Impact of Clinical Accuracy in Development and Validation of a Blood Based Test for Early Detection of Alzheimer’s Disease

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Summary
A new blood test, ADtect®, has been developed that can aid the early detection of Alzheimer’s Disease (AD). The test is based on measuring the expression of select gene expression in blood and is defined as the AD-specific gene signature. ADtect® comprises a low density array of 96 selected gene assays using RNA extracted from a blood sample. The performance of each of the 96 gene assays is calculated with an algorithm resulting in a positive or negative test score. In a multicenter study of 412 subjects the test is able to discriminate AD subjects from cognitively healthy controls with a 72% overall agreement with the clinical diagnosis. The test performance is confirmed in two independent validation studies and shows a similar and consistent good performance. However, clinical diagnosis is hardly ever completely in agreement with true pathology. This is also true for the diagnosis of AD. Assuming a clinical accuracy of 80% in the multicenter study, suggests an overall test accuracy of 85-90%. Of 18 samples where also CSF was available 15 were correctly predicted further suggesting that an agreement with clinical diagnosis likely understimates agreement with Alzheimer pathology.

Introduction
Early and accurate detection of Alzheimer’s disease (AD) is critical for implementing active management strategies which may delay the onset of the more debilitating symptoms of AD. In the development of biomarkers for early detection of AD clinical diagnosis is most often used as the standard of truth. However, it has been shown in several studies that the agreement between clinical diagnosis and true pathology can vary significantly and may mask a diagnostic test’s accuracy. To estimate an expected agreement with true pathology a simulation model was generated together with a MLE (maximum likelihood estimate) of diagnostic test performance. The accuracy of the ADtect® test would be improved by as much as 13% (see Box 3).

Calibration and Validation of the test
During calibration of the ADtect® test, the disease algorithm was developed using partial least square regression on a balanced set of samples from Alzheimer’s disease patients (N=103) and cognitively healthy controls (N=103) recruited from Norwegian and Swedish hospitals. Leave-one-out cross validation showed a 71.6% agreement of the ADtect® test with the consensus approved clinical diagnosis (Box 1). The final disease algorithm was built into the ADtect® software for patient reporting.

Two independent validation studies of the ADtect® separated in time with 1 year test was performed. The first validation included blood samples collected at 4 sites in Norway. All samples (N=74, 32 AD, 42 cognitively healthy controls) were independent from the calibration study, and 3 of the recruitment sites were independent from the calibration (Box 1). The second and extended validation study included blood samples collected at 11 sites in Norway and Sweden. All samples (N=130, 68 AD, 62 cognitively healthy controls) were independent from the calibration study, and 6 of the recruitment sites were independent from the calibration (Box 1). The validation part of these studies showed a 71.6% and 71.5% agreement of the ADtect® test with the clinical diagnosis (Box 1).

Discussion
We have developed a novel gene expression test to aid in the diagnosis of mild to moderate Alzheimer’s disease in individuals with memory complaints. On two independent validations separated in time with a year the same agreement with clinical diagnosis of 71.6% is found. This is also the same level as found in the initial calibration study. The level of the observed test performance is high but still is lower than the 80% minimum suggested by the Alzheimer’s Association and the National Institute on Aging Working Group. Neurobiol Aging. 19, 109-16.

The ADtect® test procedure
Sample Preparation (See Box 2)
Whole blood is collected from individuals in PaxGene™ Blood RNA tubes and processed according to manufacturers instructions. Total RNA is extracted from blood samples using PAXgene™ Blood RNA kit and quality assessed by Nanodrop spectrophotometer and Agilent 2100 Bioanalyzer. Samples are prepared using the high-capacity cDNA reverse transcriptase kit and the Universal PCR Master Mx reagent from Applied Biosystems. Up to 10 individual samples are applied to the ADtect® MFC.

The ADtect® test procedure

Overall performance characteristics for ADtect®

<table>
<thead>
<tr>
<th>Agreement with clinical diagnosis</th>
<th>Calibration (%)</th>
<th>Validation (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall agreement</td>
<td>71.6 +/- 6.1</td>
<td>71.6 +/- 10.3</td>
<td>71.6 +/- 4.4</td>
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<tr>
<td>Agreement with positive outcome</td>
<td>71.8 +/- 6.7</td>
<td>71.9 +/- 15.6</td>
<td>70.6 +/- 10.8</td>
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<tr>
<td>Agreement with negative outcome</td>
<td>71.4 +/- 8.6</td>
<td>71.4 +/- 13.7</td>
<td>72.6 +/- 11.1</td>
</tr>
<tr>
<td>Positive likelihood ratio (PLR)</td>
<td>2.51</td>
<td>2.52</td>
<td>2.57</td>
</tr>
<tr>
<td>Area under the curve (AUC)</td>
<td>0.77</td>
<td>0.74</td>
<td>0.74</td>
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</table>

Expected Test Accuracy

Box 3

Modelling of expected test accuracy when clinical accuracy is 80%

References