Precision Medicine for Delirium

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DF/HCC Proteomics Core
Div. of Interdisciplinary Medicine and Biotechnology
Beth Israel Deaconess Medical Center
Harvard Medical School
What is Precision Medicine?
Precision Medicine

The Promise: Advanced Molecular Diagnostic Will Tailor Medical Management & Treatment Based on the Individual Characteristics of Each Patient

Optimized Therapeutic Benefit
Less Adverse Reactions
The end of “one-size-fits-all”?
New Disease Concepts Transform Medicine

One Size Fits All

One Size Fits One

Multi-Omics enables Implementation of Precision Medicine

Disease-Centric

Patient-Centric

Presented at NIDUS Delirium Boot Camp 2017, Posted with permission.
Exceptional Success When Treatment Matched to Driver Mutation

Sequencing enables patient-specific recommendation of targeted therapies with improved outcome

Without Precision Medicine
Some benefit, many do not

With Precision Medicine
Each patient receives right medicine

**Lung Cancer Patients**

- **Normal EGFR**
  - No response

- **Mutant EGFR, Normal K-Ras/N-Ras**
  - Response

- **Mutant EGFR, Mutant K-Ras/N-Ras**
  - Shorter survival

Sequencing

Treatment with EGFR Inhibitor

Presented at NIDUS Delirium Boot Camp 2017, Posted with permission.
Approaches for Biomarker Discovery and Precision Medicine for Delirium
SAGES Study Design

- **Plasma collection at 4 timepoints (before, during and after)**
  - pre-operation (PREOP)
  - post-operation (post-anesthesia care unit) (PACU)
  - post-operation day 2 (POD2)
  - post-operation day 30 (POD30)

- **Matched case:control design**
  - delirium versus no delirium
  - 6 matching factors

- **Carefully selected patient population (N =560; 24% delirium rate)**
  - dementia-free
  - >70 years
  - elective, non-cardiac surgery

- **Biomarkers to assess**
  - Risk
  - Guide diagnosis
  - Management
  - Pathogenesis

- **Objective**: Identify reliable blood-based postoperative delirium biomarkers, delirium pathophysiology & new therapeutic targets

- **Targeted and untargeted biomarker discovery & validation**
Overall Multi-Omics Approach for Biomarker Discovery for Delirium
What defines a good biomarker?

• Specificity to the disease

• Reliability
  • low false positive rate
  • low false negative rate

• Does it inform about the underlying biological processes involved?
  • Can we predict new therapeutic targets based on revealed pathophysiology?
Potential Uses for Delirium Biomarkers

- **Risk predictor:**
  - Measurable before delirium onset
  - Identifies individuals at risk

- **Disease marker:**
  - Changes (up or down) with delirium onset
  - Returns to pre-surgery levels with delirium resolution

- **Prognostic marker:**
  - Measurable before or after delirium onset
  - Alterations in measured level is proportional to long term “consequences”
Types of Molecules Used as Biomarkers

- **Proteins**/peptides
  - Post-translational modifications (PTMs)
- Metabolites
- Lipids
- Cells
- DNA sequence
  - Entire genome
  - Specific genes, SNPs
Protein Biomarkers for Delirium
Why use plasma?

• Minimally invasive
• Easily obtained
• Widely used clinically
• A source for good representation of proteins released from many tissues in the body
• Plasma, serum, and urine are being used in the diagnosis of many diseases
• Opportunities for home diagnostics

But: CSF may be more informative
Targeted Proteomics
Cytokines and Postoperative Delirium in Older Patients Undergoing Major Elective Surgery

Sarinnapha M. Vasunilashorn,1,2,3* Long Ngo,1,3* Sharon K. Inouye,1,2,3 Tawia A. Libermann,1,3 Richard N. Jones,2,5 David C. Alsop,2,4 Jamey Guess,3 Sandra Jastrzebski,7 Janet E. McElhaney,8 George A. Kuchel,7** and Edward R. Marcantonio1,2,3**

1Harvard Medical School, Boston, Massachusetts. 2Aging Brain Center, Institute for Aging Research, Hebrew SeniorLife, Boston, Massachusetts. 3Department of Medicine, and 4Department of Radiology, Beth Israel Deaconess Medical Center, Boston, Massachusetts. 5Department of Psychiatry and Human Behavior, Warren Alpert Medical School, Brown University, Providence, Rhode Island. 7UConn Center on Aging, University of Connecticut Health Center, Farmington. 8Advanced Medical Research Institute of Canada, Sudbury, Ontario, Canada.
### Patient Characteristics In 2 Matched Cohorts (Discovery And Replication) And Pooled Cohort used for Biomarker Verification

GCP=general cognitive performance, a composite measure of neuropsychological measures reflecting cognitive domains vulnerable to delirium.  
ApoE= presence of an ApoE ε4 allele (i.e., ApoE ε carrier) has been associated with increased risk of Alzheimer’s Disease.  
Vascular comorbidity: present if patient had a myocardial infarction, congestive heart failure, peripheral vascular disease, cerebrovascular disease, hemiplegia, diabetes, and diabetes with end organ damage.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Discovery (39 pairs)</th>
<th>Replication (36 pairs)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Delirium (n=39)</td>
<td>No Delirium (n=39)</td>
</tr>
<tr>
<td>Age (M, SD)</td>
<td>77.3 (5.0)</td>
<td>76.8 (4.7)</td>
</tr>
<tr>
<td>Female (%)</td>
<td>54</td>
<td>54</td>
</tr>
<tr>
<td>GCP (M, SD)</td>
<td>55.2 (5.6)</td>
<td>56.4 (5.6)</td>
</tr>
<tr>
<td>Type of surgery (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orthopedic</td>
<td>92</td>
<td>92</td>
</tr>
<tr>
<td>Vascular</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Vascular comorbidity (%)</td>
<td>38</td>
<td>38</td>
</tr>
<tr>
<td>ApoE ε4 carrier (%)</td>
<td>13</td>
<td>13</td>
</tr>
</tbody>
</table>

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Luminex Analysis of 12 Cytokines in Plasma
Median paired difference between delirium and matched control

<table>
<thead>
<tr>
<th>Cytokine (pg/mL)</th>
<th>PREOP</th>
<th>PACU</th>
<th>POD2</th>
<th>POD1M</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>0.26</td>
<td>0.28</td>
<td>0.31</td>
<td>0.27</td>
</tr>
<tr>
<td><strong>IL-2</strong></td>
<td>0.99*</td>
<td>0.77*</td>
<td>1.07**</td>
<td>0.73*</td>
</tr>
<tr>
<td>IL-4</td>
<td>7.13</td>
<td>0.54</td>
<td>-1.56</td>
<td>-2.32</td>
</tr>
<tr>
<td>IL-5</td>
<td>0.19</td>
<td>0.19</td>
<td>-0.52</td>
<td>0.57</td>
</tr>
<tr>
<td><strong>IL-6</strong></td>
<td>1.01</td>
<td>7.17*</td>
<td>39.35**</td>
<td>0.49</td>
</tr>
<tr>
<td>IL-8</td>
<td>0.86</td>
<td>0.68</td>
<td>0.89</td>
<td>-0.18</td>
</tr>
<tr>
<td>IL-10</td>
<td>0.00</td>
<td>0.10</td>
<td>0.27</td>
<td>-0.11</td>
</tr>
<tr>
<td>IL-12</td>
<td>-2.64</td>
<td>-1.73</td>
<td>-2.88</td>
<td>-4.24*</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.03</td>
</tr>
<tr>
<td>GMCSF</td>
<td>-0.58</td>
<td>-0.49</td>
<td>-0.45</td>
<td>-0.22</td>
</tr>
<tr>
<td>TNF-α</td>
<td>2.12</td>
<td>2.52</td>
<td>3.22</td>
<td>3.10*</td>
</tr>
<tr>
<td>VEGF</td>
<td>3.50</td>
<td>-0.34</td>
<td>4.10*</td>
<td>0.83</td>
</tr>
</tbody>
</table>

*p < .05; **p < .01.
Untargeted Proteomics
Higher C-Reactive Protein Levels Predict Postoperative Delirium in Older Patients Undergoing Major Elective Surgery: A Longitudinal Nested Case-Control Study


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http://dx.doi.org/10.1016/j.biopsych.2016.03.2098
Global Proteomics using Mass Spectrometry
Quantitative Shotgun Proteomics for Unbiased Biomarker Discovery

Plasma from Matched Case-Control Patients at 4 Time Points

Depletion of Abundant Proteins Using Agilent Antibody Columns

Tryptic Digestion C1 C2 C3 C4 D1 D2 D3 D4

iTRAQ Labeling of tryptic peptides
Isobaric Tags

Combine iTRAQ labeled peptides from 8 samples

UltiMate™ Capillary/Nano LC System
2D Fractionation of Peptides
1. SCX
2. RP

Identification of Differentially Expressed Proteins

8-Plex iTRAQ MS/MS Spectra

Database Search

Dynamic Range ~3 logs

ABI 4800

Identification and Quantitation of Proteins

MALDI-TOF/TOF

Protein Analysis

MALDI Plate

Plasma from Matched Case-Control Patients at 4 Time Points

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MALDI-TOF/TOF

Protein Analysis

MALDI Plate

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Biomarker Discovery Phase
iTRAQ Quantitative Mass Spectrometry Identifies Consistently Higher Levels of CRP in Patients who Develop Delirium

Heat map of iTRAQ relative quantitation for 10 proteins in 5 matched case-control samples across four timepoints (PREOP, PACU, POD2 and POD1M)

Annotation
- C-reactive protein (CRP)
- Heparin cofactor 2 (SERPIND1)
- Pigment epithelium-derived factor (SERPINF1)
- Coagulation factor XII (F12)
- Serum amyloid P-component (APCS)
- Tetranectin (CLEC3B)
- Extracellular matrix protein 1 (ECM1)
- CD44 antigen (CD44)
- Gelsolin (GSN)
- Glutathione peroxidase 3 (GPX3)
Biomarker Verification Phase

ELISA of CRP in Whole Matched Case-Control Cohort Confirms Statistically Significant Higher CRP Levels in Patients with Delirium

Time-specific median of paired differences (MPD) of ELISA CRP concentrations between delirium cases and no-delirium controls at 4 timepoints in the discovery, replication, and pooled cohorts

<table>
<thead>
<tr>
<th>Time of Blood Draw</th>
<th>Discovery (39 pairs)</th>
<th>Replication (36 pairs)</th>
<th>Pooled (75 pairs)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MPD (mg/L)</td>
<td>IQ range</td>
<td>P-value</td>
</tr>
<tr>
<td>PREOP</td>
<td>1.97</td>
<td>(-1.02, 7.75)</td>
<td><strong>0.02</strong></td>
</tr>
<tr>
<td>PACU</td>
<td>2.83</td>
<td>(-2.29, 10.68)</td>
<td>0.06</td>
</tr>
<tr>
<td>POD2</td>
<td>71.97</td>
<td>(5.05, 139.82)</td>
<td>&lt;<strong>0.01</strong></td>
</tr>
<tr>
<td>POD1M</td>
<td>2.72</td>
<td>(-1.85, 7.16)</td>
<td>0.06</td>
</tr>
</tbody>
</table>

MPD=Median of paired differences (delirium case minus no-delirium control)
ELISA=enzyme-linked immunosorbent assay
IQ=interquartile
PREOP= preoperative, PACU= postanesthesia care unit, POD2=postoperative day 2, POD1M=30 days postoperation

p-values obtained from nonparametric signed-tank test. Bold indicates significant at p<.05 level

Presented at NIDUS Delirium Boot Camp 2017, Posted with permission.
Median CRP Concentrations by Delirium Status at 4 Timepoints in Pooled Cohort (75 Matched Pairs)
High C-Reactive Protein Predicts Delirium Incidence, Duration, and Feature Severity After Major Noncardiac Surgery

Sarinnapha M. Vasunilshorn, PhD, Simon T. Dillon, PhD, Sharon K. Inouye, MD, MPH, Long H. Ngo, PhD, Tamara G. Fong, MD, PhD, Richard N. Jones, ScD, Thomas G. Travison, PhD, Eva M. Schmitt, PhD, David C. Alsop, PhD, Steven D. Freedman, MD, PhD, Steven E. Arnold, MD, Eran D. Metzger, MD, Towia A. Libermann, PhD, and Edward R. Marcantonio, MD, SM

JAGS 65:e109–e116, 2017
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<table>
<thead>
<tr>
<th>CRP measure (mg/L)</th>
<th>Delirium Incidence</th>
<th>Delirium Duration (per day)*</th>
<th>Sum CAM-S (per point)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RR (95% CI)</td>
<td>Days (95% CI)</td>
<td>Score (95% CI)</td>
</tr>
<tr>
<td><strong>CRP PREOP</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartiles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1 (≤0.95)</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Q2 (0.95-2.56)</td>
<td>1.4 (0.8-2.3)</td>
<td>0.3 (0.2-0.5)</td>
<td>1.5 (0.9-2.1)</td>
</tr>
<tr>
<td>Q3 (2.56-6.39)</td>
<td>1.7 (1.0b-2.7)</td>
<td>0.3 (0.2-0.5)</td>
<td>2.5 (1.8-3.2)</td>
</tr>
<tr>
<td>Q4 (≥6.39)</td>
<td>1.8 (1.2-2.9)</td>
<td>0.4 (0.2-0.5)</td>
<td>3.6 (2.9-4.3)</td>
</tr>
<tr>
<td>p-trend*</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>High-risk cutpoint*</td>
<td>≥3 vs. &lt;3</td>
<td>0.2 (0.1-0.4)</td>
<td>2.6 (2.1-3.2)</td>
</tr>
<tr>
<td><strong>CRP POD2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartiles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1 (≤127.53)</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Q2 (127.53-177.05)</td>
<td>1.1 (0.6-1.7)</td>
<td>0.1 (0.1-0.2)</td>
<td>1.2 (0.6-1.8)</td>
</tr>
<tr>
<td>Q3 (177.05-235.73)</td>
<td>1.5 (1.0-2.3)</td>
<td>0.2 (0.0-0.4)</td>
<td>3.5 (2.9-4.2)</td>
</tr>
<tr>
<td>Q4 (≥235.73)</td>
<td>1.5 (1.0-2.4)</td>
<td>0.2 (0.0-0.4)</td>
<td>4.5 (3.8-5.2)</td>
</tr>
<tr>
<td>p-trend*</td>
<td>.02</td>
<td>.02</td>
<td>&lt;.01</td>
</tr>
</tbody>
</table>

Associations of CRP across the entire SAGES cohort with postoperative delirium, delirium duration, & delirium feature severity (sum of all CAM-S scores)
Summary

• IL-6 levels increase in patients experiencing delirium

• CRP is elevated before surgery and is a potential predictive biomarker for delirium, delirium duration, & delirium severity

• Pre-Inflammatory status prior to surgery may increase risk of postoperative delirium

• CRP and IL-6 involved in many diseases

• Can we identify more specific and novel biomarkers for delirium?
Challenges in Delirium Biomarker Discovery (Serum/Plasma/CSF)

Blood Test for Delirium?

- Proteins from various tissues/cells released into circulation
- Exosomes with proteins from various tissues/cells including brain released into circulation

Challenges
- Biomarker proteins present at low concentrations
- Many other more abundant blood proteins
- Exosomes present at low concentrations

Presented at NIDUS Delirium Boot Camp 2017, Posted with permission.
How Do We Measure Simultaneously a Broad Range of Low Abundant Proteins?

Molecular & Cellular Proteomics 2003, Anderson and Anderson 2 (1): 50

12-13 Logs Differences in Protein Expression
Ideal Proteomics Platform for Protein Biomarker Discovery: SOMAscan

- Highly multiplexed, sensitive, specific, quantitative proteomic tool

- Measures simultaneously 1305 proteins/sample in only 65μl of human serum/plasma/urine; 6μg of protein from tissue/cell lysate/exosomes

- Dynamic range >8 logs (femtomolar to micromolar)

- Reproducibility (~5% median %CV)

- Protein-capture SOMAmer (Slow Off-rate Modified Aptamer) reagents

- SOMAmers: protein affinity-binding reagents and unique nucleotide sequences recognizable by specific DNA hybridization probes
Within-Person Stability of Plasma Protein Expression Patterns Over 1 Year

- Each patient clusters with itself across the 2 time points
- Blood drawn from a patient at different time points is very similar
- Every person has a different fingerprint of proteins

ICC or Spearman $r \geq 0.4$ for 91% of proteins

Nurses Health Study Cohort

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Differences in BMI Easily Captured by SOMAscan:

Elevated Inflammatory Proteins Correlate with BMI

- BMI < 25 Kg/m²
- BMI ≥ 25 to < 30 Kg/m²
- BMI ≥ 30 Kg/m²

Heatmap of Proteins Comparing Individuals with BMI < 25 vs. ≥25 to < 30 vs. ≥30 kg/m²

*FDR-adjusted P-value < 0.05
SOMAscan perfectly differentiates plasma proteins before (PREOP) and after surgery (POD2)

Stress & Inflammation linked proteins are increased by surgery

POD2 vs. PREOP (BH corrected paired t-test p<0.01) L1OXV: 100%
SOMAscan Ideal for Neuroinflammation & Immune System Biomarker Discovery

• Covers large portion of immune system proteins
  • CD antigens
  • Cytokines
  • Chemokines
  • Soluble Receptors
  • Coagulation
  • Complement
  • Checkpoints

• Covers many inflammation and neuroinflammation proteins
  • Cytokines
  • Chemokines
  • Soluble Receptors
  • Acute Phase Proteins
Postoperative Delirium Plasma Biomarker Discovery

Delirium (12)

No Delirium Controls (12)

Plasma at PREOP & POD2

SOMAscan

PREOP Controls

POD2 Controls

POD2 Delirium

PREOP Delirium

POD2 Control

POD2 Del No CD

POD2 Del LTCD

PREOP Control

PREOP Del No...

PREOP Del LTCD

RFU

PREOP Con

PREOP Del

POD2 Con

POD2 Del
SOMAscan Accurately Discriminates Between Delirium & No Delirium at PREOP

Hierarchical Clustering of 12 Proteins

L1OXV: 91.67%

Principal Component Analysis of 12 Proteins
Delirium Metabolome/Lipidome Platforms

Targeted Metabolomics
- AB/SCIEX 5500 QTRAP triple quadrupole

Untargeted Metabolomics/Lipidomics
- Thermo Scientific Q Exactive HF/Plus
- Ultra fast & ultra sensitive

Serum
Biopsies
Stool

>250 metabolites

>2000 lipids

MetaboAnalyst Pathway Enrichment

5,000-20,000 metabolites

- High resolution
- Extremely fast scan speeds
- Quantitative
Metabolomics Analysis of Plasma Samples at POD2 Reveals Delirium-Specific Alterations

11 Metabolites

No Del  Del
Lipidomics Analysis of 12 Matched Pairs of Plasma Samples at PREOP and POD2 Reveals Delirium-Specific Alterations

PREOP

No Del

38 Lipids

Del

POD2

No Del

56 Lipids

Del
Delirium Immunome Platform: CyTOF **Mass Cytometry**
Massively Multi-Parametric Detection System for Single Cell ImmunoPhenotyping

**Key Advantages of CyTOF**
- Phenotypically & functionally profile all immune cell subsets at single-cell resolution for up to 100 different cell surface and intracellular signaling proteins by using antibodies coupled to metal isotopes
- Discrete isotope peaks without significant overlap, enabling higher multiplexing than FACS
- Innovative software tools (viSNE, SPADE, Citrus) incorporate pattern recognition approaches to enable detection of finely tuned cell subsets (clusters of single cells with similar expression patterns)

<table>
<thead>
<tr>
<th>Patient</th>
<th>% CD11c+CD14+ Monocytes PREOP</th>
<th>% CD11c+CD14+ Monocytes POD1</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>9.86</td>
<td>58.71</td>
</tr>
<tr>
<td>23</td>
<td>22.08</td>
<td>35.78</td>
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<tr>
<td>24</td>
<td>26.19</td>
<td>53.63</td>
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<td>18</td>
<td>36</td>
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<td>2</td>
<td>20.77</td>
<td>47.21</td>
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<td>59</td>
<td>8.27</td>
<td>36.28</td>
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<td>62</td>
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<tr>
<td>median</td>
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<td>47.2</td>
</tr>
<tr>
<td>t-test</td>
<td>0.009</td>
<td></td>
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</tbody>
</table>
Future Delirium Biomarkers will be Multi-Modal

Combination of:
- Lipids
- Metabolites
- Proteins (expression, isoforms, PTMs)
- RNAs (mRNA, miRNA, lncRNA, splicing)
- DNAs (CNVs, SNPs, methylation)
- Single Immune Cells
The Next Revolution: Single Use Health & Wellness Chip on Laptop or iPhone

- Disease Predisposition
- Early Disease Detection e.g. Cancer, Cardiovascular Disease
- Disease Predisposition e.g. Alzheimer's, Breast Cancer, Diabetes
- Targeted Drug Selection e.g. Mutated Driver Genes & Signaling Pathways
- Untreatable Disease Predisposition e.g. Huntington
- Drug Metabolism e.g. Cyt P450

Benefit: Earlier Detection, Precise Diagnosis, & Targeted Treatment

Improved Outcomes

Oxford Nanopore