Validating Field Methods for Measuring Protein Analytes in Blood Samples of Older U.S. Adults

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Health involves coordinated action of biomarkers.

How do we maximize the number of analytes measured from a single field sample?

Inflammation is mediated by cytokines and chemokines in many chronic conditions (e.g. diabetes and depression).
Inflammatory Biomarkers: Cytokines and Chemokines

- Typically measured singly or as the “pro-inflammatory triad”
  - TNF-α, IL-1, and IL-6
- Many more are involved in complex inflammatory processes.
- How do we handle and analyze data on multiple analytes?
The National Social Life, Health, and Aging Project (NSHAP)

- 2010-2011, $N = 3,377$
- 62-91 years old
- 55% women
- In-home assessments
- Health and chronic disease
Our Goals

• Simultaneously measure 18 chemokines and cytokines in a large population survey.
  • IFN-γ, IL-10, IL-1b, IL-6, MCP-1, TGF-α, TNF-α, GM-CSF, IL-12, IL-13, IL-1ra, IL-2, sIL-2ra, IL-3, IL-4, IL-5, TNF-β, and VEGF

• We also measured molecules involved in lipid and glucose metabolism (Adiponectin, Fibrinogen, Apo-b, NGAL).

• Maximize the number of samples producing numerical values.
Whole Blood Collection and Assay

K$_2$EDTA coated Microtainers™ ➔ PLASMA

Luminex xMAP® technology ➔ 18 Analyte Assay Panels
Preliminary Results for 18 Cytokines and Chemokines

How about we omit the dilution??
Study 1: Optimizing Assay Protocol

• Wave 2 (2010-2011); subset N = 78; 72.7 ± 8.4 years of age; 61.5% women.

• 250 µl of capillary whole blood collected in K$_2$EDTA coated Microtainer® and stored as plasma.

• 18-plex assays with Luminex ® xMAP technology and Bio-Plex® software.

• Each sample was assayed twice: standard dilution (1:2) and without dilution (“neat”).
Study 1: “Neat” Increases Sensitivity

16 of the 18 analytes increased 10 - 120%

Percent Change

Neat vs. Dilute

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Neat vs. Dilute</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-3</td>
<td>-14% - 4%</td>
</tr>
<tr>
<td>VEGF</td>
<td>10%</td>
</tr>
<tr>
<td>IL-1ra</td>
<td>12%</td>
</tr>
<tr>
<td>IL-12</td>
<td>13%</td>
</tr>
<tr>
<td>TNF-a</td>
<td>18%</td>
</tr>
<tr>
<td>MCP-1</td>
<td>24%</td>
</tr>
<tr>
<td>IFN-5</td>
<td>27%</td>
</tr>
<tr>
<td>IL-1b</td>
<td>38%</td>
</tr>
<tr>
<td>IL-13</td>
<td>39%</td>
</tr>
<tr>
<td>IL-5</td>
<td>39%</td>
</tr>
<tr>
<td>IL-10</td>
<td>41%</td>
</tr>
<tr>
<td>IL-4</td>
<td>58% - 60%</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>78%</td>
</tr>
<tr>
<td>TGF-a</td>
<td>85%</td>
</tr>
<tr>
<td>IL-6</td>
<td>91%</td>
</tr>
<tr>
<td>sIL-1Ra</td>
<td>120%</td>
</tr>
<tr>
<td>TNF-b</td>
<td></td>
</tr>
<tr>
<td>IL-2</td>
<td></td>
</tr>
</tbody>
</table>
Study 2: Validating Field vs. Clinical Methods

• NSHAP field methods differ from typical clinical methods in two observable ways: type of sample and timing of sample preparation.

• Undetectable values may be an artifact of the NSHAP field methods, or be true values in community dwelling older US adults.
Study 2: Sample

- N = 60
- Age matched to NSHAP Wave 2
- 75% women
- University of Chicago Senior Outpatient Center at South Shore
## Study 2: Methods

### Blood Collection Method

<table>
<thead>
<tr>
<th>Time from Blood Draw to Freeze (hours)</th>
<th>Plasma</th>
<th>Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 - 72</td>
<td>NSHAP</td>
<td>TBA</td>
</tr>
<tr>
<td>4</td>
<td>TBA</td>
<td>Clinic</td>
</tr>
</tbody>
</table>
Difference in Percent Numerical: NSHAP vs. Clinical

Note: * = p<0.05, **=p<0.01, ***=p<0.001
Study 3: Cytokine and Chemokine Patterns in Older U.S. Adults

• Describe the distributions of cytokines among the general population of older U.S. adults

• Develop multivariate methods for analyzing multiple analytes within individuals (N=2,745)

• Study the association between inflammatory profiles and health conditions
## Results of Cluster Analysis Using 80th Percentile of Each Analyte

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>642</td>
<td>23.39%</td>
</tr>
<tr>
<td>2</td>
<td>454</td>
<td>16.54%</td>
</tr>
<tr>
<td>3</td>
<td>305</td>
<td>11.11%</td>
</tr>
<tr>
<td>4</td>
<td>285</td>
<td>10.38%</td>
</tr>
<tr>
<td>5</td>
<td>239</td>
<td>8.71%</td>
</tr>
<tr>
<td>6</td>
<td>224</td>
<td>8.16%</td>
</tr>
<tr>
<td>7</td>
<td>213</td>
<td>7.76%</td>
</tr>
<tr>
<td>8</td>
<td>195</td>
<td>7.10%</td>
</tr>
<tr>
<td>9</td>
<td>188</td>
<td>6.85%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>2745</strong></td>
<td><strong>100.00%</strong></td>
</tr>
</tbody>
</table>

### Quantile regressions
- 80\textsuperscript{th} percentile

### K-means cluster analysis
- Jaccard similarity coefficient
Greater than 60% have elevated levels of:

- IL-10
- IL-13
- IL-2
- IL-4
- IL-6
- TNF-β

Likely to be white men with colds

AA: $RR = 0.57, p = 0.007$
Hispanic: $RR = 0.40, p < 0.0001$
Female: $RR = 0.64, p = 0.001$
Cold during interview: $RR = 2.23, p = 0.002$
At least 60% have elevated levels of:

- IL-1β
- TNF-α

More likely to have diabetes ($RR = 1.77, p = 0.003$)
100% have elevated levels of MCP-1

Less likely to have Frequent Depressive Symptoms ($RR = 0.58, p = 0.015$)
Conclusions

• Multiple cytokines can be measured in a single blood sample collected in home-based surveys.

• Running samples “neat” or undiluted significantly increased assay sensitivity (16 of 18 analytes).

• Our field method had significantly more numerical values than the standard clinical protocol (14 of 18 analytes).

• These methods can be used in either large scale field surveys or laboratory studies.
Distinct cytokine profiles are identifiable among the general population of U.S. older adults, which are associated with:

1. presence of a cold or acute infection
2. gender and ethnicity
3. depression
4. diabetes
Thank you!
Any Questions?

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• We thank colleagues in NSHAP and respondents who participated in this study
NSHAP Condition:
Percent of Samples with Numerical Values
Study 2: Validated Field Methods Increase Sensitivity

Field and clinical methods highly correlated

Note: Pearson $r = 0.86$, $p<0.001$; EXCLUDING OUTLIER (N=59): Spearman rank $rho = 0.82$, $p<0.001$; Pearson $r = 0.79$, $p<0.001$