Diagnosis of Alzheimer’s Disease through the Eye and its Correlation with Cognitive Tests and Brain Imaging

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Abstract

Despite decades of research, there is no definite pre-mortem diagnosis for Alzheimer’s disease (AD). Clinical diagnosis of the disease, even with newer biomarkers such as CSF and amyloid PET is not considered definite; it can only be confirmed post-mortem through histopathological identification in the brain of its characteristic features including beta-amyloid (Aβ) plaques. There is a clear need for a technique capable of improving the in-vivo diagnosis of AD at early stages.

We review here our previously published clinical studies using a Fluorescent Ligand Eye Scanning (FLES) System to differentiate between patients with probable AD and healthy volunteers (HV) as well as some new analyses. FLES is a combination of a fluorescent ligand and a laser eye scanning device. The fluorescent ligand was dosed topically to the inside of the lower eyelid of the study eyes the evening before the day of the measurement with the device.

Our published phase 2 trial showed by clinical diagnosis, the AD and HV groups could be differentiated with sensitivity of 85% and specificity of 95%, (p<0.001). Further, all but five of the clinically diagnosed AD patients were amyloid PET positive and all but three of HV were amyloid negative supporting the validity of their diagnoses. In addition, the results in the eye showed significant correlation with the Standard Uptake Value ratios (SUVr) obtained by amyloid brain imaging Positron Emission Tomography (PET) using florbetapir. We also report here new analyses showing the correlations between FLES eye findings and cognitive tests, as well as the effect of apolipoprotein (APOE) gene.

ABBREVIATIONS

AD: Alzheimer’s Disease; Aβ: Beta Amyloid; PET: Positron Emission Tomography; HV: Healthy Volunteer

INTRODUCTION

There is a dear need for a simple scalable point of care (office based) technology capable of improving the diagnosis of Alzheimer’s disease (AD) with high resolution sensitivity and specificity. The uncertainty in diagnosis is noted through the misdiagnosis rate in post mortem studies [1-5].

The definite diagnosis of AD is performed post mortem through histopathological changes in the brain and identification of abnormal aggregates of beta amyloid (Aβ) in the form of plaques and tau in the form of neurofibrillary tangles [6-8]. Improving Alzheimer’s disease diagnostic accuracy by detecting the presence of hallmark pathology (e.g. protein Aβ) in the brain
has been the major goal of brain imaging using amyloid and tau positron emission tomography (PET) tracers [9-12]. Amyvid™ (florbetapir F 18), Vizamyl™ (flutametamol), and Neuraceq™ (florbetaben F18) are radioactive tracers, recently approved by the U.S. Food and Drug Administration for positron emission tomography (PET) imaging of the brain estimating beta-amyloid plaque density in patients who are being evaluated for cognitive impairment. While Amyloid PET has a high negative predictive value for discriminating AD from non-amyloid dementias, the positive predictive value remains low for application at an individual patient level. Tau PET remains investigational at present. Further, the expense and radiation exposure from PET remain limitations for large scale application of PET for routine screening purposes or application in developing countries.

Detection of Aβ in the eye

Over the past decade, studies have demonstrated AD related molecular and pathological changes in the eye [13-24] and detected the presence of β-amyloid in the lens [14,17-19,22-24] and the retina [15,16,20,21]. Fredrikse et al. demonstrated that Alzheimer’s precursor protein (β APP) and Aβ are present in the interior fiber cells of the mammalian ocular lens [17]. Such proteins are formed in β-sheet arrays organized in an amyloid protein supramolecular order. Goldstein et al. identified Aβ1-40 and Aβ1-42 in the supranuclear region of the lens at similar concentrations levels as have been found in the brain [19]. Additional studies in transgenic mice and individuals with Down Syndrome support the hypothesis linking the AD-associated pathology in the brain and lens [22, 23]. Moreover, deep cortical cataract has been documented in transgenic mice expressing human Aβ [24].

A first exploratory clinical trial was conducted using FLES to compare 5 patients clinically diagnosed with probable AD to 5 healthy volunteers (HV). The study demonstrated the safety of the FLES technique [25]. Noting that the study included a small number of participants, sensitivity and specificity of 80% were obtained separating both groups.

Based on the promising exploratory study results, a larger study was performed in forty participants (20 probable AD and 20 HV). Both FLES and amyloid florbetapir F 18 PET imaging were performed for comparison with clinical diagnosis. The results have been published [26]. Our findings showed that the FLES was capable of differentiating two groups with sensitivity and specificity of 85% and 95%, respectively.

The aims of this article are to report on additional analyses: 1) report on ROC curves for eye test versus PET amyloid imaging, 2) examine correlations of eye tests with clinical cognitive function tests, 3) examine correlation of eye tests with APOE e4 genotype.

MATERIALS AND METHODS

Detailed Methods have been published previously [26]. The study was conducted in accordance with the current revision of Declaration of Helsinki guiding physicians in medical research involving human subjects. The Western Institutional Review Board (IRB), Olympia, WA, approved the protocol to initiate the clinical trial. The participants provided their signed written consent form. Consent was obtained by subjects or their Legally Authorized Representative should the subject not have the capacity to consent.

Study design

The study was an observer-masked, age-matched case control study of AD patients and HV [26]. The reader of the PET images was also masked as to the clinical diagnosis of the subject.

FLES System

The FLES system, as described previously [26] is a combination product of ligand-device that detects the fluorescent signature of the applied ligand bound to Aβ in the supranucleus of the lens. The ligand, aftobetin hydrochloride, is an ophthalmic ointment that is applied topically to the eye. It measures the emitted fluorescence signal of aftobetin-HCl bound to Aβ aggregates in the supranuclear region of the lens. The device scans the supranuclear region of the lens in 1 second and provides a numerical output, which is referred as Fluorescence Uptake Value (FUV). Contrary to other techniques, the device does not require operator’s (expert) read or any analysis of images.

Amyloid PET Imaging

PET imaging sessions were performed on all participants with Amyvid™ (Florbetapir F18), a radioactive tracer, used to estimate the Aβ plaque density in patients’ brain and rate images as positive or negative, as described previously [27- 29].

PET image interpretation was performed by a single blinded, certified reader at Banner Alzheimer’s Institute. PET SUVR segmentation methods have been reported previously but in brief were based on coregistering with MRI and standardized template. For all analyses Global SUVR (average of 6 cortical regions) was used as the metric.

Statistical analysis

Receiver operating characteristics (ROC) analysis was used to determine the decision threshold, or level of separation, between the AD and HV groups. The threshold is set to obtain a balance between sensitivity and specificity and calculate the area under the curve (AUC). The same analysis was used in comparing results between the two methods FLES and PET. The Cohen’s kappa coefficient (κ) was calculated as a statistical measure for the agreement between the results obtained by the either PET or FLES and the clinical diagnosis of AD.

Detailed entry criteria have been described previously [26] and in brief were as follows:

**Inclusion Criteria for AD subjects**

- Clinical Dementia Rating (CDR) between 0.5 and 2, with a rating of at least 1 on the memory sub-scale
- Mini-Mental State Exam (MMSE) score between 14 and 24 inclusive
- National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA) criteria for Probable AD
- Diagnostic and Statistical Manual-IV (DSM-IV) criteria for Dementia of the Alzheimer’s Type
Inclusion Criteria for HV subjects

- Cognitively normal, based on an absence of significant impairment in cognitive functions or activities of daily living
- CDR-Global score of 0 and CDR-Memory score of 0
- Mini-Mental State Exam (MMSE) score between 26-30
- Psychiatically healthy, no depression in the clinical judgment of Investigator
- Normal memory function documented by scoring within the education adjusted ranges on the Logical Memory II subscale (Delayed Paragraph Recall, Paragraph A only) from the Weshlser Memory Scale-Revised (the maximum score is 25)

RESULTS AND DISCUSSION

Participants

Figure 1 and 2 summarizes demographics and clinical/cognitive scores for both groups. Twenty AD subjects (45% females) with median age of 76 years old met the NINCDS-ADRDA criteria for Probable AD and DSM-IV criteria for Dementia of the Alzheimer’s Type. Twenty HV subjects (45% females) with median age of 72 years old were recruited with no evidence of cognitive impairment. Both groups have same number of education years. Apolipoprotein (APOE) e4 was more prevalent among the AD group (40%) than the HV group (15%).

ROC curves for FLES prediction of Clinical Diagnosis

As reported previously [26], the ROC analysis of the data demonstrate the prediction of clinical diagnosis by FLES with sensitivity of 85% and specificity of 95% using the Cohen’s Kappa test ($\kappa = 0.80$, 95% CI =0.61-0.99, p<0.001, AUC=0.915) [26]. The threshold line obtained by ROC was 0.367, the average FLES score for the HV group was 0.23, which is almost half that of the AD group (0.50). Moreover, the results of the AD group had a range of 0.82 compared to that of the HV group of 0.45 [26].

Amyloid PET prediction of Clinical Diagnosis

Similar statistical analysis to the FLES test was performed for the AmyvidPET, both visual and quantitative readings, which were obtained on the same cohort in the study. The statistical results of visual AmyvidPET PET (positive or negative scans) predicting clinical diagnosis show both sensitivity and specificity of 80% ($\kappa = 0.60$, 95% CI =0.35-0.85, p<0.001).

The statistical analysis performed on the quantitative AmyvidPET predicting clinical diagnosis yielded a sensitivity of 75% and specificity of 85% ($\kappa = 0.60$, 95% CI =0.35-0.85, p<0.001). Figure 3 is a scatter plot of the data obtained by quantitative AmyvidPET PET for all subjects tested in the clinical study. The threshold obtained from the ROC curve on the quantitative AmyvidPET in this cohort was 1.26, which is different than the published threshold used in differentiation between groups using AmyvidPET amyloid PET imaging[29].

Color coded dots in Figure 3 represent results obtained by FLES based on clinical diagnosis as positive (above threshold) or negative (below threshold) as described in the previous section.

In this study cohort, 11 subjects were APOE e4 heterozygotes and 29 were noncarriers. By clinical diagnosis, 40% of the AD group tested positive with APOE e4 and 85% of the HV group tested negative (Figure 1).

The FLES System correlated with APOE e4 allele test in 55% of the subjects who tested positive (APOE e4 detected) and in 59% of the subjects who tested negative (APOE e4 not detected) ($\kappa = 0.11$, 95% CI =0.21-0.43, p=0.253) (Table 3).

CONCLUSION

The findings reported here extends our previous report from the first multicenter phase 2 trial of FLES eye test as an aid for diagnosis AD [26]. As reported previously [26], the FLES eye test system was well tolerated and there were no serious adverse events recorded.

The concordance between amyloid PET and FLES was relatively high as reported previously [26]. The ROC curve analyses of the eye test, which was performed with the FLES system on one eye of every participant, demonstrated a high correlation with cognitive function.

Table 1: Quantitative PET Prediction of Clinical Diagnosis.

<table>
<thead>
<tr>
<th>Test Statistic</th>
<th>N=20</th>
<th>N=20</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>PET (Mean Cortical)</td>
<td>κ=0.6, π&lt;0.001</td>
<td>0.63</td>
<td>0.61</td>
</tr>
<tr>
<td>Negative</td>
<td>85%</td>
<td>17/20</td>
<td>25%</td>
</tr>
<tr>
<td>Positive</td>
<td>15%</td>
<td>3/20</td>
<td>75%</td>
</tr>
</tbody>
</table>

Table 2: Correlation factor (r) and p-values of Composite Cognitive tests (composed of CDR, MMSE) by FLES and PET.

<table>
<thead>
<tr>
<th>Test Statistic</th>
<th>CDR</th>
<th>MMSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLES</td>
<td>r=0.63</td>
<td>N=40</td>
</tr>
<tr>
<td>Quant.PET</td>
<td>r=0.53</td>
<td>N=40</td>
</tr>
</tbody>
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Sadowsky et al. (2014)  
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specificity of 85% and 95%, respectively, for separating AD from HV [26]. Interestingly, the range detected in the AD group by FLES is larger than that in the HV group, which could potentially be leveraged for assessment of disease severity; it may also help understand the evolution of Aβ aggregate depositions in the eye and their correlation to that in the brain. Composite tests of cognitive tests including MMSE and CDR showed similar correlation factors in pooled analyses of AD and HV.

This pilot trial has several strengths as well as limitations. It is the first controlled multisite study examining the feasibility, diagnostic utility and safety of the FLES eye test for discriminating AD from HV. In addition, the concordance with florbetapir PET scans suggests that FLES eye test results may serve as a promising surrogate for presence or absence of cortical beta-amyloid. Our findings should be interpreted within the context of the small sample size, multiplicity of comparisons, lack of autopsy validation and lack of active disease controls. However, given the inherent limitations of current methodologies and technologies to assist with clinical diagnosis, the FLES eye test data from this study supports its further phase 3 development. Further investigation with larger sample sizes, including patients referred for routine evaluation and groups with different degrees of severity of the disease, will be needed for further confirmatory analysis. Longitudinal studies will be needed to determine prognostic utility.

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**CONFLICT OF INTEREST**

The study was funded by Cognoptix. Paul Hartung, Dennis Nilan, and Charles Kerbage are employees of Cognoptix and own stock. Anne Cameron is a consultant to Cognoptix and owns stock. Gerald Cagle was an employee of Cognoptix and owns stock. Other authors have received research grants and/or advisory fees from several companies in this field, including Cognoptix. P. Murali Doraiswamy owns stock in several companies whose products are not discussed here. He also has unlicensed patent(s) in the AD diagnostic field.

**REFERENCES**


27. Highlights of Prescribing Information. These highlights do not include all the information needed to use Amyvid safely and effectively. See full prescribing information for Amyvid. Amyvid (Florbetapir F 18 Injection) for intravenous use Initial U.S. Approval: 2012.
