The LYMPRO® TEST is a blood assay that has reported differential mitogenic mitogen activation in lymphocytes drawn from Alzheimer’s disease (AD) subjects compared to healthy controls (HC) (1, 2).

The assay is based on the cell cycle reentry hypothesis for AD (3), which states that post-mitotic neurons in AD have inappropriately reentered the cell cycle with downstream overexpression of cyclins and increased neuronal cell death through apoptosis (4-5). This cell cycle dysregulation (CCD) is likely one of the earliest key pathologies in AD (6) and appears linked to tau hyperphosphorylation (4) and APP metabolism.

Brain CCD appears to be reflected by systemic manifestations, reported as CCD measured in white blood cells by several research groups. Dr. Thomas Arendt et al. at Leipzig University developed the specific technique (1, 2) for measuring white blood cells expression of CD69, a cellular marker related to the cell cycle. When stimulated by a non-specific mitogen, WBCs normally pass the G1/S checkpoint which increases the expression of CD69, whereas in AD WBCs abnormally pass this cell cycle checkpoint (see Figure 1).

We used this in vitro assay in lymphocytes and monocytess, obtained from AD and HC subjects to further develop a test that might in the future be useful to increase accuracy of clinical diagnosis of AD. Having a peripheral blood-based biomarker of AD would be highly desirable.

**METHODS**

Subjects were diagnosed as AD by dementia experts using NIA/AA (2011) clinical criteria for the determination of probable Alzheimer’s dementia.

**Table 1. Demographics**

<table>
<thead>
<tr>
<th>Variable</th>
<th>All</th>
<th>AD</th>
<th>HC</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>125</td>
<td>59</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td>Mean Age</td>
<td>73.1 ± 9.6</td>
<td>72.2 ± 9.0</td>
<td>69.6 ± 7.8</td>
<td>0.0001</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>50/75</td>
<td>36/33</td>
<td>24/42</td>
<td>0.02</td>
</tr>
<tr>
<td>APC4 status (≥4)</td>
<td>44/81</td>
<td>28/31</td>
<td>16/50</td>
<td>0.009</td>
</tr>
</tbody>
</table>

**Table 2. MMSE in the AD Cohort (HC was 32.8)**

<table>
<thead>
<tr>
<th>N</th>
<th>125</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean MMSE ± SD</td>
<td>16.1 ± 5.5</td>
</tr>
<tr>
<td>Range</td>
<td>0-26</td>
</tr>
</tbody>
</table>

**Figure 1: Review of Cell Cycle**

**CONCLUSIONS**

1. Multivariate analysis using random forest found 5 candidate variables that together generated the best performance results in ROC analysis.

2. This preliminary algorithm holds promise as a step in the development of a bioassay algorithm that can yield both strong sensitivity and specificity.

3. The LYMPRO test holds promise for use in evaluating patients through further clinical validation in diverse clinical samples, in conjunction with F18-florbetapir PET, is planned.

**REFERENCES**


**Figure 2: Five WBC cell subtypes analyzed**

**Figure 3: ROC Curves for AD & HC Groups**

**MULTIVARIATE RESULTS**

- Of the 66 variables, 5 were selected in multivariate analysis as together providing the best differentiation between groups.
- ROC graphs were produced using these 5 candidate features for training and test sets (see Figure 3) where AUC for AD and HC groups were good to very good.
- It is notable that all 5 candidate features were obtained from the same mitogen stimulation condition.

**DISCUSSION**

Findings from this expanded analysis of the LYMPRO test using multivariate analysis are consistent with the two prior published reports using univariate approaches. This lends further support that LYMPRO test may have utility as a blood biomarker reflective of AD pathology. More in-depth analysis of this cohort is underway. These preliminary findings are encouraging and warrant further studies to demonstrate the utility of this peripheral biomarker in the differential diagnosis of patients with cognitive impairment.

In addition, the study met its primary and secondary endpoints. Work continues on exploratory objectives.

Limitations:

- The study design was predicated upon cohort categorization (AD or HC) on blood biomarker readout and there were no biomarkers employed in the context of clinical diagnosis.

Nonetheless, our test sample performed similarly to Beach et al’s conclusion about clinical diagnosis (4)”...when optimizing for sensitivity and specificity, they report 90.0% sensitivity and 70.8% specificity.”