The LymPro® Assay: A Biomarker For Alzheimer's Disease Using Blood Samples From Clinically Diagnosed Alzheimer's Disease And Cognitively Intact Subjects

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ABSTRACT

A blood biomarker would be advantageous as an aid to diagnosis of Alzheimer's disease (AD). Mounting data suggests cell cycle dysregulation is involved in the pathogenesis of AD (4) and that this failure is systemic, affecting not only neurons but also blood lymphocytes (PBLs). This study built on two prior published reports that demonstrated that differences in PBL proliferation activity could be used as a biomarker for AD. CD69 and CD28 (surface markers of cell cycle activity), were each measured on peripheral T, B, and monocyte cells by flow cytometry after mitogenic stimulation. Blood samples were drawn in 140 subjects at 5 sites including Healthy Normal (HN) (n=69) and Probable AD (n=71). Multiple markers were significantly ($p<0.05$) different in AD subjects compared with HN subjects using univariate models with some markers achieving AUCs of 0.657-0.689, primarily exemplified by changes on CD19 cells expressing CD69. Within the AD group, results showed little correlation with the MMSE score. These findings are in line with the two prior published reports and suggest that LymPro may be a useful blood biomarker.

INTRODUCTION

The LymPro® test is a blood assay that measures differential mitogenic activation in lymphocytes from AD subjects compared to controls (1, 2). The assay is based on the cell cycle hypothesis for AD (3), which states that neurons in AD have inappropriately entered the cell cycle with downstream overexpression of cytokines and increased liability for neuronal cell death. This cell cycle dysregulation (CCD) is likely one of the earliest key pathologies in AD (6). CCD has systemic manifestations and has been measured in white blood cells by several groups. **Dr. Thomas Arendt** et al, at Leipzig University developed the specific technique (1, 2) we used here.

METHODS

Clinical: This was a single visit study in which 5 sites drew blood samples from clinically documented AD subjects and controls. All subjects were older than 55 years and AD subjects had a MMSE from 0-26 and met McKhann (2011) clinical criteria. HN subjects had a MMSE of 29 or 30 and no cognitive complaints. We excluded subjects on immune modulating medications and those with acute illness or infection.

Laboratory: Samples were collected in 8mL heparin CPT tubes and shipped at ambient to Becton Dickenson (BD, La Jolla). The buffy coat was separated, aliquoted, and incubated for 4 or 20 hours in one of two mitogens at different concentrations. Cells were stained with antibody cocktails and acquired on a BD FACSCanto II 8 channel flow cytometer. Markers included: CD3 / CD4 / CD8 / CD14 / CD19 / CD28 / CD69. Using BD FACSDiva software, the cell types were identified and cells expressing CD28 and CD69 were quantified.

Statistical: Univariate markers were evaluated using AUC-ROC. Binary categorizations of subjects based on LymPro biomarker results were referenced to their clinical category (AD or HN) to construct contingency tables. Sensitivity, specificity and odds ratios were estimated.

TOP LINE RESULTS

Demographics

Cohort Ages			
Age	All	AD	HN
N	140	71	69
SD	9.6	9.0	7.8
Min-Max	55-96	57-96	55-89

Age distribution is not matched

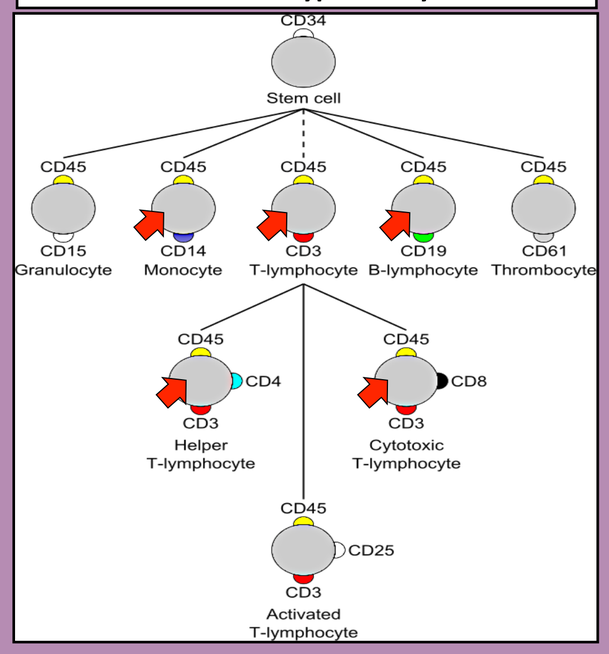
MMSE in AD subjects	
N	71
Mean	16.2
SD	5.5
Median	18
Min-Max	0 - 26

Methods

Stimulation Conditions		
Mitogen	Dose	Duration
PWM	4µg/mL	4 Hours
PHA	4µg/mL	4 Hours
PWM20	8µg/mL	20 Hours

PWM = pokeweed mitogen
PHA = phytohemagglutinin

WBC cell subtypes analyzed



Univariate results

Summary of Univariate Values			
	ROC - AUC		
Variable	PHA4 AUC	PWM20 AUC	PWM4 AUC
%CD3+ CD4+	***0.657***	***0.666***	***0.656***
%CD3+4+69+ (as a % of 3+4+)	0.542	0.590	0.551
CD3+4+69+ Mean	***0.605***	0.551	0.578
%CD3+ CD8+	0.569	0.569	0.572
%CD3+8+69+ (as a % of 3+8+)	***0.606***	***0.650***	***0.615***
CD3+8+69+ Mean	0.581	0.562	0.593
%CD19+	0.578	0.568	0.584
%CD19+69+ (as a % of CD19+)	***0.671***	***0.647***	***0.650***
CD19+69+ Mean	0.557	***0.670***	0.557
%CD14+69+ (as a % of CD14+)	0.554	***0.689***	***0.603***
CD14+69+ Mean	0.545	0.518	0.502

AUCs in bold and italic are significantly higher than random chance (0.500)

Findings are Independent of Clinical Stage

LSD Threshold Matrix		
Level	Abs Diff – LSD	p-value
HN	-3.8	1.0000
Mild AD	0.448	0.0284
Mod AD	3.177	0.0003
Severe AD	2.554	0.0062

Positive Values show pairs of means that are significantly different

Connecting Letters Report		
Level	Letter	Mean
Healthy Control	A	89.070
Mild AD	B	83.564
Moderate AD	B	81.118
Severe AD	B	78.062

Levels with different letters are significantly different

Ordered Differences Report						
Level	Comparator	Difference	Std Err Diff	Lower CL	Upper CL	p-value
HN	Severe AD	11.007	3.509	4.068	17.947	0.0021
HN	Mod AD	7.952	1.982	4.031	11.872	<.0001
HN	Mild AD	5.506	2.099	1.353	9.658	0.0097
Mild AD	Severe AD	5.501	3.772	1.957	12.961	0.1470
Mod AD	Severe AD	3.055	3.708	4.277	10.388	0.4113
Mild AD	Mod AD	2.446	2.417	2.334	7.227	0.3134

DISCUSSION

The LymPro test is a novel and potentially important blood based biomarker for the presence of Alzheimer's pathology as indicated by cell surface expression of CD69. The study was designed to understand the performance of LymPro in clinically diagnosed AD subjects and as a means to compare the current results with previously published findings. Additional analysis is ongoing to further refine the marker results and define multivariate algorithms. These findings are consistent with the two prior published reports and suggest that LymPro may be a useful blood biomarker for use in clinical trials and academic studies.

Limitations of the current study. The study design based cohort categorization (AD or HN) on clinical grounds only. There were no biomarkers employed. This has important ramifications for understanding the results reported here. While the AD subjects identified for this study utilized the McKhann (2011) clinical criteria for determining probable Alzheimer's dementia, this leaves a considerable room for misdiagnosis as observed in pathologic series. Beach, et al (4) quantified these diagnostic shortfalls comparing clinical diagnosis at Alzheimer's Research Centers to pathologic results, concluding that "…when optimizing for sensitivity and specificity, the best [clinical] result was 70.9% sensitivity and 70.8% specificity.

CONCLUSIONS

1. Some univariate variables display excellent specificity at poor sensitivity and a few display strong sensitivity but low specificity. Statistical significance was reached for multiple markers.
2. A formal algorithm development is underway by **Vadim Alexandrov** of PsychoGenics, Inc.
3. All stimulation conditions generated significant univariate performance results with a potential for an algorithm that can yield both strong sensitivity and specificity.
4. At this time, the LymPro test can be applied as a pre-screening tool for selection of patients into drug studies.

Planned future research

1. Ongoing analysis of marker performance
2. Biomarker identified Alzheimer's disease subjects with other dementia comparators.
3. Cross sectional and longitudinal studies in MCI with biomarker identified study subjects.
4. Study in pre-symptomatic Alzheimer's disease.

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