The Role of Central Laboratories in AD Trials

PEER REVIEWED

Alzheimer's disease (AD) is a debilitating neurodegenerative disorder that mainly affects the elderly population. The prevalence of AD is predicted to increase to 115.4 million by 2050 [1]. Despite having the first drug, Tacrine, approved by the FDA in 1993, there has been little progress made in the development of pharmacotherapy for AD treatment. Currently there is no cure for AD, and treatments are symptomatic only. Even with the investment of more than a billion dollars by pharmaceutical companies, there have been no reliable disease-modifying therapies discovered that have received FDA approval[2]. It is imperative to find better treatments for AD to alleviate patients' and their families' suffering, and to prevent a potential AD-associated global economic crisis. Recently, major changes were made in the diagnostic criteria for AD and encompass the latest findings in the field, which is helping to facilitate more clinical research. Already, the usual clinical interview can be coupled with brain imaging testing approved by the FDA for the diagnosis of AD in live patients. Several disease-modifying or disease-preventing therapies are also appearing in clinical trials. Central laboratories can play a key role in clinical trials for AD drug development by assisting with patient population identification and by detecting, at an early stage, drugs that are likely to fail, leading to important time and costs savings. This article will discuss the latest advances in the AD field and explain how central laboratories can contribute to the progress in the battle against this disease by supporting clinical trials through quality testing.

Alzheimer's Disease (AD) Diagnostic Criteria

A century ago, Alois Alzheimer performed an autopsy on one of his patients who had suffered from early onset of dementia. This autopsy revealed the presence of sclerotic plaques spread throughout the cortex with neurofibrillary tangles, known today as hallmarks of the disease named after him. AD is a progressive neurodegenerative disorder characterised by a gradual, irreversible impairment of cognition, memory and personality leading to dementia [3]. In general, AD affects the older population, with familial cases accounting for less than 1%. So far, AD mutations have been identified mainly in genes encoding for the proteins involved with the formation of amyloid plaques [4]. The clinical diagnosis of AD was first described in 1984 by the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and the Alzheimer's Disease and Related Disorders Association (ADRA)[5]. New developments in the AD field have initiated the revision of NINCDS-ADRDA diagnostic criteria. In 2012, new criteria and guidelines were proposed by expert workgroups spearheaded by the Alzheimer's Association (AA) and the National Institutes of Health's (NIH's) National Institute on Aging (NIA). These guidelines reflect new advances in the field aimed at facilitating clinical research. The major change from 1984 in AD diagnostic criteria is the acknowledgement of AD as a progressive disease that begins decades before the appearance of clinical symptoms, and the addition of biomarker tests. The new guidelines classify AD into three stages: 1) preclinical, asymptomatic stage (intended for research use only); 2) mild cognitive impairment; and 3) dementia, the end stage of the disease. In addition, the preclinical AD can be staged with biomarkers from stage 1 to stage 3, although it is for research use only at this moment (Table I) [6,7,8,9]. These changes in AD diagnostic criteria have an important impact on clinical trials because they open the door for early-stage drug development and AD prevention trials.

Diagnostic Tests in AD

In the past, the 1984 NINCDS-ADRDA criteria were used with a sensitivity of 81% and specificity of only 70%, with postmortem brain examination as the gold standard for AD diagnosis [5]. These criteria were not very helpful for clinical trials of drug development where the effect of drug treatments could be confirmed postmortem only. In addition, the diagnosis of AD was given when the irreversible neuronal cell death had already begun to occur, therefore limiting effective drug therapy. The new diagnostic criteria are giving a vital role to central laboratories for AD diagnosis and clinical trial structure. Although the working groups currently don't recommend the use of these diagnostic tests in the clinical settings due to the lack of standardisation and firm evidence, it is clear that they are becoming an important part of clinical trial protocols. Currently, the proposed AD biomarkers are mainly targeting the amyloid plaques and neurodegeneration in cerebrospinal fluid (CSF) and by neuroimaging techniques.

1-Radioimaging

It is well documented that amyloid plaques are composed of amyloid beta 42 (Aβ42)[10]. There are several methods that have been developed for the measurement of amyloid beta peptides. The most noticeable technique is commercially available and FDA-approved. In 2012, FDA approved flobetapir F18 (Amyvid) to become available as a diagnostic aid in the diagnosis of AD in live patients. It is a radiodiagnostic agent tagged with a radioisotope fluorine-18 that binds to amyloid plaques and can be imaged using positron emission tomography (PET). Its sensitivity and specificity is influenced by the level of the reader's training, and ranges from approximately 69%-95% for sensitivity and from 90%-100% for the specificity of the scan[11]. It is recognised as a real breakthrough in the AD field because, for the first time, amyloid plaques can be reliably detected with FDA-approved radioimaging agent in live patients. A multitude of other agents are now in development, with Pittsburg compound B (PIB) being the most used in clinical trials in the past[12,13].

2-Cerebrospinal Fluid (CSF) Testing

In the amyloid theory, the biomarker Aβ42 is the main constituent of amyloid plaques. The AD neurodegeneration is characterised by the appearance of neurofibrillary tangles in the

Table I: AD Stages, Biomarkers, Clinical Trials and Patient Population Selection.

AD Stages	Purpose	Description	Amyloid Biomarkers	Neuronal Injury Biomarkers	Cognitive Change	Clinical Trials Patient Population Selection	Clinical Trials	Currently Available Drugs
			Aβ in CSF or by PET	T-tau/P-tau in CSF, FDG-PET, sMRI	ADAS-cog, Mini-Mental State Examination, neuropsychological test battery (NTB), etc.			
Preclinical 0	For Research Only	Asymptomatic	Negative	Negative	Negative	Patient Population can include those with positive genetic markers.	Prevention Studies	No FDA-approved Therapies exist.
Preclinical 1	For Research Only	Asymptomatic with cerebral amyloidosis	Positive	Negative	Negative	Amyloidosis doesn't exclusively mean AD, but highly suggestive of progression towards AD. It will be prudent to include in this group those with genetic or other AD risk factors.	Prevention of potential clinical symptoms and disease-modifying therapies	No FDA-approved Therapies exist.
Preclinical 2	For Research Only	Asymptomatic amyloidosis with possible "downstream" neurodegeneration	Positive	Positive	Negative	Amyloidosis with neurodegeneration is highly suggestive, but doesn't exclusively imply the progression to MCI.	Prevention of potential clinical symptoms and disease-modifying therapies	No FDA-approved Therapies exist.
Preclinical 3	For Research Only	Amyloidosis with neuronal injury and subtle cognitive/behavior al decline	Positive	Positive	Positive	This late preclinical AD stage patient populations is most likely to progress towards MCI due to AD.	Prevention of potential clinical symptoms and disease-modifying therapies	No FDA-approved Therapies exist.
Mild Cognitive Impairment (MIC)	Can be used to guide clinical diagnosis	Mild changes in memory and cognition without impairment that interferes with daily activities.	Positive	Positive	Positive	This patient population is most likely to progress to dementia due to AD.	Disease-modifying therapies	No FDA-approved Therapies exist.
Dementia due to AD	Currently used in AD diagnosis	Symptomatic cognitive impairment with significant brain changes and neurodegeneration	Positive	Positive	Positive	Patients have symptomatic AD and drugs that demonstrate better tolerability can be studied in this population.	Symptomatic treatment only	5 FDA-approved drugs: Tacrine, Donepezil, Rivastigmine, Galantamine, Memantine

wbrain, which are thought to be caused by total Tau protein (T-tau) and phosphorylated Tau protein (P-tau)[14]. Many studies have shown a marked increase in CSF T-tau and P-tau, with a marked decrease in Aβ42 in AD cases with dementia[15]. Consequently, this hallmark of AD pathophysiology can be successfully quantified with commercially available enzyme-linked immunosorbent assay (ELISA) kits, such as INNOTEST ELISA (Innogenetics, Belgium), the multiplex INNO-BIA AlzBio3 kit (Innogenetics, Belgium), and bead-based multiplex xMAP Luminex platform (Luminex Corp., Tx, USA) (Table II). However, the lack of standardisation is a major limitation in the use of these biomarkers. There are no established cut-offs or reference ranges available for their measurement, but fortunately several national and international standardisation initiatives are ongoing at this time, such as the Alzheimer's Association external quality control programme, which is run in conjunction with the Clinical Neurochemistry Laboratory in Gothenburg, Sweden. They have determined that the between-laboratory coefficients of variation (CVs) can be as wide as 13-36% in 40 participating laboratories, with within-laboratory CVs as large as 2.3-26% found at six experienced laboratories[16,17]. Central laboratories can play a central role in AD clinical trials by providing the most accurate results for AD fluid biomarker measurements.

3-Volumetric MRI

The pathophysiology of AD also includes widespread brain atrophy, which can be visualised and quantified using FDA-approved automated medical device image analysis software, such as NeuroQuant (CorTechs Labs, Inc., CA, USA). The most prominent brain structure that is important for memory is a medial temporal structure called the hippocampus. The atrophy of medial temporal structures has been linked with a high risk of decline to dementia[18,19].

4-Fluorodeoxyglucose-PET (FDG-PET)

It has become increasingly clear that AD is not just an accumulation of amyloid plaques and neurodegeneration in random brain regions leading to dementia. The specific brain regions involved in AD pathophysiology have aberrant neuronal networks, synaptic dysfunction and degeneration of specific neuronal populations that are critical factors leading to known AD with dementia. Therefore, synaptic dysfunction with neuronal injury and loss will lead to a decreased metabolism in the parietal and temporal areas of the brain. This metabolic decline can be measured with FDG-PET, which is currently approved for clinical use to distinguish AD from frontotemporal dementia because the latter is characterised by hypometabolism in the frontal and not the posterior brain regions [20].

Future Perspectives and Conclusions

For years, central laboratories have helped clinical trials with drug safety monitoring. Today, as technologies become more and more complex, central laboratories can also help clinical trial sponsors select the most useful biomarkers to support the type of drug therapy being tested. Choosing the correct patient population for potential clinical trials is critical. Central laboratories can guide sponsors in selecting the most informative biomarkers to use in determining the proper patient population (Table I). In addition, it is not the measurement of isolated biomarkers that is most informative for AD diagnosis and prognosis, but their combination. It is well illustrated in studies that mild cognitive impairment (MCI) subjects with high CSF tau/Aβ42 ratios were the most likely to progress to AD dementia, while normal subjects were most likely to progress to MCI. However, the same biomarkers were completely uninformative as predictors of change in subjects that had already developed dementia due to AD [21]. The correct selection of biomarkers can demonstrate target engagement in the brain and identify drugs that will most likely fail early in the study, preventing time loss and additional costs. The currently available biomarkers are far from perfect, and the search for ones that offer earlier detection windows and are more accessible than CSF samples is ongoing[22]. Central laboratories can keep clinical trial sponsors informed about the trends in future biomarker developments.

Out of all the mental illnesses, the most progress has been made in the understanding of AD pathophysiology. Today, the AA-NIA recommendations for AD diagnosis are being tested, and the preliminary results indicate that the use of biomarkers can help in patient population staging and prognosis[23]. Although many

Table II: AD Diagnostic Tests.

Test	Amyvid	NeuroQuant	INNOTEST	INNOBIA AlzBio3
Company	Eli Lilly	CorTechs Labs, Inc.	Innogenetics	Innogenetics
Method	PET Imaging	MRI Imaging	Fluid Testing	Fluid Testing
Specimen	N/A	N/A	CSF	CSF/Plasma
Technology	Radiodiagnostic agent that binds to amyloid plaques and can be visualized using PET	Software that quantifies the volume of hippocampus and other brain regions	ELISA	Multi-analyte bead-based immunoassay and xMAPLuminex platform
Biomarker	Aβ	Specific brain region atrophy	Aβ, or T-tau, or P-tau	Aβ, T-tau, P-tau
FDA Approved?	YES	YES	NO	NO

clinical trials have failed in the development of AD disease-modifying drug therapies, the recent progress in the AD field is very encouraging. The past failures can become important learning experiences in the development of future AD clinical trials. It is clear that AD cannot be treated exclusively as a pathophysiology with amyloid plaque accumulations and neurofibrillary tangles formation, because many amyloid and Tau targeting therapeutic strategies used in isolation have failed. Moreover, not all patients with these abnormalities will develop cognitive impairments. Therefore, a combination of drugs targeting several different neuropathological pathways will likely be the most successful.

Currently, it remains difficult to delineate the useful outcome measures for clinical trials and predict drug failures in the early phases. In these situations, strong collaboration between clinical trials and central laboratories can become the key for a successful AD drug development strategy.

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Tatiana Souslova, M.Sc., Ph.D. is principal scientist at ACM Global Central Lab, which specialises in delivering high-quality testing services designed to optimise clinical trial outcomes. For more information, visit http://www.acmgloballab.com/.
Email: tsouslova@acmlab.com.