DISCERN™: MORPHOMETRIC IMAGING ASSAY FOR THE DIAGNOSIS OF ALZHEIMER'S DISEASE

Key highlights

- Simple, minimally-invasive test informing a definitive diagnosis for Alzheimer's Disease (AD) in a living patient.
- The DISCERN™ test is comprised of three assays that assess several critical factors directly related to AD that regulate memory, the formation of synaptic connections among neurons, the levels of amyloid plaques and levels of neurofibrillary tangles in the brain. In clinical trials, the morphometric imaging assay has demonstrated high sensitivity and specificity of results correlating with postmortem diagnosis as defined by the NIH Gold Standard.[20]
- Diagnostically distinguishes AD from other forms of dementia, even in early-stage AD (≤4 years of a dementia diagnosis).
- Able to diagnose AD in mixed co-morbid state with other types of dementia.
- Early detection of AD enables earlier therapeutic intervention to prevent cognitive decline.
- In clinical trials, demonstrated improved accuracy over traditional clinical diagnostic approaches for AD, even in early-stage disease.

Introduction

Alzheimer's Disease (AD) is the most common form of dementia and the sixth leading cause of death in the United States (US).[1, 2] Over 55 million people worldwide have dementia, and this number is estimated to grow to 139 million in 2050.[2] In the US, approximately 5.8 million people have AD.[3] Currently, there is no cure for AD, nor an FDA-approved tool to definitively diagnose AD in a living patient.

A challenge to correctly diagnosing AD is that many symptoms can be similar to other types of dementia.[4-6] Patients can be asymptomatic for years before showing cognitive decline, termed the preclinical stage.[7] Patients begin to display mild cognitive impairment (MCI), which includes memory loss, and visual/spatial problems.[4-8] Not all patients that display MCI progress to dementia or AD.[9] Synaptic loss and abnormality are correlated with the severity of cognitive decline in patients with AD.[10-15] Unfortunately, AD is progressive, such that cognitive and functional impairments become increasingly more severe, eventually resulting in the loss of independence and death.[8, 16] Therefore, early and definitive diagnosis is important in effective therapeutic intervention, highlighted by the fact that once patients become symptomatic, many of the currently available therapeutics are ineffective.[17] To date, the only definitive method to diagnose AD in demented patients is through post-mortem evaluation of the brain to identify the accumulation of amyloid beta-protein (Aβ) plaques and the aggregation of hyperphosphorylated tau protein (p-tau) into neurofibrillary tangles (NFT).[18, 19] This NIH gold standard, based on the 1991 Consortium to Establish a Registry for Alzheimer's Disease (CERAD),[20] was established from 142 patients, of which 119 (84%) were diagnosed with AD.[20] Clinical diagnosis of AD in living patients is an ongoing pursuit with a great focus on biomarker-based approaches.

Biomarkers were classified as a diagnostic tool in

2018 by the National Institute on Aging and Alzheimer's Association. The A/T/N classification system includes assessments of Aβ/amyloid-based markers, tau/neurofibrillary pathology, and neurodegenerative or neuronal injury markers.[17, 21, 22] These evaluations are carried out through non-invasive imaging methods such as amyloid-PET that utilizes ^{18}F-fluorodeoxyglucose (^{18}F-FDG) to measure brain amyloid deposition[23] and MRI to measure brain atrophy.[24] However, these can be costly, not highly accurate, not widely available, and not always covered by insurance.[17, 25]

Combining these methods of imaging and liquid biopsy enhances the accuracy of properly diagnosing AD. The misclassification rate is approximately 41% with routine tests, but this decreases to approximately 28% with the addition of data from MRI, FDG-PET and biomarkers found in cerebral spinal fluid (CSF).[26] The error can be partially attributed to the fluctuations of biomarkers in early disease,[17] the lack of knowledge on limits of detection, and the inability of biomarkers to identify minimal neurofibrillary changes that can be detected by neuropathic examination.[22] Furthermore, AD can be difficult to diagnose because of the development of comorbid neurological disorders associated with aging. Many clinical tests lack specificity for AD in the presence of other dementias or aging.[27, 28] Further investigation of biomarkers is necessary to accurately diagnose AD earlier, and in a more cost-effective and less invasive manner.

The Morphometric Imaging (MI) Assay: Identifying AD through Peripheral Tissue

Over a decade of research has discovered that AD pathology can be observed in peripheral tissues such as skin - introducing a new target for disease-related biomarkers. Studies have shown that Aβ can form deposits in the skin of AD patients, which noticeably changes fibroblast biology.[29-31] Deposits of neurodegenerative disease-related toxic proteins such as tau, α-synuclein have been found in the epidermal layer of skin.[32] These discoveries led to the development of the DISCERN™ test, which reliably and accurately identifies neurological changes associated with AD from a simple skin biopsy.[33] The test is comprised of 3 assays (Morphometric Imaging [MI], PKCε [i] and AD Index[ii]) performed on a single skin punch biopsy. Assays in the DISCERN™ test were granted Breakthrough Device designation by the FDA in 2018 and have Clinical Laboratory Improvement Act (CLIA) status for 49 of 50 states. Recently, the DISCERN™ Laboratory Developed Test (LDT)/CLIA certified lab test was awarded Medicare reimbursement codes 206U and 207U by the American Medical Association (AMA) and gap-fill status by CMS (Medicare).

The DISCERN™ MI assay is based on measuring an ensemble of multiple AD factors such as inflammation, synaptic growth, and neuronal death that can be detected in skin fibroblasts.[33] MI is the primary assay of the DISCERN™ test and begins with a skin biopsy of 3mm from the upper arm from which fibroblasts are harvested and isolated (Figure 1).[34] An extracellular matrix is used to stimulate the fibroblasts into forming networks, and as observed in Figure 2, the ability of skin fibroblasts from AD patients (Figure 2B) to correctly form networks over time is dysregulated, a key differentiating factor from healthy fibroblasts (Figure 2A).[33]

Results

In the study described here, fresh biopsy samples were evaluated as well as primary cells obtained from a total of 74 patients, all of which were

characterized by autopsy and family history. Cells in this study were from patients with age-matched healthy controls (N=27), AD (N=26), and non-AD dementia (non-ADD) (N=21). All samples were blinded to the operator performing the assay and the pathologists assessing the autopsies.[33]

The MI assay evaluates network formation extent and rate to diagnose AD using a natural logarithmic format ln (A/N) to measure the average area (A) per number (N) of aggregates per sample. An integrated study included 27 healthy samples determined a cut-off of 6.98, from which the sensitivity, specificity, and confidence intervals can be assessed for the assay. To measure average area (A) per number (N) of aggregates, fibroblasts were grown for 48 hours from control, AD, and non-ADD patient samples (Figure 3A). Aggregation analysis demonstrated a clear separation of ln (A/N) that definitively distinguished between control, AD, and non-ADD samples ($P<0.0001$). Control and non-ADD cells formed many small aggregates while AD cells formed bigger isolated aggregates.

The MI assay was able to capture the physiological abnormalities found in peripheral tissue that correspond to AD systemic effects. Variability - due to external factors such as fever, concomitant drugs, and infections - that is consistently observed in clinical tests utilizing blood was not observed with the MI assay.[35, 38] Results of the MI assay were consistent with autopsy validation results, unlike tests that utilize blood or CSF for testing.[35, 36] Early detection with the DISCERN™ test compared to clinical diagnosis showed a higher percentage correctly diagnosed notably in early diagnosis (≤4 years) as well as late diagnosis (>4 years). In clinical studies, the MI assay has been able to identify AD in patients with mixed dementias (Figure 3C and D).[37, 38] Taken together, the MI assay demonstrated he ability to categorically differentiate between a healthy patient, an AD patient, and a non-ADD patient (Figure3A and B) even in the early stages of disease compared to clinical diagnosis (Figure 3C and D).[35, 37, 39, 40]

Conclusions

The MI assay was able to effectively diagnose AD in a minimally invasive manner on a living patient with greater than 95% accuracy, specificity, and sensitivity overall. Diagnostic values did not overlap between AD and non-ADD patients, suggesting the ability to distinguish between AD and other forms of dementia. Early stages of AD can be definitively diagnosed with the MI assay with better accuracy than clinical tests. This provides a substantial advantage over other biomarker tests by detecting the disease before patients become severely symptomatic. The MI Assay may have the ability to detect preclinical stages providing a longer window of time for medical interventions that can improve patient prognosis. The ease of obtaining skin samples, especially in elderly patients, as opposed to an invasive lumbar puncture enables patients to have frequent sampling over the course of their disease progression and treatments. In the future, this test could be utilized in clinical trials and treatments to establish efficacy and disease control over time.

Taken together, the promising results from the MI assay could help patients to improve the regimen of therapeutic interventions to prevent cognitive decline earlier in the disease process. These findings suggest that the MI assay can distinguish AD from other types of dementia and further confirmatory studies are ongoing. Potential future applications of the MI assay may include diagnosis of other neurodegenerative disorders such as Parkinson's disease and other forms of dementia.

Figures

Figure 1.

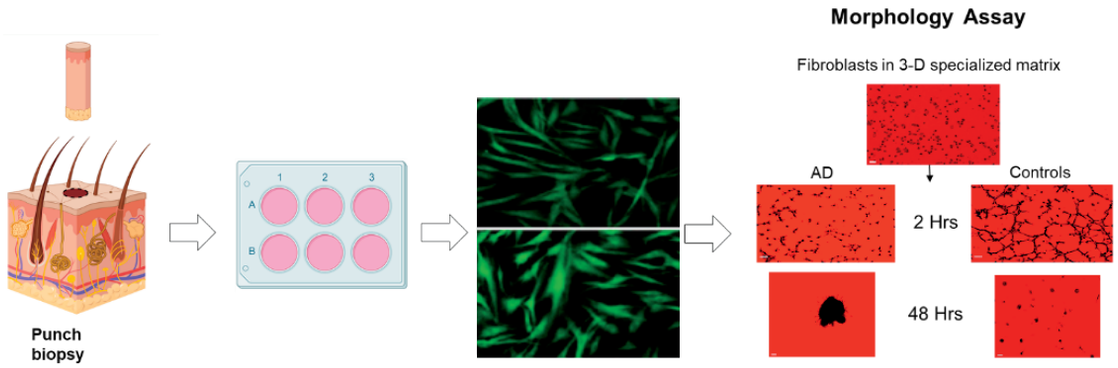

Figure 1.

Assay Process. A punch biopsy is performed, and fibroblasts are dissociated and cultured, then harvested for morphometric imaging (MI).

Figure 2.

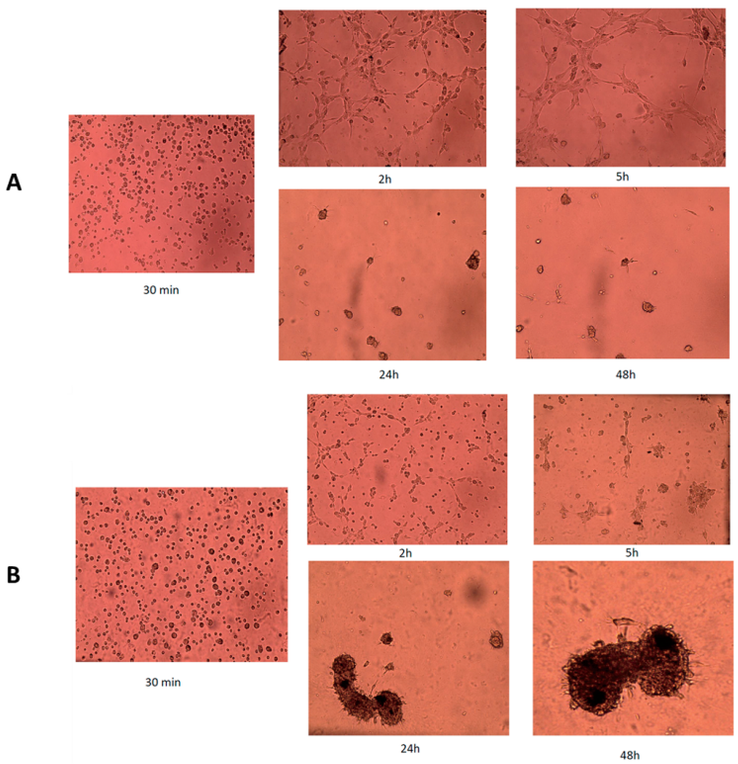

Figure 2.

Skin fibroblasts cultured in 3D Matrigel matrix at various time points A) Healthy patients B) Alzheimer's disease patients.

Figure 3.

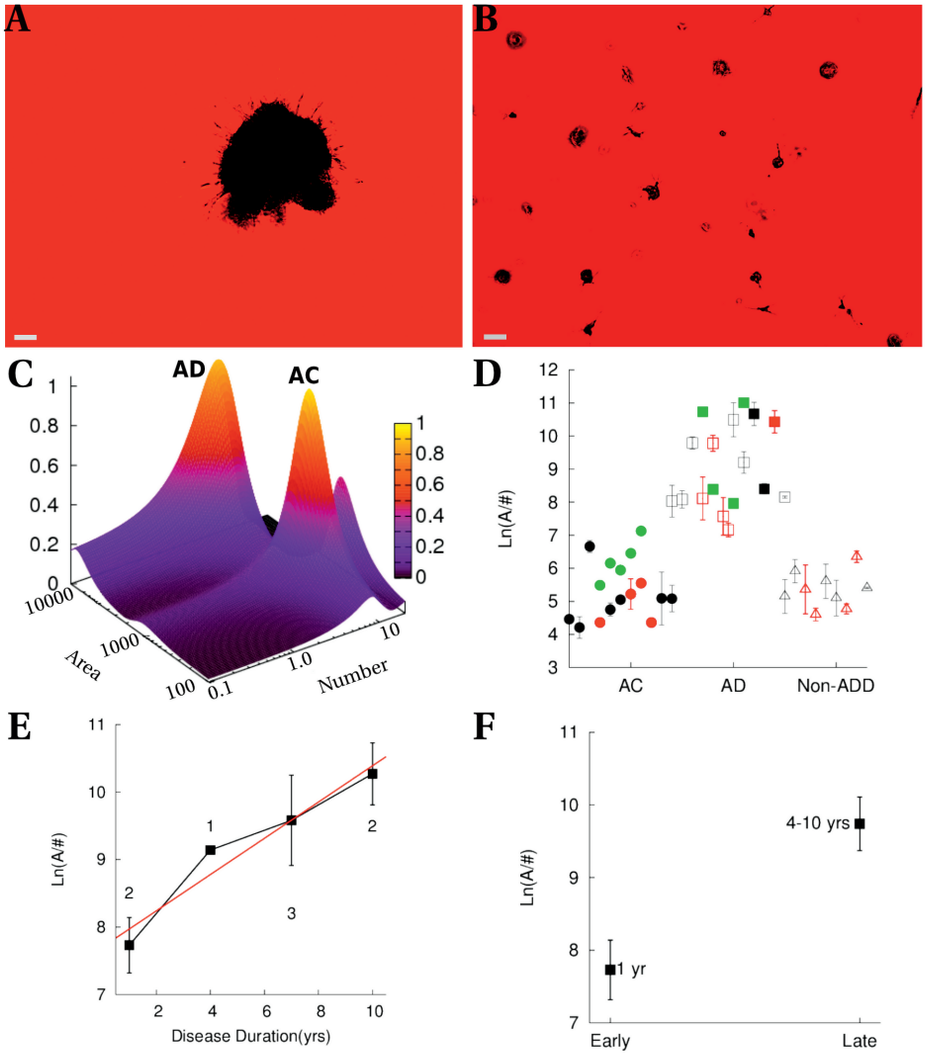

Figure 3.

Accuracy of the Morphometric Imaging Assay. A) Patients with autopsy-confirmed Alzheimer's Disease (AD) could be accurately distinguished from patients with non-AD dementias and age-matched controls. B) Natural logarithm of the ratios of aggregate areas to number, Ln (Area/#), for control (n=11, circles), AD (n=13, squares), non-ADD (n=9, triangles). Open symbols were autopsy or genetically validated samples. Red samples were analyzed under double-blind conditions. Green symbols we**re freshly harvested samples (vs banked samples) C) Aggregates increase with disease duration D) Ability for early detection of AD.**

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