Phenotypic Syndromes in Alzheimer’s/ Vascular Spectrum Dementia: The Case for Heterogeneity

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Rowan University

Clinical Heterogeneity in Alzheimer’s Disease

Focal AD Syndromes

- 
  McDonald (Brit J Psychiatry, 1969, 115, 67-271): Described groups of AD patients presenting with “parietal syndrome” presenting with dyspraxia and constructional impairment versus an “amnesic syndrome”.

- 
  Martin et al., (JEEN, 1986 8, 594-610): Described neuropsychological subgroups of pts with AD with corresponding areas of hypometabolism.

- 
  Logopenic Aphasia (Gorno-Tempini et al., Neurology, 2011, 76, 1006-1014): Dysfluency in speech, impaired repeating and naming, relatively intact comprehension.

- 

- 
  Frontal Variant AD (Woodward et al., Int J Geriatr Psychiatry, 2010, 25, 732-738; Taylor et al., Nat Clin Pract Neurol, 2008, 4, 226-232; Alladi et al., Brain 2007, 130, 2636-2645): Described as rare compared to other focal AD syndromes. The neuropsychology of this syndrome has yet to be comprehensively described.

Neuroradiological Heterogeneity in Alzheimer’s Disease

Young Noh et al., Neurology, 2014

T1 MRI scans from 152 pts with AD; pts with significant WM excluded; scans processed to measures cortical thickness

Hierarchical Cluster Analysis yielded a 3-cluster solution

- Bilateral medial temporal group
- Bilateral parietal / precuneus / bilateral DLF group
- Diffuse association cortical group

Demographic/ Clinical Characteristics

- Three groups equal for MMSE, CDR, illness duration, education, & APOE_4

Neuropsychological Differences

- Neurocognitive tests on verbal serial list learning and semantic fluency
**Neuropathological Heterogeneity in Alzheimer Disease**

Murray et al., (*Lancet Neurol* 2011, 10, 785-796)

- Reviewed 889 autopsy cases of AD and found evidence for three NFT subtypes: a ‘limbic’ group; a posterior association cortical group; and a combined limbic/ cortical group.
- Measurable amounts of Lewy Bodies and vascular pathology were found in about 25 percent in all three groups.
- Exact impact of non-AD pathologies was not assessed. No neuropsychological profiles were described.

**Neuropathological Heterogeneity in Vascular/ Alzheimer Spectrum Dementia**


- 500 brains were described from the MCR initiative;
- Mixed AD/ VaD pathology was the most common pathological presentation;
- Many pts presented with dementia in life but with comparatively modest pathological burden;
- There was a group who upon autopsy met criteria for dementia, but dementia was not present in life;
- Risk of dementia in life was higher although statistically equal for vascular versus Alzheimer’s pathology;
- While no relationship between disability and Alzheimer-type pathology was observed, the risk of functional disability was increased three-fold in those with subcortical neuronal loss.

This study concluded

“In aggregate research shows that textbook assumptions about the pathological basis of dementia as discrete disease entities are an oversimplification (p. 365)”

**Demographic Data Based on Clinical Diagnosis**

Memory Clinic Patients Diagnosed with Dementia

<table>
<thead>
<tr>
<th></th>
<th>AD (n= 128)</th>
<th>VaD (n= 95)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>age</td>
<td>78.10 (5.23)</td>
<td>80.12 (5.24)</td>
<td>&lt;.005</td>
</tr>
<tr>
<td>education</td>
<td>12.57 (2.64)</td>
<td>11.99 (2.56)</td>
<td>ns</td>
</tr>
<tr>
<td>MMSE</td>
<td>22.84 (2.78)</td>
<td>21.92 (3.61)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Johnsq LA</td>
<td>5.99 (3.77)</td>
<td>16.15 (6.45)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

*Clinical Diagnosis
AD = McKhann et al., 1984; IVD – Chui et al., Neurology 1992
Latent Class Analysis: Neuropsychological Protocol of Interest

Working Memory / Mental Search
1. Mental Control - Boston Revision WMS (Acr= month backward; alphabet rhyming; alphabet visualization)
2. letter fluency (letters 'FAS')

Language / Lexical Access
3. Boston Naming Test
4. ‘animal’ (semantic) fluency

Episodic Memory
5. P(r)VLT: delayed free recall (9-word version)
6. P(r)VLT: delayed recognition

Four Group LCA Solution: Distinct Phenotypic Syndromes

<table>
<thead>
<tr>
<th></th>
<th>moderate / mixed (n= 54; 24%)</th>
<th>mild / mixed (n= 93; 42%)</th>
<th>amnesic (n= 49; 23%)</th>
<th>dysexecutive (n= 29; 13%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mental Control</td>
<td>60.59</td>
<td>77.41</td>
<td>87.69</td>
<td>57.82</td>
</tr>
<tr>
<td>(percent correct)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>letter fluency</td>
<td>18.41</td>
<td>20.88</td>
<td>36.08</td>
<td>16.03</td>
</tr>
<tr>
<td>‘animal’ fluency</td>
<td>6</td>
<td>9</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>Boston Naming</td>
<td>22</td>
<td>43</td>
<td>44</td>
<td>39</td>
</tr>
<tr>
<td>P(r)VLT - delayed free recall</td>
<td>48</td>
<td>1.67</td>
<td>63</td>
<td>2.57</td>
</tr>
<tr>
<td>P(r)VLT - recognition discriminability (percent)</td>
<td>61.24</td>
<td>75.67</td>
<td>61.53</td>
<td>81.69</td>
</tr>
<tr>
<td>age</td>
<td>80</td>
<td>79</td>
<td>79</td>
<td>80</td>
</tr>
<tr>
<td>education</td>
<td>12</td>
<td>12</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>MMSE</td>
<td>21</td>
<td>24</td>
<td>23</td>
<td>22</td>
</tr>
</tbody>
</table>

Four Group Dementia LCA Solution: Neuroradiological Findings

<table>
<thead>
<tr>
<th></th>
<th>moderate / mixed</th>
<th>mild / mixed</th>
<th>amnesic</th>
<th>dysex</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total brain volume (mm³/TBV)</td>
<td>1,125,284.34 (217,843.64)</td>
<td>1,131,607.01 (205,484.88)</td>
<td>1,233,778.61 (216,751.43)</td>
<td>1,032,460.21 (319,706.43)</td>
<td>ns</td>
</tr>
<tr>
<td>Junque Score (0-40)</td>
<td>8.11 (5.91)</td>
<td>8.71 (7.11)</td>
<td>5.74 (4.17)</td>
<td>16.31 (7.45)</td>
<td>dysex = all groups; p&lt;.001</td>
</tr>
<tr>
<td>Hippocampal volume (mm³)</td>
<td>3,035.43 (921.49)</td>
<td>3,842.97 (1,005.96)</td>
<td>3,590.28 (679.85)</td>
<td>4,672.08 (911.98)</td>
<td>dysex = all groups; p&lt;.002</td>
</tr>
<tr>
<td>Percent Hippocampus / TBV</td>
<td>2.73 (0.85)</td>
<td>3.47 (1.00)</td>
<td>2.95 (0.57)</td>
<td>4.74 (1.12)</td>
<td>dysex = all groups; p&lt;.003</td>
</tr>
</tbody>
</table>
## Four Group LCA Solution: Mental Control and Letter Fluency Test Performance

<table>
<thead>
<tr>
<th></th>
<th>moderate/mixed</th>
<th>mild/mixed</th>
<th>amnesic</th>
<th>dysex</th>
<th>significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mental control (time to completion – secs)</td>
<td>155.83 (17.78)</td>
<td>128.15 (18.64)</td>
<td>163.99 (21.51)</td>
<td>548.74 (66.66)</td>
<td>amn &lt; all groups; p &lt; .001</td>
</tr>
</tbody>
</table>
| Mental control (slope – sq root) | 5.31 (1.56) | 4.45 (2.20) | 4.01 (2.82) | 4.38 (1.66) | 1. dysex < amn; p < .001  
2. dysex > mild/ mixed; p < .005 |

## Four Group Cluster Solution: Animal Fluency: Assessing Lexical Access/ Semantic Integrity

<table>
<thead>
<tr>
<th></th>
<th>moderate/mixed</th>
<th>mild/mixed</th>
<th>amnesic</th>
<th>dysex</th>
<th>significance</th>
</tr>
</thead>
</table>
| Animal responses | 8.44 (2.75) | 9.16 (3.21) | 11.82 (3.46) | 8.84 (3.82) | amn < all groups; p < .001  
2. dysex > mild/ mixed; p < .005  
3. mod/mixed > amn; p < .002 |
| Association index (AI) | 11.55 (2.62) | 17.17 (23.52) | 22.14 (21.34) | 16.73 (19.37) | mod/mixed > mild/ mixed; p < .001  
mod/ mixed < amn; p < .001 |
| Animal Francis/ Kucera Score | 25.54 (20.71) | 26.78 (20.66) | 27.94 (14.52) | 28.32 (16.61) | mod/ mixed > all groups; p < .001 |

## Four Group Cluster Solution: Serial List Learning Performance – Savings and Cue Recall Intrusions

<table>
<thead>
<tr>
<th></th>
<th>moderate/mixed</th>
<th>mild/mixed</th>
<th>amnesic</th>
<th>dysex</th>
<th>significance</th>
</tr>
</thead>
</table>
| Saving Index (percent) | 25.20 (18.68) | 30.20 (22.94) | 30.62 (18.72) | 30.12 (27.04) | amn < mild/ mixed; p < .019  
amn < dysex; p < .001  
mod/mixed < mild/ mixed; p < .013  
mod/ mixed < dysex; p < .001 |
| % Cue Recall Intrusion | 25.20 (26.20) | 26.78 (26.20) | 26.78 (26.00) | 25.39 (26.47) | amn < mild/ mixed; p < .015  
amn > dysex; p < .001  
mod/mixed > mild/ mixed; p < .032  
mod/ mixed < dysex; p < .001 |
| CRI – Francis and Kucera Score (median test) | 25.20 | 13.25 | 27.30 | 6.40 | amn > dysex; p < .001  
mod/ mixed > mild/ mixed; p < .028  
mod/ mixed < dysex; p < .002 |
Phenotypic Syndromes in Alzheimer’s/ Vascular Spectrum Dementia

**LCA-Determined Within Group Comparisons**

<table>
<thead>
<tr>
<th></th>
<th>moderate/mixed</th>
<th>mild/mixed</th>
<th>amnesic</th>
<th>dysexecutive</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>executive scale</strong></td>
<td>-0.34 (0.43)</td>
<td>0.17 (0.46)</td>
<td>1.12 (0.44)</td>
<td>-0.99 (0.49)</td>
</tr>
<tr>
<td><strong>language scale</strong></td>
<td>-1.06 (0.50)</td>
<td>0.20 (0.61)</td>
<td>0.68 (0.69)</td>
<td>0.00 (0.77)</td>
</tr>
<tr>
<td><strong>memory score</strong></td>
<td>-0.66 (0.51)</td>
<td>0.21 (0.72)</td>
<td>-0.61 (0.53)</td>
<td>0.69 (0.88)</td>
</tr>
</tbody>
</table>

**LCA Analysis of Vascular/ Alzheimer Spectrum Dementia: Focal Phenotypic Syndromes**

**Dysexecutive Group**
- Negative slope (“Titanic Effect”) on timed executive tests; copious and pandemic perseverations; inability to establish mental set on tests assessing verbal concept formation; relative intact access to semantic knowledge; little evidence for a primary anterograde amnesia; dysexecutive problems in memory tests.

**Amnestic Group**
- Striking anterograde amnesia on list learning tests; relatively intact access to semantic knowledge; only mild dysexecutive impairment.

**Moderate/ Mixed Group**
- Striking anterograde amnesia; striking evidence for loss of and/or inability to access semantic knowledge; some evidence for greater dysexecutive impairment.

**Mild/ Mixed Group**
- Relatively mild impairment on standard neuropsychological tests.

**Cluster Analysis of Vascular/ Alzheimer Spectrum Dementia: Imputing Neuropathology**

**Dysexecutive Group** (Senile Dementia of the Dysexecutive Type)
- A white matter dementia syndrome characterized and driven by subcortical LA likely due to disruption of downward projections from the DLPFC to the DMN of the thalamus.

**Amnestic Group** (Senile Dementia of the Amnesic Type)
- A gray matter dementia syndrome characterized by relatively restricted NFT confined to the medial hippocampus/temporal lobe.

**Moderate/ Mixed Group** (Senile Dementia of the Mixed (amnesic/semantic) Type)
- A primarily gray matter dementia syndrome characterized by GREATER involvement involving the the medial hippocampus/temporal AND adjacent anterior/posterior cortex. Dysexecutive involvement could be due to frontal/sub NFT and/or specific tract involvement between posterior/ anterior cortex.

**Mild Mixed Group** (Senile Dementia of the Mixed Type)
- A primarily less severe gray matter dementia syndrome with fewer NFT throughout the brain.
Cognitive Impairment in Older Adults

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Rowan SOM

Learning Objectives

• Discuss the benefits of early detection and diagnosis of cognitive impairment.
• Recognize the difference between routine screening and assessing/diagnosing dementia.
• Identify the diagnostic process associated with most common types of dementia.
• Identify tools and tests that can be used in the diagnosis of dementia.

Outline

• Cognitive Impairment Identification/Screening
• Cognitive Assessment
• Dementia work up
• Diagnosis
• Disclosing the diagnosis
• Dementia Management
Scope of the problem

- Cognitive impairment in older adults is severely underdetected in primary care
- Older adults who have the condition are unlikely to receive a diagnostic evaluation
- Without a diagnosis, older adults who have dementia and their families are unlikely to receive appropriate care
- Cognitive impairment complicates treatment of co-morbidities

Barriers to Diagnosis

- Brief time for office visits.
- Need to focus office time on diagnosis and treatment of the person's other physical health problems.
- Ambivalence about the value of diagnosis.
- Concerns about the negative effect of a dementia diagnosis on the person or family.
- Perceived lack of effective drug treatments
- Lack of awareness about nondrug treatments and community services and supports that have been shown to benefit people with dementia and their families.

Outline

- Cognitive Impairment Identification/Screening
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- Dementia Management
Cognitive Conversation

- Raise the topic of brain health and changes in memory and cognition that may occur in aging
- Ask about memory and cognition
- Listen for older adults’/family concerns about memory and cognition
- Add a question about memory or cognition to health risk questionnaires

Cognitive Aging

- Memory deficits are usually subtle (e.g. misplacing items, forgetting names)
- Items recalled at a later time (e.g. ‘tip of tongue phenomenon’)
- Speed of processing declines

- No evidence of progressive worsening
- Function is preserved
- Increase in wisdom and expertise

Possible Risks or Threats to Brain Health

- Some medicines or improper use of them
- Smoking
- Excessive use of alcohol
- Heart disease, Diabetes or other health problems
- Poor diet
- Lack of physical activity
- Insufficient sleep
- Little social activity and being alone most of the time


• Be physically active.
• Reduce cardiovascular risk factors (including hypertension, diabetes, and smoking).
• Manage medications.
• Be socially and intellectually active, and continually seek opportunities to learn.
• Get adequate sleep and seek professional treatment for sleep disorders, if needed

Screening for Cognitive Impairment

• USPSTF: “I” Grade for routine screening for Cognitive Impairment (Oct. 2014)
• Assess cognitive function whenever cognitive impairment or deterioration is suspected
• Screening is part of annual wellness exam.
• Brief instruments can be used to screen for cognitive impairment
  – Mini Cog
  – Informant questionnaires

“Red Flags”

• The patient is a “poor historian.”
• The patient fails to appear for scheduled appointments or comes at the wrong time or on the wrong day.
• There is sudden difficulty adhering to a medication regimen.
• The patient defers to a family member to answer questions directed to the patient
• Unexplained functional decline or new onset psychiatric symptoms are evident
Cognitive Impairment in Older Adults

Identification and Screening

- Annual Wellness Visit
- Mini Cog
- Family questionnaire

AD8 Dementia Screening

Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE) Short Form
Outline

• Cognitive Impairment Identification/Screening
• Cognitive Assessment
• Dementia work up
• Diagnosis
• Disclosing the diagnosis
• Dementia Management

Cognitive Assessment Tools

• Montreal Cognitive Assessment (MOCA)
• St Louis University Mental Status Examination (SLUMS)
• Mini Mental Status Examination (MMSE)
Outline

• Cognitive Impairment Identification/Screening
• Cognitive Assessment
• Dementia work up
• Diagnosis
• Disclosing the diagnosis
• Dementia Management

History and Physical

• Review onset, course, and nature of memory and cognitive deficits and any associated behavioral, medical or psychosocial issues
• Conduct structured mental status exam (e.g. MoCA, SLUMS, MMSE)
• Perform neurological exam focusing on focal/lateralizing signs
• Assess ADLs and IADLs
Cognitive Impairment in Older Adults

Instrumental Activities of Daily Living Scale

Diagnostics

- Lab Tests
  - Routine: CBC, lytes, BUN, Cr, Ca, LFTs, glucose
  - Dementia screening labs: TSH, B12
  - Contingent labs (per patient history): RPR, HIV, heavy metals
- Neuroimaging
  - CT or MRI

Neuropsychological Testing

- Indicated in cases of early or mild symptom presentation, for differential diagnosis, determination of nature and severity of cognitive functioning, and/or development of appropriate treatment plan
Cognitive Impairment in Older Adults

Outline

• Cognitive Impairment Identification/Screening
• Cognitive Assessment
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• Disclosing the diagnosis
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Dementia

Major Neurocognitive Disorder

• Substantial cognitive decline from a previous level of performance, both subjectively and on the basis of objective testing
• Impairment interferes with independence in activities of daily living
• Cognitive deficits are not better explained by another mental disorder or delirium
• The changes involve one or more cognitive domains: memory, reasoning and problem solving, language, visuospatial abilities, change in personality and behavior

Mild Cognitive Impairment

Mild Neurocognitive Disorder

• Memory (or other cognitive) concern, usually not noticed by others but noticed by the individual and/or those close to them
• Objective memory (or other cognitive) impairment suggestive of modest decline
• Intact instrumental activities of daily living
• Not better explained by another cause (e.g. depression, anxiety)
• The prevalence of MCI in adults aged 65 years and older is 10 to 20%, and the estimated annual conversion rate to dementia is 10 to 15%
Alzheimer’s Disease

- Most common type of dementia (60–80% of cases)
- Memory loss and impaired learning early in the disease
- Behavior and psychological symptoms are common
  - Early: Depression and apathy
  - Moderate to Severe: agitation, wandering, psychotic features

Dementia With Lewy Bodies

- Second most common type of dementia (up to 30% of cases)
- Hallmark symptoms include visual hallucinations, REM sleep disorder, significant fluctuation in cognition and parkinsonism
- Severe neuroleptic sensitivity

Frontotemporal Dementia

- Primarily affecting individuals in their 50s and 60s
- EITHER marked changes in behavior/personality OR language variant (difficulty with speech production or loss of word meaning)
- Memory deficits are often not pronounced in early stages
Vascular Dementia

- Relatively rare in pure form (6-10% of cases)
- Often coexist with AD pathology “Mixed Dementia”
- Neuropsych profile consistent with “Dysexecutive Syndrome” of slow information processing and inattention

Outline

- Cognitive Impairment Identification/Screening
- Cognitive Assessment
- Dementia work up
- Diagnosis
  - Disclosing the diagnosis
- Dementia Management

Disclosing the Diagnosis

- Include family members, friends, or other caregivers
- A majority of patients want to know if they have Alzheimer’s Disease (AD)
- Most family members appreciate the benefits of diagnosis
- Diagnosis does not cause psychological stress in most patients and their families
Delivering the Diagnosis

Talking Points

• “Memory changes are due to abnormal accumulation of proteins in the brain, which interfere with the essential functions of thinking and processing. Basically, these proteins clog things up.”

• “Most people who have dementia don’t end up in nursing homes—they can live at home with family, be reasonably content, and have nice lives.”

• “Eventually, dementia will cause a worsening in your ability to handle regular tasks, such as shopping, finances, and medications. But we’ll talk regularly, and we’ll manage that.”

• “It is important for you and your family to plan for the future. The sooner the planning starts, the more you might be able to participate. Planning includes advance directives.”

• “Dementia is not hereditary in most cases.”

• “Dementia is a progressive condition with no cure, but we have treatments for symptoms and proper care and planning can greatly alleviate the burden of dementia.”

Outline

• Cognitive Impairment Identification/Screening
• Cognitive Assessment
• Dementia work up
• Diagnosis
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Dementia Management

• Pharmacologic Treatment
• Non Pharmacologic Management
Medications

- Cognition: Donepezil, Rivastigmine patch, Galantamine and Memantine (mid-late stage)
- Mood & Behavior: SSRIs or SNRIs
- Avoid/Minimize: Anticholinergics, hypnotics, narcotics, and antipsychotics (not to be used in Lewy Body dementia)

Dementia Care Plan

- Caregiver Education and Support
- Stimulation/activity/Maximizing function
- Advance Care Planning
- Legal Planning
- Safety
  - Medication management
  - Driving
- Chronic Disease Management
  - Modify treatment goals
  - Prevent potentially harmful hospitalization

Refer to Alzheimer's Association

Cognitive Impairment and Care Plan

G0505/99483

- Cognition-focused evaluation including history and examination
- Moderate or high complexity medical decision-making
- Functional assessment, including decision-making capacity
- Use of standardized instruments to stage dementia
- Medication reconciliation and review for high-risk medications (if applicable)
- Evaluation for neuropsychiatric and behavioral symptoms, including depression
- Evaluation of safety, including motor vehicle operation
- Identification of caregiver(s), caregiver's knowledge, caregiver's needs, social support, and caregiver's willingness to give care
- Advance care planning and palliative care needs
- Creation and sharing of a care plan
- All of the specified elements must be performed by the billing physician
Cognitive Impairment in Older Adults

Summary: Role of Primary Care

[Diagram with steps and options related to cognitive impairment in older adults]
Alzheimer's disease:
new mechanisms and early blood-based diagnosis

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Founder, Chief Scientific Officer, Stockholder

**Funding**
Osteopathic Heritage Foundation
Michael J Fox Foundation
Alzheimer’s Association
Foundation Venture Capital Group
Boye Foundation
GlaxoSmithKline
National Institutes of Health
Alzheimer’s Disease
The brain shrinks because neurons die.
Why do our neurons die?
One reason neurons die: they accumulate beta-amyloid

Extracellular vs Intraneuronal Amyloid (Abeta42) Deposition

Extracellular ———————————— Intraneuronal

Normal healthy brain
AD brain
Extracellular amyloid
Intracelluar amyloid

Important to resolve which is correct for proper drug targeting
Tools used to diagnose AD:

- A detailed patient history
- Information from family and friends
- Physical and neurological exams and lab tests
- Neuropsychological/cognitive tests
- Imaging tools such as CT scan, or magnetic resonance imaging (MRI).

Problem: These assess symptoms. AD pathology can be pretty far advanced by the time symptoms appear and a definitive diagnosis can be made using these methods. In other words, it may be too late for treatments to be effective, and the cost of these tests is very high.
Our blood contains brain-reactive autoantibodies that leak into the brain upon blood-brain barrier breach and bind selectively to pyramidal neurons in regions of AD pathology.

AD brain cerebral cortex
How Many Autoantibodies are in the Blood?

Nearly 2,000 autoantibodies are detected using 9,486 human protein targets - this reflects only 1/3 of the total human proteome.

### Effects of Age and Gender on Number of Autoantibodies in the Blood

<table>
<thead>
<tr>
<th>Age</th>
<th>N</th>
<th>% Female</th>
<th>Antibody Count</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;45</td>
<td>10</td>
<td>33.3</td>
<td>1498.2 ± 545.7</td>
<td>0.0021</td>
</tr>
<tr>
<td>45 - 65</td>
<td>32</td>
<td>18.2</td>
<td>2335.6 ± 1009.5</td>
<td>0.37</td>
</tr>
<tr>
<td>&gt;65</td>
<td>15</td>
<td>60</td>
<td>2647.8 ± 1139.2</td>
<td>0.0028</td>
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</tbody>
</table>

### Summary

<table>
<thead>
<tr>
<th>Sex</th>
<th>N</th>
<th>Age</th>
<th>Antibody Count</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>18</td>
<td>57.6 ± 18.7</td>
<td>2772.5 ± 714.8</td>
<td>0.004</td>
</tr>
<tr>
<td>Male</td>
<td>39</td>
<td>53.1 ± 15.1</td>
<td>2039.3 ± 1092.7</td>
<td></td>
</tr>
</tbody>
</table>

Total 166 | 62.4 ± 16.3 | 1996.9 ± 1051.9 |

Nagele et al., 2013 PLoS ONE
Natural IgG Autoantibodies Are Abundant and Ubiquitous in Human Sera, and Their Number Is Influenced By Age, Gender, and Disease

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Abstract

The presence of self-reactive IgG autoantibodies in human sera is largely thought to represent a breakdown in central tolerance and is typically regarded as a harbinger of autoimmune pathology. In the present study, immune-response profiling of human serum from 166 individuals via human protein microarrays demonstrates that IgG autoantibodies are abundant in all human serum, usually numbering in the thousands. These IgG autoantibodies bind to human antigens from organs and tissues all over the body and their serum diversity is strongly influenced by age, gender, and the presence of specific diseases. We also found that serum IgG autoantibody profiles are unique to an individual and remarkably stable over time. Similar profiles exist in rat and swine, suggesting conservation of this immunological feature among mammals. The number, diversity, and apparent evolutionary conservation of autoantibody profiles suggest that IgG autoantibodies have some important, as yet unrecognized, physiological function. We propose that IgG autoantibodies have evolved as an adaptive mechanism for debris-clearance, a function consistent with their apparent utility as diagnostic indicators of disease as already established for Alzheimer’s and Parkinson’s diseases.

Autoantibodies are involved in the clearance of debris generated by the body on a day-to-day basis.

Hypothesis:

Autoantibodies are involved in the clearance of debris generated by the body on a day-to-day basis.

If so, then...

The presence of disease leads to production of excessive debris from the organ affected.

...and this leads to an increased abundance of autoantibodies responsible for the clearance of disease-associated debris.
Autoantibody biomarkers bind to targets.

Incubation with fluorescent 2ndary antibodies.

Analysis and identification of disease-specific autoantibody biomarkers.

Detection of disease-specific autoantibody biomarkers using Protein Microarrays.

Overall Accuracies

Early AD at mild cognitive impairment (MCI) – 100%
Mild-moderate AD – 96%
Early PD – 92%
Mild-moderate PD – 97.1%
MS – 95%
Mild-Moderate Alzheimer’s Disease

1. Detected **451 autoantibodies** showing significantly higher prevalence in AD compared to controls (p < 0.01) – these are potentially useful as AD biomarkers.

2. Selected **the top 10 autoantibodies** showing the largest difference in group prevalence as our diagnostic indicators.

3. **Using only the top 10 indicators**, AD sera were distinguished from control sera with a **sensitivity of 96.0% and specificity of 92.5%**
Diagnosis of Alzheimer’s Disease Based on Disease-Specific Autoantibody Profiles in Human Sera

Eric Nagele¹, Min Han²,³, Cassandra DeMarshall², Benjamin Belinka¹, Robert Nagele³*¹

¹ Durin Technologies, Inc., New Brunswick, New Jersey, United States of America, ² Graduate School of Biomedical Sciences, School of Osteopathic Medicine, University of Medicine and Dentistry of New Jersey, Stratford, New Jersey, United States of America, ³ New Jersey Institute for Successful Aging, School of Osteopathic Medicine, University of Medicine and Dentistry of New Jersey, Stratford, New Jersey, United States of America

Abstract

After decades of Alzheimer’s disease (AD) research, the development of a definitive diagnostic test for this disease has remained elusive. The discovery of blood-borne biomarkers yielding an accurate and relatively non-invasive test has been a primary goal. Using human protein microarrays to characterize the differential expression of serum autoantibodies in AD and non-demented control (NDC) groups, we identified potential diagnostic biomarkers for AD. The differential significance of each biomarker was evaluated, resulting in the selection of only 10 autoantibody biomarkers that can effectively differentiate AD sera from NDC sera with a sensitivity of 96.0% and specificity of 92.5%. AD sera were also distinguishable from sera obtained from patients with Parkinson’s disease and breast cancer with accuracies of 86% and 92%, respectively. Results demonstrate that serum autoantibodies can be used effectively as highly-specific and accurate biomarkers to diagnose AD throughout the course of the disease.

Citation: Nagele E, Han M, DeMarshall C, Belinka B, Nagele R (2011) Diagnosis of Alzheimer’s Disease Based on Disease-Specific Autoantibody Profiles in Human Sera. PLoS ONE 6(8): e23112. doi:10.1371/journal.pone.0023112
Early Detection and Staging of Parkinson’s Disease
Origin of autoantibodies useful for diagnostics

Early-Stage Parkinson’s Disease Diagnostic Results
Using a Panel of the Top 50 PD Biomarkers
Testing Set Data Only

<table>
<thead>
<tr>
<th>Early-stage PD (n = 51) vs.</th>
<th>STAGING</th>
<th>DISEASE SPECIFICITY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age-matched controls</td>
<td>Mild-moderate PD</td>
</tr>
<tr>
<td>n</td>
<td>55</td>
<td>29</td>
</tr>
<tr>
<td>Sensitivity%</td>
<td>94.1</td>
<td>96.1</td>
</tr>
<tr>
<td>Specificity%</td>
<td>85.5</td>
<td>100.0</td>
</tr>
<tr>
<td>PPV%</td>
<td>85.7</td>
<td>100.0</td>
</tr>
<tr>
<td>NPV%</td>
<td>94.0</td>
<td>93.6</td>
</tr>
<tr>
<td>Overall accuracy%</td>
<td>92.0</td>
<td>97.5</td>
</tr>
<tr>
<td>Overall error%</td>
<td>12.1</td>
<td>2.5</td>
</tr>
</tbody>
</table>
If autoantibody biomarkers can be used to early diagnose and stage PD, can they also be used for early diagnosis and staging of AD?

YES!
Detection of Early Stage AD
MCI or even pre-symptomatic stages

Alzheimer’s Disease (AD):
AD pathology is underway 8-10 years before symptoms emerge

Early detection allows early treatment
(prior to appearance of symptoms)

Minimental Exam

Funded by the Osteopathic Heritage Foundation
The Alzheimer’s Disease Neuroimaging Initiative

**ADNI-1: Naturalistic study of AD progression**

- 200 NORMAL 3 yrs
- 400 MCI 3 yrs
- 200 AD 2 yrs
- Visits every 6 mo

- 57 sites
- Clinical, blood, LP
- Cognitive Tests
- 1.5T MRI

Some also have
- 3.0T MRI (25%)
- FDG-PET (50%)
- PiB-PET (approx 100)

All data in public database:
UCLA/LONI/ADNI
No embargo of data
Early detection of AD at the Mild Cognitive Impairment Stage

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Age (Years)</th>
<th>Age (Range)</th>
<th>Sex (% Male)</th>
<th>Ethnicity (% Caucasian)</th>
<th>MMSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild Cognitive Impairment</td>
<td>50</td>
<td>73.0 ± 7.1</td>
<td>55-91</td>
<td>58</td>
<td>94</td>
<td>27.9</td>
</tr>
<tr>
<td>Controls</td>
<td>50</td>
<td>70.9 ± 5.1</td>
<td>62-87</td>
<td>56</td>
<td>78</td>
<td>-</td>
</tr>
<tr>
<td>Mild-Moderate Alzheimer’s disease</td>
<td>50</td>
<td>78.5 ± 8.8</td>
<td>61-97</td>
<td>42</td>
<td>88</td>
<td>16.5</td>
</tr>
<tr>
<td>Mild-Moderate Parkinson’s disease</td>
<td>25</td>
<td>73.9 ± 9.5</td>
<td>53-88</td>
<td>48</td>
<td>45</td>
<td>-</td>
</tr>
<tr>
<td>Early-Stage Parkinson’s disease</td>
<td>25</td>
<td>72.4 ± 2.9</td>
<td>67-79</td>
<td>56</td>
<td>96</td>
<td>-</td>
</tr>
<tr>
<td>Multiple Sclerosis</td>
<td>25</td>
<td>53.8 ± 6.6</td>
<td>43-67</td>
<td>40</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Breast Cancer</td>
<td>11</td>
<td>52.5 ± 0.9</td>
<td>51-54</td>
<td>0</td>
<td>100</td>
<td>-</td>
</tr>
</tbody>
</table>

Subject demographics. The number of individuals (n), age, range of age, gender, and ethnicity are listed for each disease group. For MCI and mild-moderate AD subjects, the Mini-Mental State Examination (MMSE) score is included as a measure of cognitive impairment.

DeMarshall et al., 2016
Low CSF Abeta42 Levels in MCI Subjects
A biochemical confirmation of ongoing AD pathology (95% confidence)

CSF Aβ42 levels in MCI samples. CSF Aβ42 (pg/ml) for EMCI (blue) and LMCI (red) samples.

We are detecting the pathology – independent of symptoms
Testing Set of subjects (those not used in biomarker discovery) and achieved the same 100% overall accuracy. In addition, we tested the specificity of the biomarker panel (the ability to distinguish AD-driven MCI from other diseases) and achieved specificity values exceeding 95%. These are fantastic results and we are very excited about it. This suggests that we have arrived at our final biomarker panel that will be used to design the test kit for Specific Aim 3.

### Diagnosis of Alzheimer’s Disease at the MCI Stage

**ADNI MCI subjects with Low CSF Abeta42 Levels**

100% overall diagnostic accuracy

<table>
<thead>
<tr>
<th>MCI (n = 25) vs.</th>
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<tr>
<td>n</td>
<td>25</td>
<td>25</td>
<td>*50</td>
<td>25</td>
<td>11</td>
</tr>
<tr>
<td>Sensitivity %</td>
<td>100.0</td>
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</tr>
<tr>
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<td>98.0</td>
<td>100.0</td>
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</tr>
<tr>
<td>PPV %</td>
<td>100.0</td>
<td>96.2</td>
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<tr>
<td>NPV %</td>
<td>100.0</td>
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</tr>
<tr>
<td>Overall Accuracy %</td>
<td>100.0</td>
<td>98.0</td>
<td>98.7</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Overall Error %</td>
<td>0</td>
<td>2.0</td>
<td>1.3</td>
<td>0</td>
<td>0</td>
</tr>
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</table>

This data is derived from Testing Set ADNI subjects only; i.e., subjects not involved in biomarker discovery.

*DeMarshall et al. 2015 manuscript in preparation*
Confirmation of 100% diagnostic accuracy for AD-driven MCI using only Testing Set subjects. Here, we note that our panel of 50 MCI biomarkers was derived from the analysis of 25 MCI subjects (called the Training Set). Here, as an indisputable test of diagnostic accuracy, we used the Training Set biomarkers to diagnose a Testing Set of subjects (those not used in biomarker discovery) and achieved the same 100% overall accuracy. In addition, we tested the specificity of the biomarker panel (the ability to distinguish AD-driven MCI from other diseases) and achieved specificity values exceeding 95%. These are fantastic results and we are very excited about it. This suggests that we have arrived at our final biomarker panel that will be used to design the test kit for Specific Aim 3.

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Staging of Alzheimer’s Disease

Distinguishing MCI from mild-moderate AD

98.7% overall accuracy
Confirmation of 100% diagnostic accuracy for AD-driven MCI using only Testing Set subjects. Here, we note that our panel of 50 MCI biomarkers was derived from the analysis of 25 MCI subjects (called the Training Set). Here, as an indisputable test of diagnostic accuracy, we used the Training Set biomarkers to diagnose a Testing Set of subjects (those not used in biomarker discovery) and achieved the same 100% overall accuracy. In addition, we tested the specificity of the biomarker panel (the ability to distinguish AD-driven MCI from other diseases) and achieved specificity values exceeding 95%. These are fantastic results and we are very excited about it. This suggests that we have arrived at our final biomarker panel that will be used to design the test kit for Specific Aim 3.

**Disease Specificity of MCI Biomarkers**

Distinguishing MCI from other diseases

Nearly 100% overall accuracy

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AD MCI biomarkers can distinguish MCI from early-stage PD, multiple sclerosis and breast cancer with nearly 100% overall accuracy.
Blood-Based Biomarkers

Detection of Alzheimer’s disease at mild cognitive impairment and disease progression using autoantibodies as blood-based biomarkers

Cassandra A. DeMarshall\textsuperscript{a,b,c}, Eric P. Nagele\textsuperscript{a,d}, Abhirup Sarkar\textsuperscript{a,b,c}, Nimish K. Acharya\textsuperscript{a,c}, George Godsey\textsuperscript{b,c}, Eric L. Goldwaser\textsuperscript{a,b,c}, Mary Kosciuk\textsuperscript{a,c}, Umashanger Thayasivam\textsuperscript{e}, Min Han\textsuperscript{a,b,c}, Benjamin Belinka\textsuperscript{d}, Robert G. Nagele\textsuperscript{a,c,d,*}, on behalf of the Alzheimer’s Disease Neuroimaging Initiative

\textsuperscript{a}Biomarker Discovery Center, New Jersey Institute for Successful Aging, Rowan University School of Osteopathic Medicine, Stratford, NJ, USA
\textsuperscript{b}Graduate School of Biomedical Sciences, Rowan University, Stratford, NJ, USA
\textsuperscript{c}Department of Geriatrics and Gerontology, Rowan University School of Osteopathic Medicine, Stratford, NJ, USA
\textsuperscript{d}Durin Technologies, Inc., New Brunswick, NJ, USA
\textsuperscript{e}Department of Mathematics, Rowan University, Glassboro, NJ, USA
Capabilities of Our New AD and PD Diagnostic Tests

Early Diagnosis
Patient Management
Test of Drug Efficacy

1. Early detection and diagnosis of AD and PD.

2. Staging the disease - allows physicians to follow disease progress in individual patients.

3. Evaluate patient response to drug therapy by monitoring patient progression through clinical stages.


5. Allows early enrollment into clinical trials.

6. Evaluate patient response to therapy (drug efficacy) in clinical trials of new drugs.
Multiple Sclerosis (MS)

- Demyelinating disease of the central nervous system
- 4 distinct subtypes
  - ~80% of all patients initially diagnosed as relapsing-remitting subtype
    - 2/3 of those patients progress to secondary progressive subtype

Results: 95% overall accuracy
Can distinguish relapsing-remitting from secondary progressive subtypes
We can identify MCI in hip fracture repair patients with >95% accuracy.
Concussion and Traumatic Brain Injury

Work Underway – Panel of TBI biomarkers are being tested
Collaboration with the University of Pennsylvania and VA Hospital
A Major Change in Disease Diagnostics

Allows simultaneous, early, pre-symptomatic disease detection of multiple diseases

**Long-term goal:** Construct a Multi-Disease Diagnostic Blood Test

Only 20-50 protein targets are needed for each disease diagnostic. Each array has space for thousands of protein targets. Thus, plenty of room for hundreds of diagnostic tests on a single microarray.

Various cancers

Neurodegenerative diseases

Alz    Park    MS    Breast    Lung    Pancreatic
If treatments are effective, there should be less disease-associated debris production and a corresponding decrease in autoantibody biomarkers in the blood.

Concept:

If treatments are effective, there should be less disease-associated debris production and a corresponding decrease in autoantibody biomarkers in the blood.

Compare blood samples before and at intervals after drug treatment. Resolution of disease leads to reduction in titer of disease-specific biomarkers.
With Profound Thanks

Current Nagele Lab
Nimish Acharya, PhD
Mary Kosciuk, PhD
Cassandra DeMarshall, PhD
Eric Nagele, DO, Neurology Resident
Eric Goldwaser, PhD
Abhirup Sarkar, PhD Student
George Godsey, PhD Student
Benjamin Belinka, PhD
Uma Thayasivam, PhD

Recent Past Nagele Lab Members
Peter Clifford, DO-PhD
Gilbert Siu, DO-PhD
Eli Levin, DO-PhD
Min Han, MD-PhD

Honorary Lab Members
Benjamin Belinka, PhD, Durin Tech.
Gerald Carey, Rowan SOM

Collaborators
RowanSOM
NJISA
David Libon, PhD
Martin Forsberg, MD
Johns Hopkins - Delirium
Frederick Sieber, MD
Esther Oh, MD
Univ. of Penn - TBI
Randel Swanson, DO-PhD
Kings College London - Psychosis
Thomas Pollak, PhD
City Univ. of NY – AD mechanisms
Hoau-Yan Wang, PhD

Funding
The Osteopathic Heritage Foundation
Michael J Fox Foundation
Foundation Venture Capital Group
Boye Foundation
Alzheimer’s Association
National Institutes of Health
THANK YOU!