**Supplemental Digital Content 1: Detailed Proteomics Methods**

**Sputum and Plasma Protein Digestion**

For sputum, acetone precipitation of proteins was performed using 400 µL of sputum to 1600 µL ice cold 100% acetone. The samples were incubated for 3 hours at -20° C and then centrifuged at 15,000 X g at 4°C for 15 minutes to pellet proteins. The protein pellets were then re-solubilized in 8 M urea containing 5 mM of dithiothreitol (DTT) and then transferred to an Amicon Ultra 0.5 ml ultrafiltration device (EMD Millipore, Billerica, MA) with a 3 kDa MW cut-off to buffer-exchange/concentrate proteins into 100 mM Tris pH 8.0.

50 µL of plasma from the same 6 subjects as done for sputum was then immunodepleted using a Seppro® IgY 14 (Sigma-Aldrich, St. Louis, MO) liquid chromatography column using the manufacturer’s recommended protocols. The flow-through fractions from the column were collected, combined, and applied to an ultrafiltration device (Amicon-Ultra 4, EMD Millipore) to buffer-exchange/concentrate proteins into 100 mM Tris pH 8.0. A Bradford protein assay was then performed for protein concentration estimation. 10 µgs of total protein from each sputum or plasma sample was aliquotted into a fresh Eppendorf tube and digested for mass spectrometry analysis. Briefly, the samples were denatured with 8M urea and then incubated with 5 mM DTT for 1 hour at room temperature to reduce cysteine residues. After reduction, the samples were incubated with 30 mM iodoacetamide for 1 hour at room temperature in the dark to alkylate the cysteine residues. 4% Lys-C/trypsin (w/w) mix was then added to the samples and incubated for 2 hours at 37°C. After this initial 2 hour incubation, samples were diluted with 100 mM Tris pH 8.0 until the final concentration of urea was below 1.5 M and the samples were then incubated at 37°C overnight to fully digest the proteins into peptides. The reaction was stopped by the addition of formic acid to a 0.1% final concentration. The peptide samples were cleaned using Cerex octadecyl C18 heavy load solid phase extraction (SPE) columns (Chrom Tech, Apple Valley, MN) according to manufacturer’s instructions and then concentrated to dryness using a speed-vac.

**LC-MS/MS and Candidate Biomarker Selection Methods**

Peptides (500 ng) were eluted from a C18 pre-column (100-μm id × 2 cm, Thermo Fisher Scientific) onto an analytical column (75-μm ID × 10 cm, C18, Thermo Fisher Scientific) using a 5% hold of solvent B (acetonitrile, 0.1% formic acid) for 5 min, followed by a 5-7% gradient of solvent B over 5 min, 7-15% gradient of solvent B over 45 min, 15-35% gradient of solvent B over 60 min, 35-40% gradient of solvent B over 28 min, 40-85% gradient of solvent B over 5 min, 85% hold of solvent B for 10 min and finally a return to 5% in 1 minute and another 10 minute hold of 5% solvent B. All flow rates were 400 nl/min. Solvent A consisted of water and 0.1% formic acid. Three replicate analyses were performed per sample. Data dependent scanning was performed by the Xcalibur v 2.1.0 software (Andon et al. 2002) using a survey mass scan at 60,000 resolution in the Orbitrap analyzer scanning m/z 400-1600, followed by collision-induced dissociation (CID) tandem mass spectrometry (MS/MS) of the fourteen most intense ions in the linear ion trap analyzer. Precursor ions were selected by the monoisotopic precursor selection (MIPS) setting with selection or rejection of ions held to a +/- 10 ppm window. Dynamic exclusion was set to place any selected m/z on an exclusion list for 45 seconds after a single MS/MS. All tandem mass spectra (MS/MS) were searched against human proteins downloaded from Uniprot on August 06, 2013 (http://www.uniprot.org) using Thermo Proteome Discoverer 1.3 (Thermo Fisher Scientific) considering fully tryptic peptides with up to two missed cleavages. Iodoacetamide derivatives of cysteine residues, and oxidation of methionines, were specified as variable modifications. At the time of the search, this reference proteome set contained 88,323 protein entries. Sputum and plasma proteins were identified at 95% confidence with XCorr score cut-offs (Qian et al. 2005) as determined by a reversed database search. The protein and peptide identification results from the LC-MS/MS experiment were also interrogated and visualized with Scaffold v 4.2.1 (Proteome Software Inc., Portland OR), a program that relies on various search engine results (i.e.: Sequest, X!Tandem, MASCOT) and which uses Bayesian statistics to reliably identify more spectra (Keller et al. 2002). Sputum proteins were accepted that passed a minimum of two peptides identified at 0.13% false discovery rate (FDR) at the peptide level and 0.6% FDR at the protein level. Plasma proteins were accepted that passed a minimum of two peptides identified at 0.01% FDR at peptide level and 1.0% FDR at the protein level.

Label-free quantitation and meta-analysis was conducted using Scaffold. Total spectral counts were used for quantitative analysis. Proteins needed at least a 2-fold change of diesel and/or B75 levels from baseline levels to be further considered as a candidate marker. After a 2-fold change was identified, the protein had to be identified in at least 4/6 subjects for sputum and 3/6 subjects for plasma. The motivation for selecting certain markers to validate depended largely on the availability of good quality ELISA assays and if the protein has been previously reported to have a role in inflammation.

**References Cited**

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