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

Kristina Pagel, Philip Schumm, Kristen Wroblewski, Joscelyn Hoffmann, Hannah You, Elbert Huang, Louise Hawkley, Martha McClintock

The University of Chicago

NIA-Sponsored Biomarker Network Meeting

April 26th, 2017

Biomarkers in Field or Survey Research

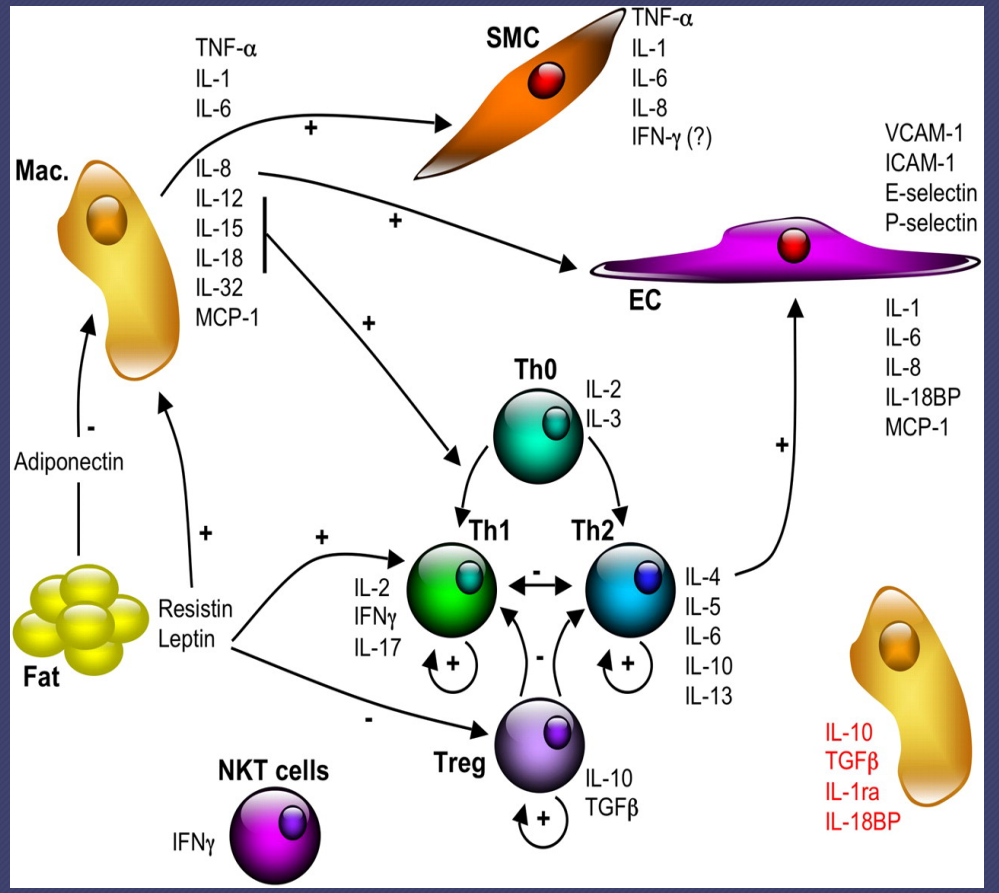
- Health involves coordinated action of biomarkers.

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

THE NATIONAL SOCIAL LIFE HEALTH & AGING PROJECT

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.

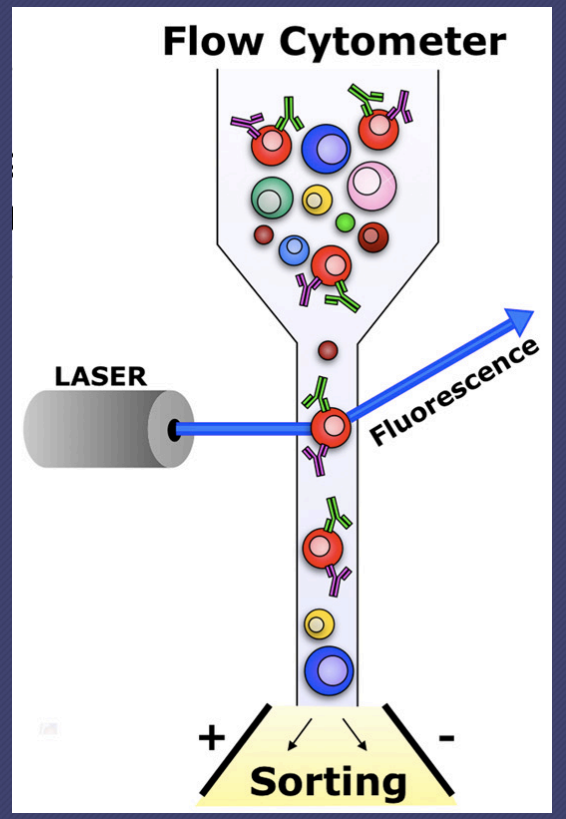
Whole Blood Collection and Assay

K_2EDTA coated Microtainers ™

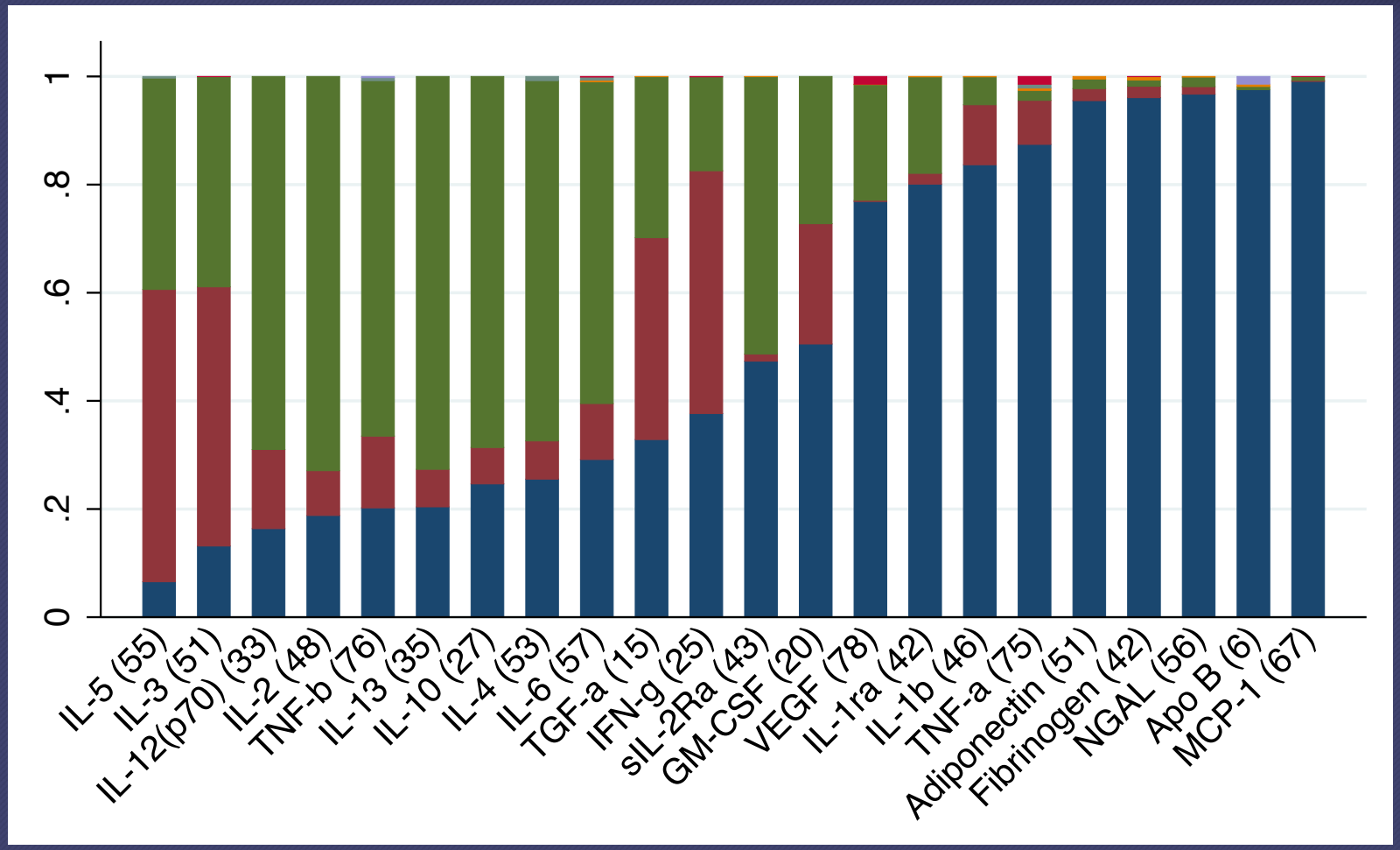
➔ PLASMA

Luminex xMAP® technology

➔ 18 Analyte Assay Panels



Preliminary Results for 18 Cytokines and Chemokines



Numerical

- On Standard Curve
- Extrapolated

Categorical

- OOR Low
- OOR High

How about we omit the dilution??

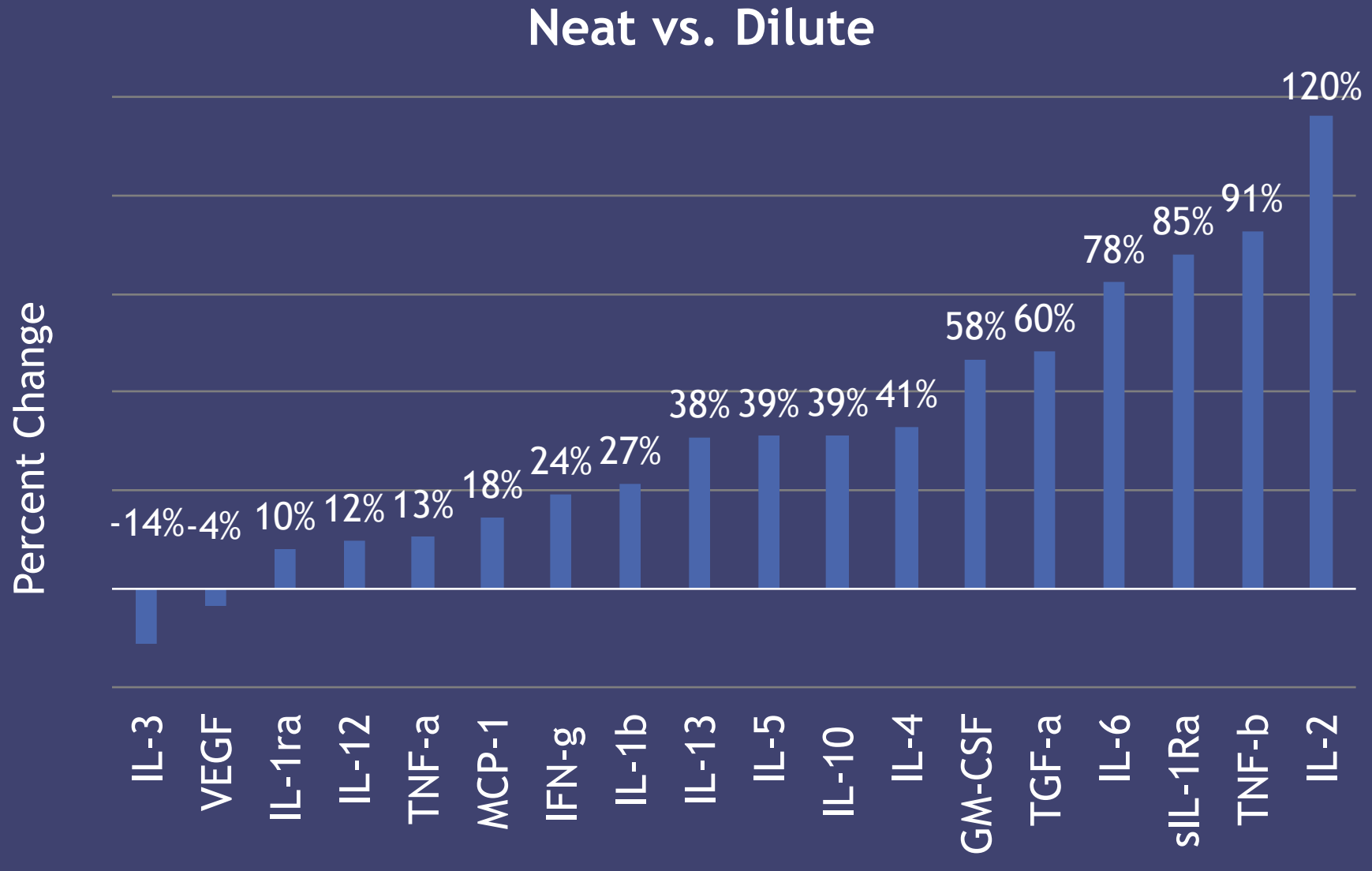
Study 1: Optimizing Assay Protocol

- 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_2EDTA 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

$$\frac{\#\text{Neat} - \#\text{Dilute}}{\#\text{Dilute}}$$

16 of the 18 analytes increased 10 - 120%



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

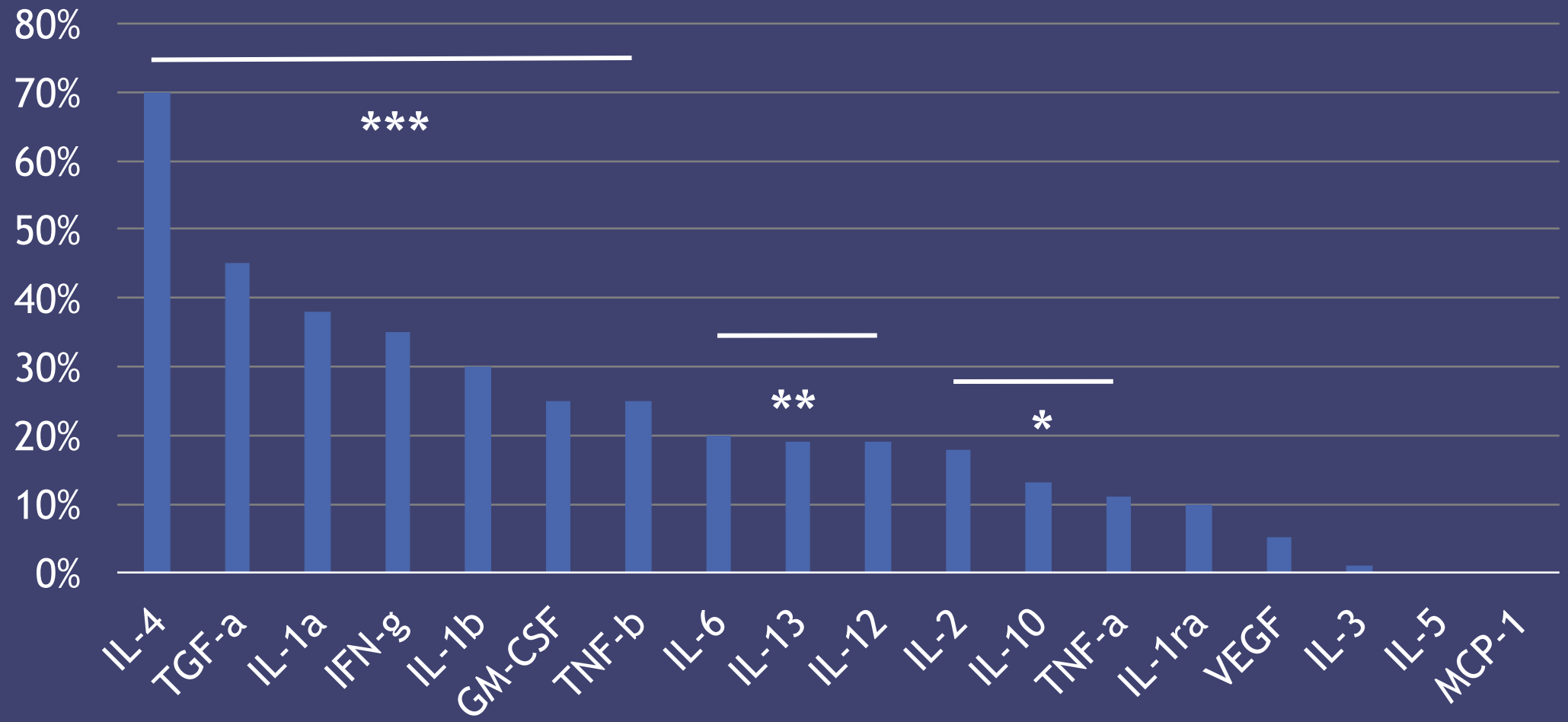
- N = 60
- Age matched to NSHAP Wave 2
- 75% women
- University of Chicago Senior Outpatient Center at South Shore

Study 2: Methods

Blood Collection Method

Time from Blood Draw to Freeze (hours)	Plasma	Serum
24 - 72	NSHAP	TBA
4	TBA	Clinic

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

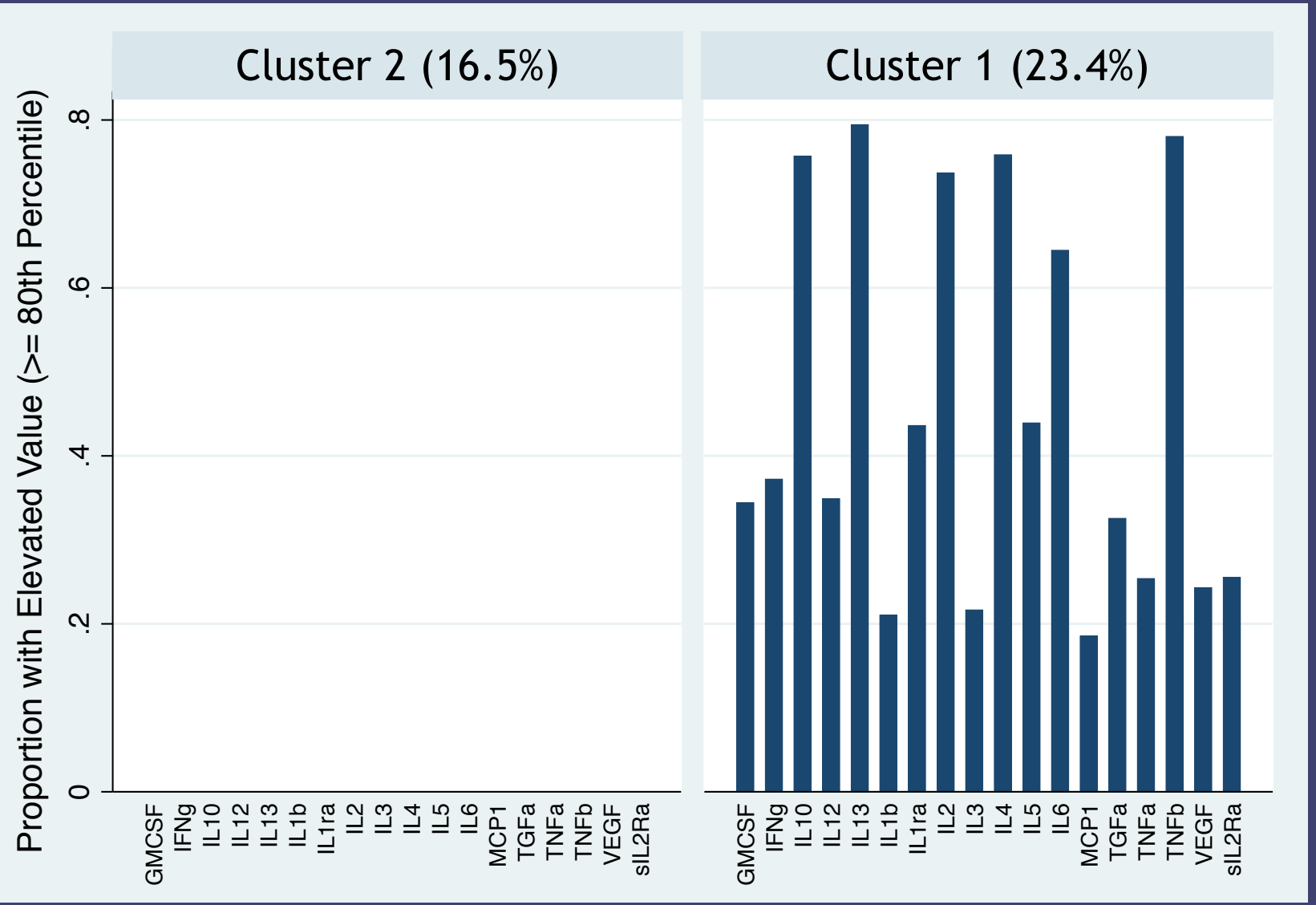
Quantile regressions

- 80th percentile

K-means cluster analysis

- Jaccard similarity coefficient

Cluster	Frequency	Percent
1	642	23.39%
2	454	16.54%
3	305	11.11%
4	285	10.38%
5	239	8.71%
6	224	8.16%
7	213	7.76%
8	195	7.10%
9	188	6.85%
Total	2745	100.00%

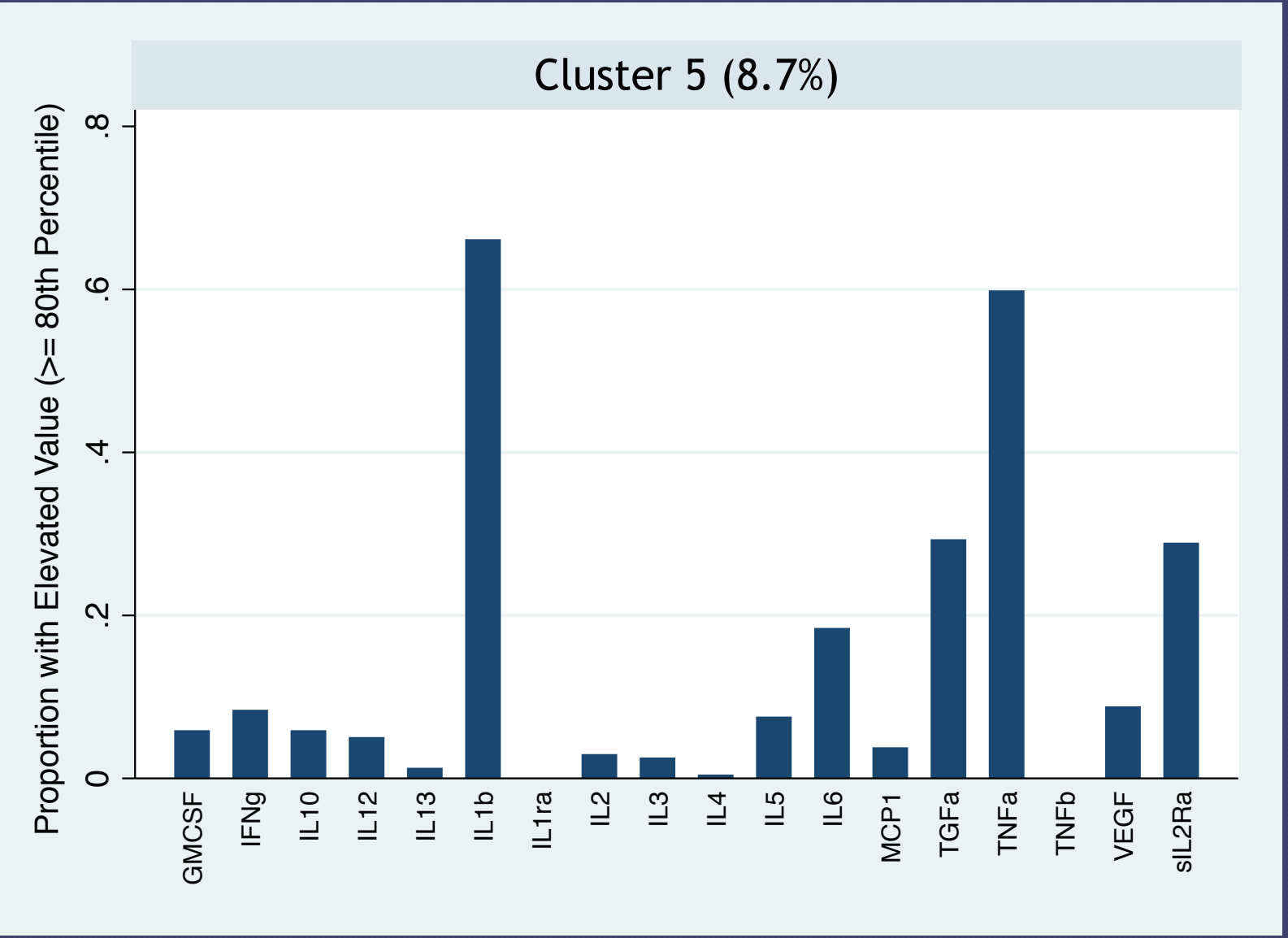


Greater than 60% have elevated levels of:

IL-10	IL-13
IL-2	IL-4
IL-6	TNF-β

Likely to be white men with colds

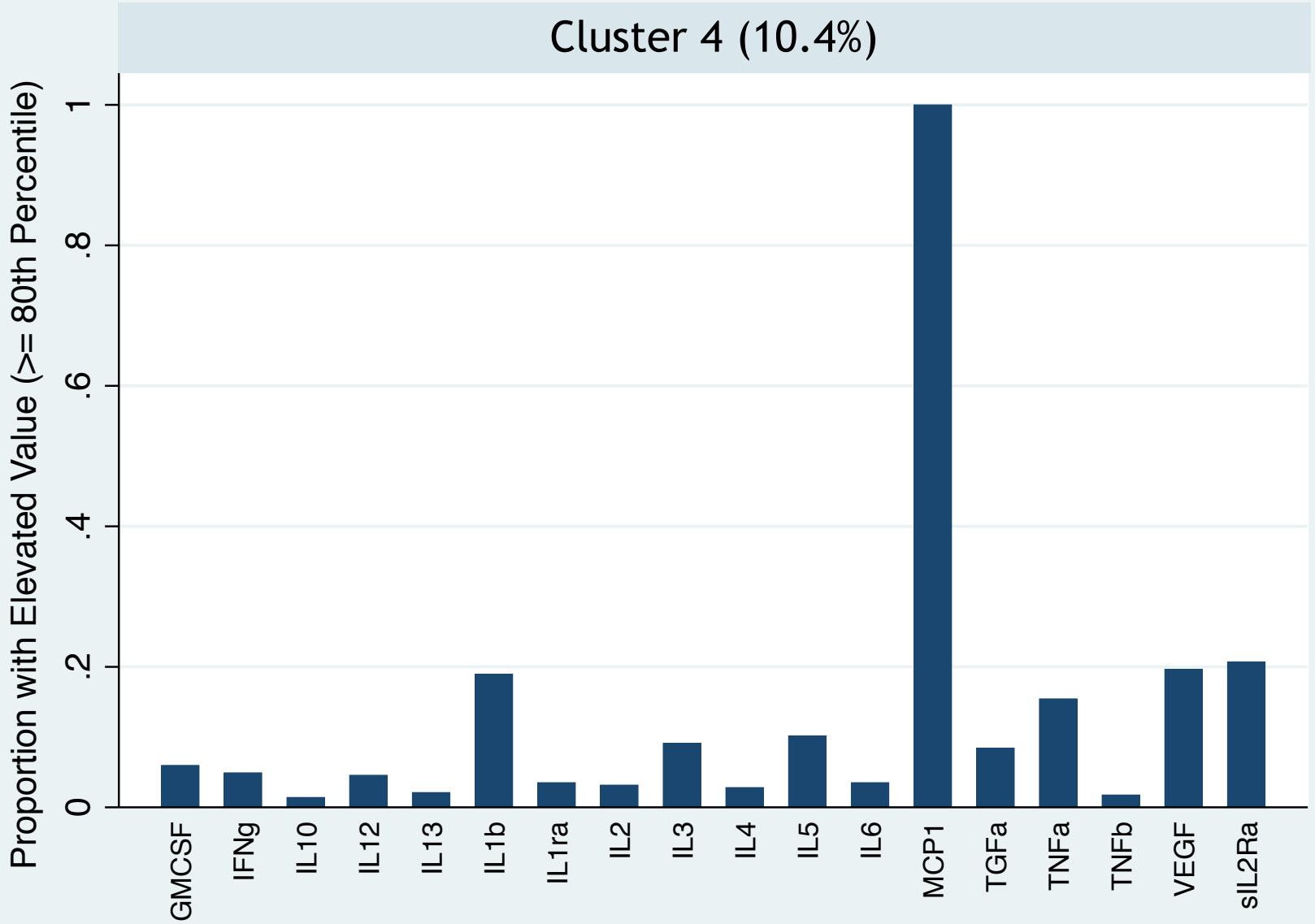
AA: $RR = 0.57$, $p = 0.007$
Hispanic: $RR = 0.40$, $p < 0.0001$
Female: $RR = 0.64$, $p = 0.001$
Cold during interview: $RR = 2.23$, $p = 0.002$



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.

Conclusions

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

Reference

Waite, Linda J., Kathleen Cagney, Benjamin Cornwell, William Dale, Elbert Huang, Edward O. Laumann, Martha McClintock, Colm A. O'Muircheartaigh, and L. Philip Schumm. National Social Life, Health, and Aging Project (NSHAP): Wave 2 and Partner Data Collection. ICPSR34921-v1. Ann Arbor, MI: Inter-university Consortium for Political and Social Research [distributor], 2014-04-29.

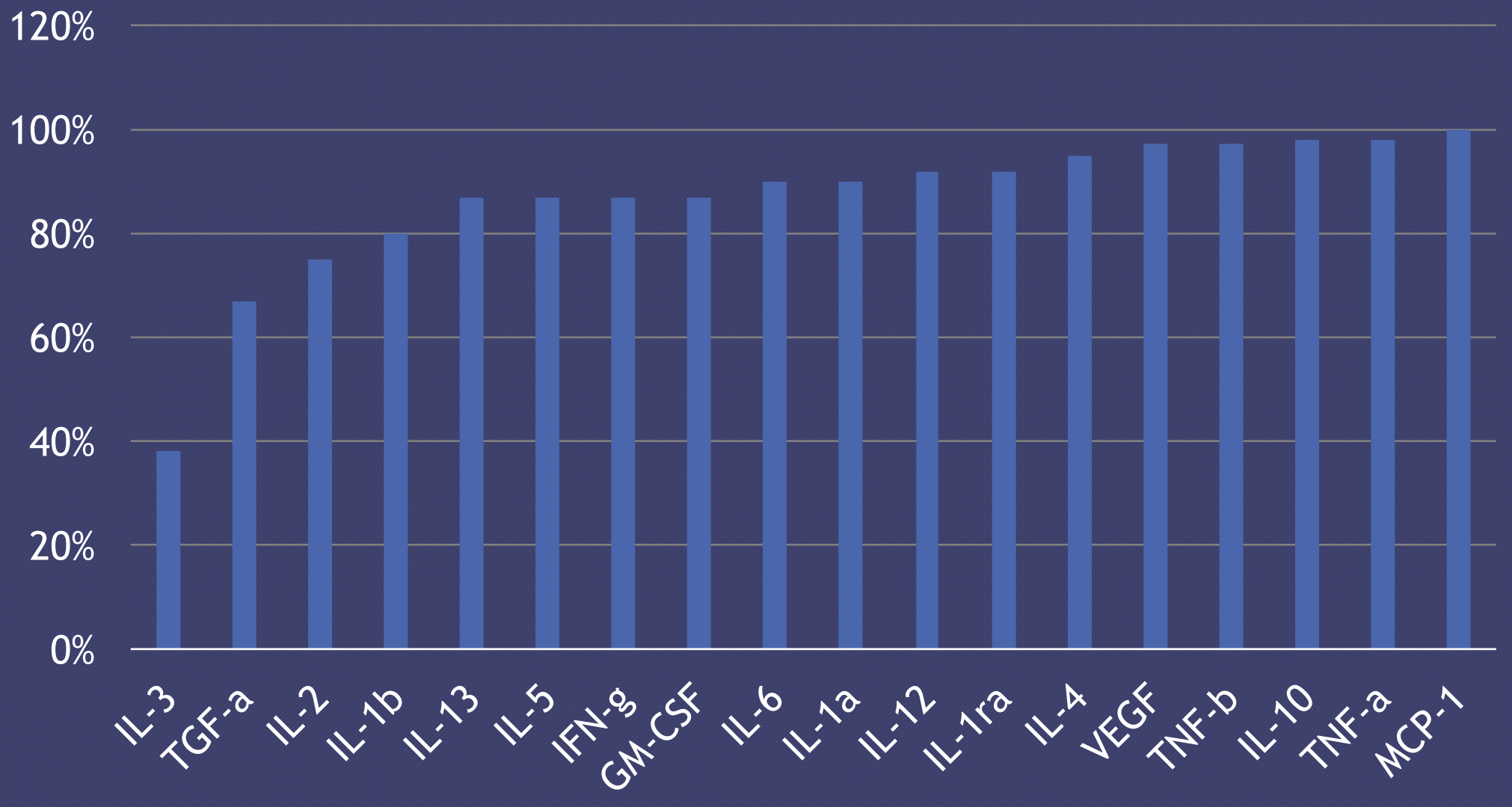
Thank you!
Any Questions?

- This research was supported by funding from the National Institute on Aging (T32AG000243; P30AG012857)

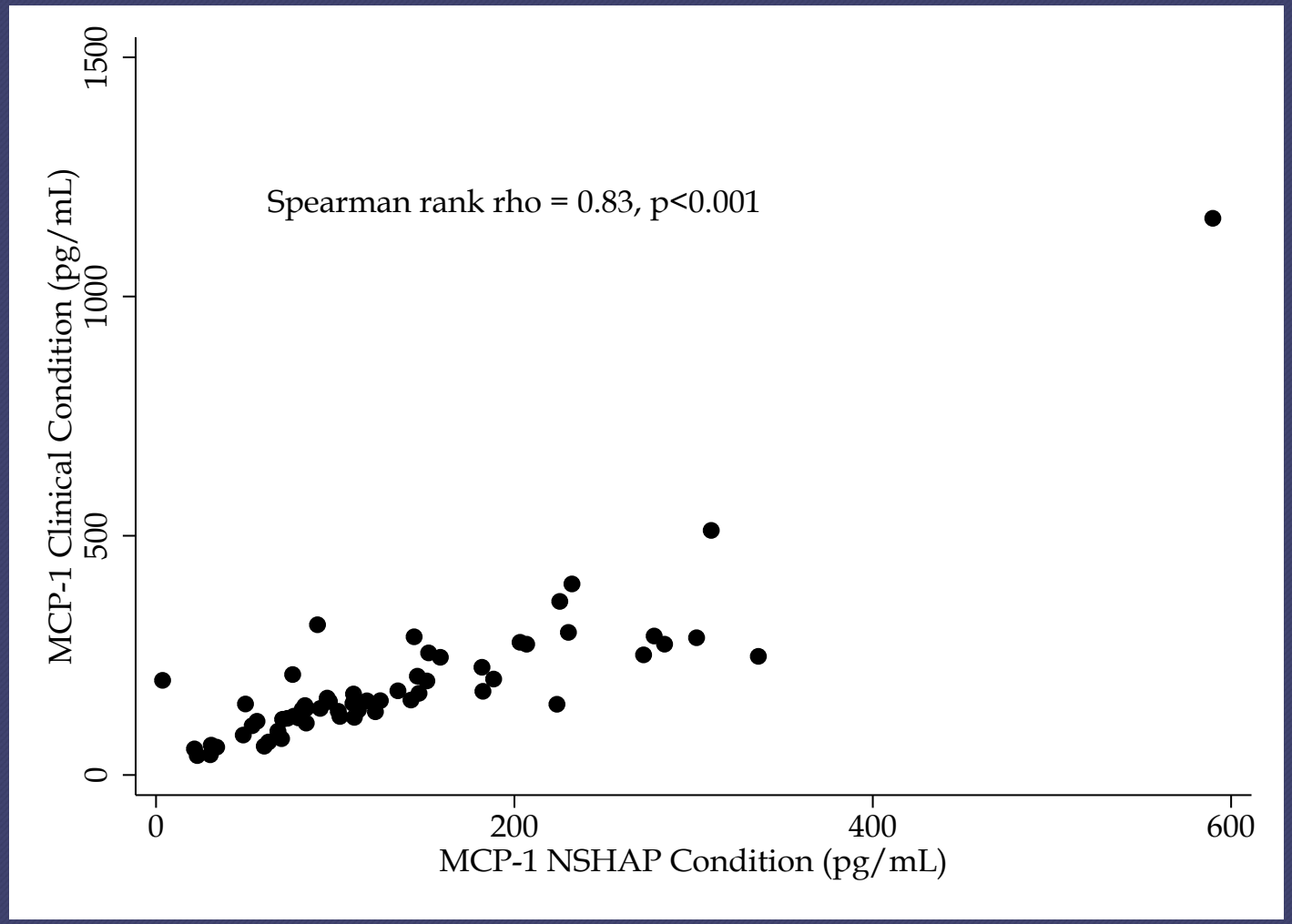
- NSHAP is supported by the National Institute on Aging and the National Institutes of Health (R37AG030481; R01AG033903). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health

- 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