also be a factor of variability in the clinical manifestation of AD [56, 57].

Finally, demographic variables, such as age, sex, and ethnicity, can influence the onset of AD [58].

If the onset of AD follows a deterministic model, the various clinical trials conducted using anti-Aβ drugs, interrupting the amyloid cascade in the early or prodromal stages should prevent the onset of symptoms of the disease. This concept still needs validation [59–61]. It is more likely that the complexity of neurodegenerative diseases, such as AD, will require multiple therapeutic strategies, as happens with several chronic conditions, such as hypertension, diabetes, etc.

All these observations suggest that a probabilistic model better explains the reality we observe daily in clinical practice in which lifestyles and demographic and environmental factors can modulate the phenotypic expression of AD [15].

In the probabilistic model, amyloid deposition is still a key factor in the pathophysiology of AD but its role is considered especially important in cases where genetic risk factors, such as autosomal dominant AD and *APOE ε*4-related sporadic AD, are also present [15].

The conceptual framework expressed by the 2021 recommendations of the IWG [7] supports a probabilistic model and fits well with what we observe in our patients.

Recommendations

The above concepts suggest some recommendations for transferring the diagnostic criteria created for research into clinical practice.

We suggest that the diagnosis of AD should be understood both as clinical and pathological. It should require the presence of both a clinical phenotype of AD (phenotype positive) and biomarker evidence of AD pathology (amyloid-positive and tau positive) [7]. The positivity of both amyloid and tau biomarkers is required because an amnestic phenotype with only amyloid positivity is not specific to AD and is seen in other neurodegenerative diseases [7].

Recommended biomarker measures for Aβ pathology are low CSF Aβ$_{42}$, or high tracer retention in amyloid PET. For tau pathology, are recommended high CSF phosphorylated tau (not total tau because of low specificity) or increased ligand retention in tau PET.

We propose that the diagnosis of AD should be established only in the presence of specific clinical presentations and of the demonstration of a contextual Aβ and tau pathology. In particular, clinical phenotypes commonly associated with the disease are the amnestic syndrome of the hippocampal type [62], the posterior cortical atrophy variant [63], and the logopenic variant primary progressive aphasia [64] (Table 3, adapted from Dubois and colleagues [7]).

In the case of atypical clinical presentations (behavioral or dysexecutive variants, corticobasal syndrome, and semantic or non-fluent variants of primary progressive aphasia) even in the presence of

Table 3
Alzheimer's disease diagnosis in clinical setting and likelihood of Alzheimer's disease as a primary diagnosis

	COMMON AD Phenotypes Amnestic variant, logopenic variant of primary progressive aphasia and posterior cortical atrophy	UNCOMMON AD Phenotypes Behavioral or dysexecutive variant, corticobasal syndrome, non-fluent variant of primary progressive aphasia, and semantic variant of primary progressive aphasia	OTHER Phenotypes (e.g., dementia with Lewy bodies, Richardson syndrome, Huntington disease, and amyotrophic lateral sclerosis)
A + T+	Highly probable- established	Probable	Unlikely
A + T?	Probable	Possible	Unlikely
A + T–	Probable	Possible	Unlikely
A? *T*+	Possible	Unlikely	Unlikely
A- *T*+	Possible	Unlikely	Unlikely
A- T?	Unlikely	Highly unlikely – excluded	Highly unlikely – excluded
A? T–	Unlikely	Highly unlikely – excluded	Highly unlikely – excluded
A-- T–	Highly unlikely – excluded	Highly unlikely – excluded	Highly unlikely – excluded
A? T?	Not assessable	Not assessable	Highly unlikely – excluded

Reprinted/adapted from *Lancet Neurology*, Vol. 20, Dubois et al., Clinical diagnosis of Alzheimer's disease: recommendations of the International Working Group, pp. 484–496, 2021 [7], with permission from Elsevier. A + /T+, Amyloid positive/Tau positive; A– /T–, Amyloid negative/Tau negative; A? /T?, Amyloid unknown/Tau unknown.

positive biomarkers, the diagnosis of AD cannot be made with certainty (AD co-pathology or atypical forms of AD) (Table 3).

In these situations, it would be advisable to follow the patient over time to identify the final diagnosis.

Conversely, we propose that the investigation of pathophysiological biomarkers in cognitively unimpaired individuals should be avoided (unless for research purposes), since a biomarker positive status (amyloid-positive and tau-positive) alone, lacks a predictive value for subsequent progression to cognitive decline and ultimately to dementia.

In this regard, we argue that, given the multifactorial and heterogeneous nature of AD, progresses in the capability to identify those individuals who are at higher risk of developing AD are more likely to happen if a less rigid framework for AD pathophysiology will be adopted.

Conclusion

Some limitations of the research framework for CSF AD biomarker analysis have been highlighted, and suggestions are made that could foster a harmonized transition from research criteria to clinical practice. In addition, the use of biomarkers is limited by the invasiveness of CSF procedure while blood biomarkers tests are advancing rapidly. The clinical validity and utility of AD biomarkers are still being developed to better understand the relationship of biomarkers to underlying pathology.

This review supports the thesis that the diagnosis of AD must consider the clinical phenomenology and the direct relationship with the patient, suitably integrated with the knowledge of the most recent and modern biomarkers.

DISCLOSURE STATEMENT

Authors' disclosures available online (https://www.j-alz.com/manuscript-disclosures/21-5432r2).

DISCLAIMER

The views expressed in this publication are the sole responsibility of the authors and do not necessarily reflect the views of the affiliated organizations.

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