Biomarkers for Delirium

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What is a biomarker?

- A biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process, or of a condition or disease.
What defines a good biomarker?

1. Specificity to the disease

2. Reliability
   i. low false positive rate
   ii. low false negative rate

3. Does it inform about the underlying biological processes involved?
   i. Can we predict new biomarkers based on revealed pathophysiology?

Best practices to search for new biomarkers

• Well controlled studies with defined criteria

• Properly collected and stored biological samples
  – Overall goal: avoid introducing bias into samples

• How good and appropriate are your control samples?
  – Study design is usually comparative
  – Ideal situation: each person would be their own control, so need collection timepoint(s) before disease onset
Delirium Challenges

- Delirium: clinical diagnosis only right now
  - no measurable biomarkers exist to inform diagnosis, management

- Dynamic nature
  - acute onset in response to surgery/trauma
  - fluctuates (subsyndromal)
  - can resolve quickly
  - some patients experience long-term consequences

- Biomarkers: insights into the biology of delirium
  - pathophysiology—important to design targeted prevention, treatment
  - understand subtypes, high risk groups

Tailor study design to incorporate dynamics

- Classic approach:
  - measure biomarkers at a single time point
  - “diseased” and “non-diseased”

- Delirium methodology:
  - measure biomarkers over several time points
    - before, during and after
  - consider both “disease” effects and “time” effects in selecting samples for biomarker discovery
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:**
  - Changes after delirium onset
  - Alterations in measured level is proportional to long term “consequences”

Heterogeneity of Delirium
Specific Challenges I

- **Clinical, not pathological, diagnosis**

- **Wide spectrum of psychomotor phenotypes:**
  - highly agitated to nearly comatose

- **Wide spectrum of severity:**
  - Mild to very severe
  - Subsyndromal delirium

- **Is all delirium actually the same disease?**
Strategies to reduce heterogeneity

- Carefully characterize clinical phenotypes, including measures of disease severity
  - Enables potential subset analyses
  - Exclude subsyndromal cases, at least at first

- Focus on settings that reduce heterogeneity
  - Example: elective surgery is relatively "clean" population that enables measurement before, during, and after episode of delirium
  - Analyze a few or one surgery type for discovery phase of study

Confounding factors
Specific Challenges II

- Delirium occurs in a complex patient “setting”
  - Many potential confounders:
    - Older patients exhibit multiple comorbidities
    - Acute illnesses exhibit varying etiology and severity
    - Multiple treatments including medications

- How do you know if you are identifying biomarkers of delirium or a confounding factor(s)?
Overcoming/minimizing confounders

- **Restrict study sample**
  - Exclude patients with certain comorbidities
  - Can be done up front, or at analytic stage

- **Matched case:control design**
  - Match on key confounding variables (age, sex)
  - Impossible to match on more than a few

- **Statistical adjustment**
  - Measure confounders and adjust for them
  - Challenge: under controlling, over controlling

Types of Molecules Used as Biomarkers

- **Proteins**/peptides
  - Post-translational modifications (PTMs)
- **Small-molecules**
- **Lipids**
- **Cells**
- **DNA sequence**
  - Entire genome
  - Specific genes, SNPs
Sources of Biomarkers

- **Body fluids**
  - blood (invasive) -> serum/plasma
  - cerebrospinal fluid (CSF) (invasive)
  - urine
  - saliva

- **Tissue**
  - blood cells (invasive)
  - biopsies (invasive)

Protein Biomarkers for Delirium

**Why use blood?**

- Minimally invasive
- Easily obtained
- Wide use in clinical assays
- A source of proteins released from tissues in the body
- Potential for at-home diagnostics
Rationale for blood as a biomarker source

Since blood carries oxygen and other nutrients to all organs, proteins from cells are released into the circulation

Complexities:
- Biomarker proteins present at low concentrations
- Dominant blood proteins obscure lower level potential protein biomarkers

Methods to search for new protein biomarkers

- **Targeted:**
  - Based on previous knowledge assay a limited, defined set of candidate protein biomarkers
  - Measure in both Disease and Control samples, compare levels

- **Untargeted:**
  - Measure as many proteins as technology will allow
  - Compare Disease vs. Control
  - Employ multiple patient cohorts to ensure robustness
  - For large-scale validation use a more economical targeted technology/strategy
SAGES study design

- Blood collection at four (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
  - six (6) matching factors

- Carefully selected patient population
  - dementia-free
  - > 70 years
  - elective, non-cardiac surgery

- Targeted and untargeted biomarker discovery techniques
Targeted approach

Cytokines and Postoperative Delirium in Older Patients Undergoing Major Elective Surgery

Sarinnapha M. Vasunilashom,1,2,3* Long Ngo,1,3* Sharon K. Inouye,1,2,3 Towia A. Libermann,1,5 Richard N. Jones,2,6 David C. Alsop,2,4 Jamey Guess,7 Sandra Jastrzebski,7 Janet E. McElhaney,8 George A. Kuchel,7,** and Edward R. Marcantonio1,2,3***

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## Six matching factors in 75 patient pairs

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pooled (75 Pairs)</th>
<th>Delirium (n = 75)</th>
<th>No Delirium (n = 75)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Age, Years, Mean (SD)</td>
<td>77.6 (4.7)</td>
<td>77.2 (4.5)</td>
<td></td>
</tr>
<tr>
<td>2. Female Sex, %</td>
<td>56</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>3. GCP, Mean (SD)</td>
<td>54.5 (5.3)</td>
<td>55.5 (5.2)</td>
<td></td>
</tr>
<tr>
<td>4. Type of Surgery, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orthopedic</td>
<td>88</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>Vascular</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>7</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>5. Vascular Comorbidity, %</td>
<td>44</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>6. APOE ε4 Carrier, %</td>
<td>20</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

## 12 cytokines measured in blood

### 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>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.
IL-6 levels in Discovery and Replication cohorts

A

Discovery cohort

B

Replication cohort

Untargeted approach
Key Challenges for Untargeted Delirium Protein Biomarker Discovery

• 100,000s of different protein isoforms
• Dynamic range of protein concentrations >12 logs
  • More abundant proteins obscure lower level proteins
• Finding multiple protein biomarkers for sensitive and specific personalized medicine
• Most technologies require compromising sensitivity vs. number of proteins detected vs. sample consumed
• Hypothesis: Most accurate delirium biomarkers will involve more than one biological pathway

SAGES study paper

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
Unbiased Protein Biomarker Discovery and ELISA Verification Strategy: From iTRAQ–Mass Spectrometry to Antibody-Based Confirmation

C-reactive protein (CRP) is increased in delirium
iTRAQ relative quantitation method for 5 matched pairs
CRP levels in patient plasma
4 timepoints: 75 matched pairs

Delirium protein biomarkers -> pathophysiology

- **Interleukin-6 (IL-6)**
  - plays a key role in regulating inflammation
  - controls CRP gene expression
  - known to disrupt the blood brain barrier (BBB)

- **C-reactive protein (CRP)**
  - key end-point biomarker of inflammation
  - biological function is to bind to phospholipids
  - animal model studies have implicated CRP in disruption of BBB
Delirium Protein Biomarkers
SAGES study: found so far …

- Risk predictor (CRP and …)
  - Measurable before delirium onset
  - Identifies individuals at risk

- Disease marker (IL-6 and CRP and …)
  - Changes (up or down) with delirium onset
  - Returns to pre-surgery levels with delirium resolution

- Prognostic marker (still searching)
  - Changes after delirium onset
  - Change in measured level is proportional to long term “consequences”

How would insults to the body affect brain function?

Blood → CSF → Brain
Summary

- IL-6 levels increase in patients experiencing delirium
- CRP is elevated before surgery and is a potential predictive biomarker for delirium
- CRP levels increase during delirium, above the surgery effect
- Inflammation is increased before and during delirium
- Can we identify other biomarkers distinct from the IL-6 /CRP pathway to generate a more robust multi-marker panel test?

Questions?