RESEARCH ARTICLE

PEDF, a pleiotropic WTC-LI biomarker: Machine learning biomarker identification and validation

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Abstract

Biomarkers predict World Trade Center-Lung Injury (WTC-LI); however, there remains unaddressed multicollinearity in our serum cytokines, chemokines, and high-throughput platform datasets used to phenotype WTC-disease. To address this concern, we used automated, machine-learning, high-dimensional data pruning, and validated identified biomarkers. The parent cohort consisted of male, never-smoking firefighters with WTC-LI (FEV₁, %Pred < lower limit of normal (LLN); n = 100) and controls (n = 127) and had their biomarkers assessed. Cases and controls (n = 15/group) underwent untargeted metabolomics, then feature selection performed on metabolites, cytokines, chemokines, and clinical data. Cytokines, chemokines, and clinical biomarkers were validated in the non-overlapping parent-cohort via binary logistic regression with 5-fold cross validation. Random forests of metabolites (n = 580), clinical biomarkers (n = 5), and previously assayed cytokines, chemokines (n = 106) identified that the top 5% of biomarkers important to class separation included pigment epithelium-derived factor (PEDF), macrophage derived chemokine (MDC), systolic blood pressure, macrophage inflammatory protein-4 (MIP-4), growth-regulated oncogene protein (GRO), monocyte chemoattractant protein-1 (MCP-1), apolipoprotein-AII (Apo-AII), cell membrane metabolites (sphingolipids, phospholipids), and branched-chain amino acids. Validated models via confounder-adjusted (age on 9/11, BMI, exposure, and pre-9/11 FEV₁, %Pred) binary logistic regression had AUC_{ROC} [0.90(0.84–0.96)]. Decreased PEDF and MIP-4, and increased Apo-AII were associated with increased odds of WTC-LI. Increased GRO, MCP-1, and simultaneously decreased MDC were associated with decreased odds of WTC-LI. In conclusion, automated data pruning identified novel WTC-LI biomarkers; performance was validated in an independent cohort. One biomarker—PEDF, an antiangiogenic agent—is a novel, predictive biomarker of particulate-matter-related lung disease. Other biomarkers—GRO, MCP-1, MDC, MIP-4—reveal immune cell
involvement in WTC-LI pathogenesis. Findings of our automated biomarker identification warrant further investigation into these potential pharmacotherapy targets.

Author summary

Disease related to air pollution causes millions of deaths annually. Large swaths of the general population, as well as certain occupations such as 1st responders and military personnel, are exposed to particulate matter (PM)—a major component of air pollution. Our longitudinal cohort of FDNY firefighters exposed to the World Trade Center dust cloud on 9/11 is a unique research opportunity to characterize the impact of a single, intense PM exposure by looking at pre- and post-exposure phenotype; however, PM-related lung disease and PM’s systemic effects are complex and call for a systems biological approach coupled with novel computational modelling techniques to fully understand pathogenesis. In the present study, we integrate clinical and environmental biomarkers with the serum metabolome, cytokines, and chemokines to develop a model for early disease detection and identification of potential signaling cascades of PM-related chronic lung disease.

Introduction

Globally, air pollution (of which particulate matter [PM] is a significant component) contributes to pulmonary and vascular disease yielding a devastating 7 million annual deaths. Lung injury due to inhalational exposure is a major health concern not only for 1st responders and military personnel but also for large swaths of the population. PM is a significant component of ambient air pollution and prominent in WTC-PM exposure. The destruction of the World Trade Center (WTC) complex pulverized 1.2 million tons of construction material. Particulate analysis showed that metals, such as chromium, nickel, and iron, powdered concrete, calcium carbonate, fibrous glass, asbestos, components of jet fuel, fire retardants, dioxins and silicates were components of WTC-PM. Therefore, our findings in the WTC-exposed FDNY cohort fit into a larger set of studies demonstrating the association of lipids, inflammation, and pulmonary injury and repair after toxin exposure.

The Fire Department of New York (FDNY) rescue/recovery workers exposed to WTC-PM have developed obstructive airways disease (OAD). The role of classic cardiovascular risk factors in the development of pulmonary disease has been a topic of considerable interest. In a WTC-exposed case-cohort study, pulmonary artery to aorta diameter (PA/A) was also associated with early serum biomarkers of vascular disease and predictive of lung disease known as WTC-lung injury (WTC-LI). Metabolic syndrome (MetSyn) phenotypic characteristics also predicted WTC-LI and airway hyperreactivity (AHR).

Recently, we focused on discovering metabolic and vascular disease-associated bioactive pathways associated with WTC-LI. Through the use of high-throughput omics technologies, our group has assessed the metabolome of WTC-LI patients. We explored methods to identify a metabolic signature unique to WTC-LI. Automated machine learning techniques exceeded in their ability to address potential multicollinearity, and their robustness to false positive and negative discoveries compared to traditional analyses that are based on significance testing. We identified several bioactive classes of lipid and amino acid metabolites.

The objective of this study was to develop and validate a multivariate predictive model of WTC-LI by integrating the metabolome with clinical, cytokine, chemokine, and

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environmental data to improve early identification of disease compared to single-platform models. This process is key to identification of significant biologically active pathways. In this investigation, we have implemented a machine learning approach to first identify and then validate analytes, such as Pigment epithelium-derived factor (PEDF), and their associated pathways that most accurately classify future WTC-LI. While PEDF has been identified as a biomarker of OAD, we have now identified PEDF as a novel, predictive biomarker of the negative health effects of PM exposure.[30]

Methods

Ethics statement

Subjects provided written consent to research including biomarker analysis at enrollment (Institutional Review Board approved protocols at Montefiore Medical Center (#07-09-320) and New York University (#16–01412)).

Study design

The baseline cohort (N = 801) was obtained from symptomatic subjects referred for subspecialty pulmonary examination (SPE) between 10/1/2001 and 3/10/2008, and underwent pulmonary function testing as previously described.[19,31,32] The parent cohort consisted of subjects who were male never-smokers with normal pre-9/11/2001 (9/11) lung function, reliable NHANES-predicted FEV\textsubscript{1}, and pulmonary function tests available within 200 days after 9/11, with WTC-LI (n = 96; defined as FEV\textsubscript{1}, %Pred < the lower limit of normal (LLN) at SPE) and randomly selected controls (n = 127) selected as previously described, with the additional criterion of having serum available for analysis.[19,31,32] Subjects (n = 15/group) in the metabolomics cohort were chosen from the parent cohort for untargeted metabolome assessment if they maintained stable case assignment as previously described, Fig 1.[27] This cohort functions as a training set.

The clinical biomarkers and serum cytokines and chemokines identified in the metabolomics cohort were fully available for validation in (n = 43/96) cases of WTC-LI and (n = 71/127) controls that did not overlap with the metabolomics cohort. For clarity, we will refer to this subset of the parent cohort as the validation cohort.[31] Note that the metabolomics and validation cohorts are disjoint subsets of the parent cohort by design, Fig 1.

Demographics and clinical data were obtained from the WTC-Health Program (WTC-HP). Exposure intensity is categorized as per the FDNY-WTC Exposure Intensity Index and is based on first arrival time at the WTC site, as described.[15,33,34] Specifically, subjects are considered highly exposed if they arrived the morning of 9/11, intermediate exposure if they arrived the afternoon of 9/11, and low exposure if they arrived on or after September 12, 2001.[28]

Analytical methods of high throughput OMICs platforms

Biomarkers. Serum collected within 200 days after 9/11 was processed and stored as previously described.[19,23,31,32,35] Serum was thawed once and assayed on the following commercially available multiplexed kits (Millipore and R&D) according to manufacturer’s instructions on a Luminex 200IS (Luminex Corporation, TX): cardiovascular (HCVD1-67AK, Millipore), neurodegenerative (HNDG2-36K, Millipore), metabolic (HMH-34K, Millipore), 39-plex (MPXHCYTO-60K, Millipore), soluble receptors (HSCR-32K, Millipore), TIMP (R&D Systems), and Apolipoprotein (APO-62K, Millipore).[19,20,31,36] Samples were processed in approximately 2:1 ratio of controls to cases to avoid batch bias, and analyzed with MasterPlex QT (Version: 1.2; MiraiBio). Serum was quantified in the parent cohort.
Metabolomics. Serum aliquots were at -80°C until metabolite quantification. Compounds were matched to corresponding library entries of retention index, mass, and spectral data as previously described.\cite{37-40} Qualified metabolites were detected in 80% or more of subjects per group with a relative standard deviation of 15% or greater.\cite{41} In qualified metabolites, missing data was imputed by using the minimum observed value of each compound, as previously described.\cite{27}

**Analysis pipeline.** Global metabolomic profiling was performed on the metabolomics cohort (n = 15/group). Curated data of the qualified profile (n = 580 metabolites) incorporated with serum analytes quantified via Luminex (n = 106 cytokines, chemokines) and clinical biomarkers (n = 5) into random forests (RF) models consisting of 5,000–500,000 trees in increments of 5,000 trees to determine the minimal amount of trees required to achieve rank stability, defined as zero pairwise differences in prospective refined profile membership among 10 replicate random forests (randomForest 4.6.14, R-Project) similar to previously

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**Fig 1. Study Design.** From a parent cohort of WTC-LI cases (n = 96) and controls (n = 127), a metabolomics subcohort (n = 15/group) and a validation cohort (n = 114) were drawn as described. https://doi.org/10.1371/journal.pcbi.1009144.g001
Variables ranking within the top 5% of mean decrease accuracy scores were included in the refined profile. Of the 10 replicate models, we report the mean decrease accuracy of the model with the lowest out-of-bag classification error rate. To account for the potential confounding effects of multicollinearity on classical permutation importance, we measured the permutation importance of each variable conditioned on all other variables in this random forest (i.e. the Conditional Permutation Importance) using a version of the R package permimp 1.0.1 that was customized in-house to support parallel processing. To assess the classification accuracy of the refined profile as the out-of-bag error rate, a second iteration of RF was run. The number of trees ranged from 500–15,000 in increments of 500; 10 replicate forests were grown at each increment. Furthermore, we measured variable importance in forests trained using only the refined profile to gain insight into individual biomarker contribution in a lower-dimensional setting. For all random forests, at each node, the square root of the number of total model variables were sampled with replacement. The R packages doParallel 1.0.16, doRNG 1.8.2, and foreach 1.5.1 assisted with parallel processing.

Principal component analysis (PCA) (SPSS 23, IBM) of the correlation matrix of mean-centered, normalized attributes was employed to visualize potential mechanistic relationships between metabolites and other data. The number of components retained was determined based on analysis of the scree plot. Unsupervised two-way hierarchical clustering was performed on the refined profile’s data matrix using Spearman correlation and average linkage (Matlab R2018a). Linkage thresholds of 0.78 and 1.10 were used to define clusters of metabolites and subjects, respectively.

Validation. Cytokines, chemokines, and clinical biomarkers identified in the refined profile of the metabolomics cohort were fully available for validation of a multivariate predictive model of WTC-LI in the non-overlapping parent cohort via binary logistic regression. There are several ways to build predictive models of disease based on a subset of important features. These include support vector machines which allow flexible modelling of nonlinear effects and interactions among biomarkers. We chose binary logistic regression to construct a linear combinator of identified biomarkers for differentiating cases from controls that would be interpretable. Least absolute shrinkage and selection operator (LASSO) was used to identify an optimal subset of serum analytes in a binary logistic regression (R package glmnet 4.1). Additional variables were included based on high mean decrease accuracy in the RF of the metabolomics cohort. Variables were dichotomized using Youden’s index. Multicollinearity was assessed on continuous variables via Pearson correlation, and, where significant (p < 0.05), handled via generation of composite variables post-dichotomization. Performance of logistic regressions was assessed via AUC_{ROC} (R package pROC 1.16.2). We have opted to construct receiver-operator characteristic (ROC) curves and report AUC_{ROC} as the main criteria of the model because ROC curves and AUC_{ROC} are appropriate in the setting of balanced classes. The final model underwent 5-fold cross-validation to assess generalizability (R package boot 1.3.25). All logistic regressions were confounder-adjusted for age on 9/11, BMI at SPE, exposure, and Pre-9/11 FEV_{1}, %Pred.

Statistics, database management and multivariate model development

SPSS 23 (IBM) was utilized for data storage and handling. Data analysis was performed in R (3.6.0 and 4.0.3, R-Project). Continuous and ordinal variables were expressed as median and inter-quartile range. Wilcoxon test was used to compare continuous and ordinal data. For categorical data, count and proportions were used to summarize and Pearson’s χ^2 was used for comparison. For all tests, p < 0.05 was considered significant. Correlations were calculated using the R package Hmisc 4.4.2. SPSS file formats were read and written in R haven 2.3.1.
Additionally, the R package matrixStats 0.57.0 was used to perform some row- and column-wise computations. Finally, ggplot2 3.3.2 was used for tune result visualizations.

Computing resources

Big Purple (https://hpcmed.org/resources) was used for computing and includes Cray CS500, Skylake 6148, 20-core, 2.4GHz, 150W processors, 32GB DDR4-2666 DIMMs (DUAL RANKED), 384 GB/node, 9.6 GB/core, 2TB SATA disk, 2TB NVMe SSD and an EDR 100Gb/s Infiniband network interface for MPI and Data traffic. Additional, hardware details can be found at (https://hpcmed.org/resources/bigpurple/hardware).

Results

Demographics

The metabolomics cohort has been previously comprehensively described in terms of clinically available lipids, leukocyte differentials, and metabolic biomarkers.[27] The parent cohort has also been previously described in terms of its available clinical characteristics.[20] In the present study, we did not consider differential expression of serum cytokines and chemokines in the parent cohort. We additionally provide clinical characteristics and serum cytokines and chemokines of the metabolomics and validation cohorts used in this paper, Table 1.

Table 1. Clinical measures for primary endpoint.

|                      | WTC-LI (N = 43) | Control (N = 71) | WTC-LI (N = 15) | Control (N = 15) |
|----------------------|-----------------|------------------|-----------------|------------------|
| Age on 9/11 (y)      | 41 (36–46)      | 41 (37–44)       | 39 (37–46)      | 42 (38–46)       |
| Race                 |                 |                  |                 |                  |
| Caucasian            | 39 (91%)        | 70 (99%)         | 15 (100%)       | 15 (100%)        |
| African American     | 4 (9%)          | 1 (1%)           | 0 (0%)          | 0 (0%)           |
| BMI at SPE (kg/m²)   | 29.41 (27.47–34.20) | 29.74 (26.90–31.57) | 30.28 (27.80–31.42) | 25.66 (24.40–27.98) |
| BMI at WTC-HP (kg/m²)| 28.97 (27.26–32.45) | 28.59 (26.97–30.85) | 29.29 (25.82–31.24) | 25.84 (25.10–27.37) |
| Pre-9/11 FEV₁ (%Pred)| 95 (83–105)     | 104 (94–113)     | 85 (83–90)      | 97 (92–105)      |
| SBP (mmHg)           | 120 (110–132)   | 120 (110–128)    | 119 (108–129)   | 110 (100–112)    |
| Exposure             |                 |                  |                 |                  |
| Low                  | 7 (16%)         | 8 (11%)          | 4 (27%)         | 1 (7%)           |
| Intermediate         | 24 (56%)        | 49 (69%)         | 8 (53%)         | 11 (73%)         |
| High                 | 12 (28%)        | 14 (20%)         | 3 (20%)         | 3 (20%)          |
| PEDF (pg/cL)         | 1.70 (1.12–3.86) | 4.03 (1.41–5.03) | 1.14 (0.71–4.24) | 5.29 (4.39–5.73) |
| MDC                  | 1517.59 (1254.37–1988.01) | 1428.08 (1074.94–1837.64) | 1899.30 (1649.77–2312.16) | 1334.72 (892.12–1395.58) |
| MIP-4 (ng/mL)        | 155.65 (105.07–201.04) | 198.08 (123.85–1036.74) | 137.74 (106.58–283.08) | 1207.36 (194.90–1674.58) |
| GRO                  | 707.87 (460.34–1094.75) | 708.49 (567.38–929.57) | 811.81 (671.70–1143.64) | 485.20 (412.83–615.32) |
| MCP-1                | 543.18 (366.42–765.20) | 544.07 (403.57–654.20) | 589.41 (495.64–1087.35) | 398.23 (314.11–493.11) |
| sIL-2Rt              | 555.01 (462.04–741.69) | 535.63 (390.08–787.68) | 573.39 (534.55–736.32) | 419.91 (273.28–580.45) |
| Amylin               | 53.45 (35.40–67.48) | 58.39 (46.37–147.64) | 53.45 (46.20–55.12) | 63.25 (55.12–153.36) |
| sCD40L               | 9032.94 (5223.64–16242.28) | 8306.12 (4893.05–15116.64) | 15834.16 (8255.89–28494.18) | 5070.92 (712.82–9163.09) |
| MMP-1                | 402.79 (141.08–767.15) | 681.41 (337.84–1441.27) | 130.70 (75.64–633.73) | 647.26 (286.43–1368.21) |
| sVEGFR1              | 457.86 (343.76–626.35) | 457.86 (343.76–627.62) | 480.31 (249.97–749.74) | 377.41 (292.12–420.08) |
| EGF                  | 64.14 (29.11–125.71) | 64.88 (43.50–132.55) | 117.37 (33.49–246.65) | 47.23 (23.42–74.38) |
| Leptin               | 7756.10 (4638.00–13557.20) | 6428.99 (4021.21–10479.97) | 8377.03 (5583.98–18744.24) | 4344.83 (1964.47–6250.28) |
| Apo AII (μg/mL)      | 1818.81 (632.93–2479.78) | 780.70 (418.96–1594.96) | 913.40 (319.21–4298.69) | 673.30 (538.10–1554.70) |
| sIL-2R   α           | 61.89 (9.98–117.47) | 74.65 (8.94–133.06) | 12.65 (8.93–101.87) | 113.16 (2.00–177.31) |
| MMP-13               |                 |                  |                 |                  |

Values are in Median (IQR) or N (%) as indicated; p calculated by Wilcox test or Chi-Square as appropriate; Apo AII available for 14 controls in metabolomics cohort. Analytes are pg/mL unless otherwise stated. Apo AII is shown in units μg/mL for readability but analyzed in ng/mL.

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In the metabolomics cohort, cases of WTC-LI had significantly elevated SBP, BMI at WTC-HP and SPE, MDC, GRO, MCP-1, sIL-2Rα, sCD40L, EGF, and Leptin, and significantly decreased Amylin compared to controls. Additionally, cases of WTC-LI and controls were not significantly different in Apo-AII.

In the validation cohort, cases of WTC-LI had significantly elevated Apo AII compared to controls; however, cases of WTC-LI and controls were not significantly different in BMI at WTC-HP or SPE, systolic blood pressure (SBP), MDC, GRO, MCP-1, sIL-2Rα, Amylin, sCD40L, EGF, or Leptin. Finally, there were no observed differences in clinical biomarkers or serum analytes between controls in the validation cohort and those in the parent cohort, nor were there differences in clinical biomarkers or serum cytokines and chemokines between cases of WTC-LI in parent and validation cohorts.

In both the metabolomics and validation cohorts, cases of WTC-LI had significantly decreased pre-9/11 FEV1, %Pred, PEDF, MIP-4, and MMP-1 compared to controls; however, there were no significant differences in age on 9/11, race, exposure, sVEGFR1, and MMP-13 between cases of WTC-LI and controls in either cohort.

Finally, controls in the validation cohort significantly differed from controls in the metabolomics cohort in BMI at WTC-HP and SPE, SBP, PEDF, MDC, GRO, MCP-1, sIL-2Rα, sCD40L, and Leptin; however, cases of WTC-LI in the validation cohort did not differ from cases of WTC-LI in the metabolomics cohort in clinical biomarkers or serum cytokines and chemokines. Furthermore, relative expression of cases of WTC-LI compared to controls were preserved for these biomarkers across both cohorts, with the exceptions of BMI at SPE, SBP, GRO, and MCP-1, which were elevated in cases of WTC-LI compared to controls in the metabolomics cohort, but had a fold change of approximately 1 in cases of WTC-LI compared to controls in the validation cohort.

Metabolomics

We have previously characterized 765 detected and 580 qualified metabolites.[27] We included the qualified metabolites in RF with serum, clinical, and environmental biomarkers to assess the most discriminative variables as those with a mean decrease accuracy score within the top 5% of scores. The tuning process determined the minimum number of trees (n = 345,000) that resulted in 0 average pairwise unique elements among prospective refined profiles of 10 replicate random forests, S1A Fig. This yielded a refined profile of 19 metabolites (largely sphingolipids, phospholipids, fatty acids, and amino acids), 14 serum biomarkers (including protease/antiprotease, metabolic inflammatory, innate immunity inflammatory, and soluble receptor biomarkers), and 2 clinical biomarkers (BMI at WTC-HP entry and SBP), Fig 2A. Membership in the refined profile was identical using the variable importance ranking produced by conditional permutation importance, S1B Fig. A second RF trained on only the refined profile achieved a 0% estimated out-of-bag error rate with 1,500 trees, S2A Fig. Furthermore, an additional analysis of variable importance was performed using the smallest forest (1,500 trees) to achieve the minimal error rate. Variable importance ranking derived from both classical and conditional permutation importance were similar to each other (Spearman’s rank correlation of 0.942, p<0.001) and to the variable importance ranking derived using the qualified profile (Spearman’s rank correlations of 0.812, p<0.001 and 0.770, p<0.001, respectively), S2B and S2C Fig.

Clustering of the data matrix of the refined profile identified 5 clusters (C1-5) reflective of potential mechanistic relations, Fig 2B. C1—elevated in controls—contained vascular biomarkers, as well as amino acids and peptides. In C2, amino acids predominated and were decreased in WTC-LI. Fatty acids primarily comprised C3, many of which were elevated in
cases of WTC-LI, and in controls. Variables in C4 were also generally elevated in WTC-LI, and included vascular biomarkers (sphingolipids, SBP). Finally, C5 contained sVEGFR1 and GRO, which were elevated in cases of WTC-LI.

PCA of the refined metabolite profile captured 73.3% of variance in the 7 components retained based on examination of the scree plot. The PCA loading weights plot provides an alternative, low-dimensional view of the variables in C1-5, and the clustering patterns observed in the loading weights plot are reflective of the structure of C1-5.

Validation of serum and clinical biomarkers in WTC-LI vs. controls

RF analysis uncovered several previously unidentified serum biomarkers in the metabolomics cohort. Using binary logistic regression, we aimed to validate these serum biomarkers in the validation cohort.

Biomarkers identified by integrated MultiOMICs approach

To analyze the relationship between all cytokines, chemokines, and clinical biomarkers identified in RF as continuous variables and potential confounders, we transformed exposure into dummy variables and determined an optimal model using LASSO set to maximize 5-fold cross-validated AUC\textsubscript{ROC}. Metabolites were not fully available in the validation cohort and so were not included in the use of LASSO. MIP-4, MMP-1, Apo AII, MDC, Amylin, Pre-9/11...
FEV$_1$, %Pred, and intermediate exposure remained in the model with AUC$_{ROC}$ (Standard Error) of 0.728(0.066), Table 2 and S3 Fig.

To handle potential multicollinearity, we then included other variables that were important predictors in the metabolomics cohort, but were significantly correlated ($p<0.05$) with other variables in the initial regression, Fig 2. Here, we considered the top 6 variables by mean decrease accuracy in the refined profile—there was appreciable drop in mean decrease accuracy after these variables.

Univariate regression analysis was conducted to characterize the relationship between WTC-LI and the analytes of interest identified via LASSO or the refined biomarker profile—PEDF, MDC, MCP-1, MIP-4, GRO, SBP, MMP-1, and Apo AII, Table 3.

This process provides insight into the contribution and performance of single biomarkers as measured by OR and AUC$_{ROC}$, Table 3. MMP-1 and MIP-4 were significant in univariate, confounder-adjusted models.

In building the final model, we optimized biomarker cutpoints via Youden’s Index. To account for significant correlations observed in Fig 2B, we created composite variables. The relationship between these variables and WTC-LI was analyzed in confounder-adjusted binary logistic regressions, which are summarized along with AUC$_{ROC}$ as a performance measure, Table 3. Apo AII $>1794.22$ μg/mL, MMP-1 $>832.48$ pg/mL, (PEDF $<$ 3.94 pg/cL and

### Table 2. LASSO regression.

| Analyte      | Coefficient  |
|--------------|--------------|
| MIP-4        | -1.427 e-04  |
| MMP-1        | -3.168 e-04  |
| Apo AII      | 1.498 e-08   |
| MDC          | 1.659 e-04   |
| Amylin       | -9.472 e-04  |
| Pre-9/11 FEV$_1$, %Pred | -3.010 e-02 |
| Exposure Intermediate | -8.066 e-02 |

Analyte units: MIP-4 and Apo AII ng/mL; MMP-1 pg/mL.

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### Table 3. Univariate and composite models.

| Analyte      | Univariate OR (95% CI) | Univariate p | AUC$_{ROC}$ (95% CI)  |
|--------------|-------------------------|--------------|-----------------------|
| GRO          | 1.000 (0.999–1.001)     | 0.495        | 0.711 (0.608–0.815)   |
| SBP (mmHg)   | 0.991 (0.957–1.026)     | 0.612        | 0.719 (0.616–0.822)   |
| MCP-1        | 1.000 (0.998–1.002)     | 0.776        | 0.710 (0.607–0.812)   |
| MDC          | 1.001 (1.000–1.001)     | 0.100        | 0.717 (0.615–0.819)   |
| Apo AII (ng/mL) | 1.000 (1.000–1.000)     | 0.077        | 0.741 (0.644–0.837)   |
| MMP-1        | 0.999 (0.998–1.000)     | 0.012        | 0.755 (0.661–0.849)   |
| MIP-4 (ng/mL) | 0.998 (0.996–0.999)     | 0.017        | 0.783 (0.697–0.869)   |
| PEDF (pg/cL) | 0.824 (0.665–0.978)     | 0.058        | 0.743 (0.647–0.838)   |
| Apo AII $>$ 1794.22 (μg/mL) | 8.044 (3.086–22.931) | $< 0.001$  | 0.812 (0.729–0.895)   |
| MMP-1 $>$ 832.48 | 0.173 (0.062–0.440) | $< 0.001$  | 0.799 (0.713–0.884)   |
| SBP $>$ 127.5 (mmHg) | 1.280 (0.460–3.539) | 0.633       | 0.714 (0.610–0.817)   |
| GRO $>$ 580.28, MCP-1 $>$ 279.07, and MDC $<$ 1756.99 | 0.327 (0.127–0.787) | 0.015       | 0.760 (0.665–0.854)   |
| PEDF $<$ 3.94 (pg/cL) and MIP-4 $<$ 368.70 (ng/mL) | 5.252 (2.150–13.900) | $< 0.001$  | 0.797 (0.712–0.882)   |

Analytes are pg/mL unless otherwise stated. All models were adjusted for the potential confounders age on 9/11, BMI at SPE, pre-9/11 FEV$_1$, %Pred, and exposure.

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MIP-4 < 368.70 ng/mL, and (GRO > 580.28 pg/mL, MCP-1 > 279.07 pg/mL, and MDC < 1756.99 pg/mL) were significant in univariate, confounder-adjusted models, while SBP > 127.5 mmHg was not significant.

The variables initially identified via LASSO were then transformed to dichotomous variables using Youden’s index. These variables, along with dichotomous and composite variables corresponding to those with the highest mean decrease accuracy score within the refined profile, were included in a final, confounder-adjusted, binary logistic regression, Table 4 and Fig 3.

PEDF < 3.94 pg/mL and MIP-4 < 368.70 ng/mL, (GRO > 580.28 pg/mL, MCP-1 > 279.07 pg/mL, and MDC < 1756.99 pg/mL), and Apo AII > 1794.22 μg/mL, were significant in this model, which achieved an AUC ROC of 0.902 (95% CI 0.842–0.961), Fig 3. Furthermore, we validated the final multivariate model using 5-fold cross-validation. The estimated bias-corrected prediction error was 0.160.

Discussion

Lung injury is heterogeneous in cause, process, and outcome. The biomarkers found to be important to the development of WTC-LI similarly are heterogenous.[19,20,22–28,31,32,34–36,50–54] The integration of high-throughput platforms with classical clinical measurements, serum cytokines, and chemokines represent a promising avenue to characterization of disease and identification of therapeutic targets for lung injury; however, approaching such a high-dimensional dataset may be restrictively complex. In this paper, we presented an automated data pruning method and successfully integrated metabolomics data with a longitudinal dataset of serum cytokines and chemokines, and other clinical measures in 9/11 FDNY rescue and recovery workers exposed to WTC-PM.

We discovered several novel biomarkers of WTC-LI—including PEDF—in a cohort with untargeted metabolomics, and analyzed biomarker relationships in a hypothesis-generating fashion to identify potential cellular signaling cascades. We then validated the predictive ability of the cytokines, chemokines and clinical biomarkers in a broader cohort.

PEDF—the most important variable in RF of the metabolomics subcohort and one of the most predictive in univariate, confounder-adjusted binary logistic regressions—is a pleiotropic glycoprotein that belongs to the serpin superfamily of serine protease inhibitors.[55] PEDF is a multifunctional protein with important roles in regulation of inflammation and angiogenesis,
and has been implicated in several lung injury patterns. It is produced by various cell types, including endothelial cells. In pulmonary fibrosis, PEDF is an angiostatic factor. Furthermore, PEDF expression contributes to modulation of the inflammatory and angiogenic phenotype of the lung endothelium, which is key to several conditions such as pulmonary hypertension. This non-inhibitory protein plays critical roles in many physiological and pathological processes by acting through multiple high affinity ligands and cell receptors. 

PEDF is notably involved in organogenesis and the homeostatic maintenance of adult tissues/organs. The mRNA that encodes PEDF (SERPINF1 mRNA) is expressed in most tissues/organs. In addition to regulating fibrosis in the lung, PEDF regulates angiogenesis in the lung, pancreas, kidney, and eye, as well as lipid metabolism in the liver. PEDF also plays a role in bone cell differentiation. Deficiencies or defects of PEDF protein expression can lead to abnormal organ development and are closely associated with the progression of angiogenic diseases. Because of PEDF’s abundance and variety of functions in the body, PM-associated PEDF alterations could be a mediator of systemic effects secondary to oropharyngeal aspiration of or inhalational exposure to PM. Clinical studies have shown that PEDF is correlated with idiopathic pulmonary fibrosis (IPF), COPD, lung cancer, MetSyn, and diabetes. Specifically, elevated PEDF levels are involved in the pathogenesis of COPD and IPF. In a study by Li et al., PEDF expression levels were upregulated in both cigarette smoke extract-stimulated epithelial cells and cigarette smoke-exposed rat lung. Additionally, a significant, negative correlation between PEDF levels and lung function was shown, with plasma PEDF levels in COPD patients significantly higher than those in both the healthy nonsmoking and

Fig 3. Predictive performance of Final Model. ROC of the final, confounder-adjusted, multivariate binary logistic regression shows its predictive performance as well as that of its constituents when entered separately in confounder-adjusted binary logistic regressions.

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smoking subjects. These results indicate the elevation of PEDF plays a role in the pathogenesis of COPD by mediating inflammatory signaling processes.[30]

Reduced PEDF expression facilitates the progression of lung cancer due to the loss of PEDF-related suppression of tumor growth and motility.[60] PEDF is an adipocyte-secreted protein that acts as a pro-inflammatory factor by activating inflammatory signaling in several cell types. Therefore, PEDF contributes to the onset and maintenance of chronic inflammation in obesity and obesity-induced insulin resistance, and related complications such as MetSyn and type 2 diabetes mellitus.[61,62] Serum levels of PEDF are higher in patients with MetSyn and type 2 diabetes.[62,63] PEDF expression is elevated in proportion to the accumulation of the number of components of MetSyn in the general population. Nakamura et al. demonstrated that waist circumference, triglycerides, and creatinine were significant independent determinants of serum PEDF levels in diabetics; [63] however, strong, well-known risk factors such as age, blood pressure, and smoking were not related to PEDF.[63] PEDF has been shown to have neurotrophic and neuroprotective effects, and has been implicated in the pathogenesis of Alzheimer’s Disease.[64]

Additionally MMP-1, a protease which has been implicated in lung fibrosis, interstitial pneumonia, and cancer, was found to be important in our model.[65,66] Proteases have a prominent role in cancer, coronary disease, and OAD.[67–71] Increased protease activity is a component of many diseases, including cigarette-induced chronic lung disease and other causes of accelerated lung function decline.[67–70] MMPs’ central role in lung remodeling and pathogenesis of OAD has been of particular interest. MMPs are a family of Zn$^{2+}$-dependent proteases that can catabolize and degrade the extracellular matrix. Levels of MMPs are affected by environmental factors such as hypoxia, inflammation, and oxidative stress; MMPs as biomarkers of lung disease severity and prognostic indicators have been investigated in several studies.[67,72–74]

A metabolic hotspot

In C4, sphingolipids (sphinganine, sphingosine) displayed similar expression as other regulators of vascular proliferation and metabolic indicators (BMI, Leptin) and several immune-cell signaling molecules ([IL-2Ra, sCD40L, MCP-1, and MDC]). Biomarkers of inflammation such as MDC and of MetSyn, were observed in serum drawn within 6 months of WTC exposure, and predicted the post-9/11 loss in FEV$_1$ in this cohort of WTC-exposed FDNY firefighters. [19,31] The proximity of drivers of vascular proliferation to markers of metabolic dysregulation in this cluster is interesting in the context of the potential interaction between angiogenesis and adipogenesis.[75] Furthermore, the presence of immune-cell signaling molecules in this cluster could be reflective of a phenotype of chronic inflammation concomitant with obesity that has been observed in this cohort. Our prior works have found that triglycerides ≥150mg/dL and BMI ≥30kg/m$^2$ impart more risk on development of WTC-LI than smoking or exposure alone. We also know that there is a dose-response with increasing number of MetSyn characteristics and risk of WTC-LI.[19,22,24–28] Many of these mediators are also important in acute lung injury and fibrosis.[76,77]

In contrast to C4, C1 displayed an opposite elevation pattern. Containing PEDF, the decreased expression of C1 further supports a claim of potentially excessive angiogenesis that has been associated with lung diseases, including asthma, as well as PEDF’s previously discussed myriad consequences of dysregulation. Furthermore, in C5, sVEGR1’s moderate elevation in WTC-LI is consistent with this picture.

C2 was the most similar cluster to C1 and contained Amylin and MIP-4. These biomarkers are metabolically active (Amylin) and mediators of inflammation in adaptive immunity (MIP-
4). Therefore, their clustering proximity is of interest in the context of the interaction of metabolic health, inflammation, and pulmonary health. MIP-4 is a chemokine and involved in both innate and adaptive immunity. MIP-4 (CCL18) is an early promoter of Treg differentiation and may generate an anti-inflammatory counter-regulatory response.[20,78,79]

Additional biomarkers were identified as being associated with WTC-LI. Similar to MCP-1, MDC, and MIP-4, GRO was also found to be important in our WTC-LI model and further revealed immune cell involvement in WTC-LI pathogenesis.[80] GRO—also relevant in acute and chronic lung inflammation such as fibrosis—is part of a chemokine receptor system that mediates neutrophil recruitment.[81–84] In addition, lipids are a diverse group of bioactive compounds that have been implicated in the development of lung disease. Elevated levels of Apo AII were associated with pulmonary arterial hypertension in Sickle cell disease, suggesting a role in pulmonary vascular injury.[85] This is in line with our prior observation that dyslipidemia predicts poor outcome after WTC dust exposure.[19,20]

In light of the integrated metabolomics and the added granularity in phenotyping, the findings of our final multivariate binary logistic regression show the strength of early-identification of disease that may predict a non-resolving pathology. In comparison to prior work, the integrated multiplatform model developed here had 6.7% improved performance compared to a metabolomics-only model of disease in this cohort.[27] Furthermore, the final model based on serum and clinical biomarkers identified by the data analysis pipeline in the present study had higher $\text{AUC}_{\text{ROC}} (0.858 \text{ vs. } 0.902)$ compared to previous work in a similar cohort.[20] Future investigation will include contemporary assessment of the metabolome, and should also include additional assessment of serum cytokines and chemokines. Such research could determine the degree to which present-day omics displays features predicted by early-disease biomarkers, and the potential these features have as therapeutic targets both proximal to exposure and years later.

**This study has several limitations**

The metabolome is only assessed at a single time point, and therefore limits our ability to understand how longitudinal metabolomic variations relate to the development of WTC-LI. Given the size of the metabolomics analysis cohort, we attempted to minimize confounding effects by selection from a homogeneous subject pool; cases of WTC-LI and controls in the metabolomics cohort only differed in BMI, SBP, and HR, but were no different in other metabolic and inflammatory biomarkers. In our metabolomics cohort, we controlled for baseline effects due to BMI variations via percent-predicted-based case definitions.

Finally, the limitations of the present analysis pipeline have been previously described.[27,29] Briefly, any machine learning model is specific to its training data. Given the size of the metabolomics cohort, we have used machine learning methods that avoid overfitting. We cannot support claims of causality from the present analysis, but we can identify potentially important associations and support our findings with relevant literature. While we lack an external validation cohort for the trends observed in metabolomics, we have validated the serum, clinical, and environmental biomarkers via the binary logistic regression model built on the validation cohort. Note that the metabolomics and validation cohorts are subject to the same pre-analytical and analytical biases, and thus our biomarkers have yet to be evaluated in a truly independent test cohort. To address multicollinearity in the high-dimensional integrated dataset of metabolites, clinical, and other serum biomarkers, we assessed variable importance via conditional permutation importance, but found no differences in refined profile membership compared to classical permutation importance. To handle multicollinearity in the final model, we used dichotomous and composite variables. Dichotomization can
reduce statistical power. So, we assessed generalizability of our model using 5-fold cross validation. [86]

Conclusion
Automated data pruning successfully identified a set of maximally discriminative biomarkers in a high-dimensional systems biology dataset and validated these biomarkers in an independent cohort using standard regression techniques. Additionally, we have used pattern recognition methods to visualize associations between maximally predictive metabolites and biomarkers, and potentially elucidate mechanistic relations. PEDF may be an important mediator in WTC-LI and OAD. Future research to discover PEDF’s role in pathogenesis will include targeted molecular imaging and transcriptional quantification to determine PEDF’s activity at relevant tissues and organs.

Supporting information
S1 Fig. A. Random Forests Hyperparameter Tuning. Variable rank stability among the top 5% of important variables by mean decrease accuracy was assessed and the minimal number of trees required to achieve stability, defined as no differences among prospective refined profile membership among 10 replicate models, was used. The vertical line intersects the horizontal axis at 345,000 trees. B. Conditional Permutation Importance. Using the random forest that derived mean decrease accuracy in Fig 2A, conditional permutation importance was assessed to determine if multicollinearity affected variable ranking. (TIF)
S2 Fig. Tuning process using refined profile. A. Mean estimated out-of-bag error rate for 10 replicate forests grown at each increment of forest size, with forest size ranging from 500 to 15,000 trees. B. Mean decrease accuracy and C. Conditional permutation importance for the smallest forest that achieved the minimum estimated out-of-bag error rate. (TIF)
S3 Fig. LASSO tuning procedure. Tuning process maximized 5-fold cross-validated AUC as a function of log(λ). (TIF)
S1 Table. Overview of Analytes and Representative References. (DOCX)

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References
1. Peters A, Dockery DW, Muller JE, Mittleman MA. Increased particulate air pollution and the triggering of myocardial infarction. Circulation. 2001 Jun 12; 103(23):2810–5. https://doi.org/10.1161/01.cir.103.23.2810 PMID: 11401937
2. Peters A, von Klot S, Heier M, Trentinaglia I, Hormann A, Wichmann HE, et al. Exposure to traffic and the onset of myocardial infarction. N Engl J Med. 2004 Oct 21; 351(17):1721–30. https://doi.org/10.1056/NEJMoa040203 PMID: 15496621
3. Wellenius GA, Schwartz J, Mittleman MA. Air pollution and hospital admissions for ischemic and hemorrhagic stroke among medicare beneficiaries. Stroke, a journal of cerebral circulation. 2005 Dec; 36(12):2549–53. https://doi.org/10.1161/01.STR.0000198987.78760.47 PMID: 16254223
4. Wellenius GA, Yeh GY, Coull BA, Suh HH, Phillips RS, Mittleman MA. Effects of ambient air pollution on functional status in patients with chronic congregate heart failure: a repeated-measures study. Environ Health. 2007; 6:26. https://doi.org/10.1186/1476-069X-6-26 PMID: 17845720
5. Wellenius GA, Coull BA, Batalha JR, Diaz EA, Lawrence J, Godleski JJ. Effects of ambient particles and carbon monoxide on supraventricular arrhythmias in a rat model of myocardial infarction. Inhalation toxicology. 2006 Dec; 18(14):1077–82. https://doi.org/10.1080/08958370600945473 PMID: 17050344
6. Wellenius GA, Schwartz J, Mittleman MA. Particulate air pollution and hospital admissions for congestive heart failure in seven United States cities. The American journal of cardiology. 2006 Feb 1; 97(3):404–8. https://doi.org/10.1016/j.amjcard.2005.08.061 PMID: 16442405
7. Dominici F, Peng RD, Bell ML, Pham L, McDermott A, Zeger SL, et al. Fine particulate air pollution and hospital admission for cardiovascular and respiratory diseases. JAMA: the journal of the American Medical Association. 2006 Mar 8; 295(10):1127–34. https://doi.org/10.1001/jama.295.10.1127 PMID: 16529132
8. Simkhovich BZ, Kleinman MT, Kloner RA. Particulate air pollution and coronary heart disease. Curr Opin Cardiol. 2009 Nov; 24(6):604–9. https://doi.org/10.1097/HCO.0b013e32833161e5 PMID: 19696664
9. Organization WH. 9 out of 10 people worldwide breathe polluted air, but more countries are taking action. 2018 May 2, 2018.
10. Caplan-Shaw CE, Yee H, Rogers L, Abraham JL, Parsia SS, Naidich DP, et al. Lung pathologic findings in a local residential and working community exposed to World Trade Center dust, gas, and fumes.
Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2011 Sep; 53(9):981–91.

11. King MS, Eisenberg R, Newman JH, Tolle JJ, Harrell FE Jr., Nian H, et al. Constrictive bronchiolitis in soldiers returning from Iraq and Afghanistan. N Engl J Med. 2011 Jul 21; 365(3):222–30. https://doi.org/10.1056/NEJMoa1101383 PMID: 21774710

12. Organization WH. WHO Global Ambient Air Database (update 2018). 2018.

13. McGee JK, Chen LC, Cohen MD, Chee GR, Prophete CM, Haykal-Coates N, et al. Chemical analysis of World Trade Center fine particulate matter for use in toxicologic assessment. Environ Health Perspect. 2003 Jun; 111(7):972–80. https://doi.org/10.1289/ehp.5930 PMID: 12782501

14. Rom WN, Reibman J, Rogers L, Weiden MD, Oppenheimer B, Berger K, et al. Emerging exposures and respiratory health: World Trade Center dust. Proc Am Thorac Soc. 2010 May; 7(2):142–5. https://doi.org/10.1513/pats.200908-092RM PMID: 20427588

15. Prezant DJ, Weiden M, Banauch GI, McGuinness G, Rom WN, Aldrich TK, et al. Cough and bronchial responsiveness in firefighters at the World Trade Center site. N Engl J Med. 2002 Sep 12; 347(11):806–15. https://doi.org/10.1056/NEJMoa021300 PMID: 12226151

16. Aldrich TK, Gustave J, Hall CB, Cohen HW, Webber MP, Zeig-Owens R, et al. Lung function in rescue workers at the World Trade Center after 7 years. N Engl J Med. 2010 Apr 8; 362(14):1263–72. https://doi.org/10.1056/NEJMoa0901087 PMID: 20375403

17. Zeig-Owens R, Webber MP, Hall CB, Schwartz T, Jaber N, Weakley J, et al. Early assessment of cancer outcomes in New York City firefighters after the 9/11 attacks: an observational cohort study. Lancet. 2011 Sep 3; 378(9794):898–905. https://doi.org/10.1016/S0140-6736(11)60989-6 PMID: 21890054

18. Prezant DJ, Levin S, Kelly KJ, Aldrich TK. Upper and lower respiratory diseases after occupational and environmental disasters. The Mount Sinai journal of medicine, New York. 2008 Mar-Apr; 75(2):89–100. https://doi.org/10.1002/msj.20028 PMID: 18500710

19. Naveed B, Weiden MD, Kwon S, Gracey Ej, Comfort AL, Ferrier N, et al. Metabolic syndrome biomarkers predict lung function impairment: a nested case-control study. Am J Respir Crit Care Med. 2012 Feb 15; 185(4):392–9. https://doi.org/10.1164/rcrcc.2011109-1672OC PMID: 22095549

20. Weiden MD, Naveed B, Kwon S, Cho SJ, Comfort AL, Prezant DJ, et al. Cardiovascular biomarkers predict susceptibility to lung injury in World Trade Center dust-exposed firefighters. Eur Respir J. 2013 May; 41(5):1023–30. https://doi.org/10.1183/09031936.0077012 PMID: 22903969

21. Kwon S, Crowley G, Mikhail M, Lam R, Clementi E, Zeig-Owens R, et al. Metabolic Syndrome Biomarkers of World Trade Center Airway Hyperreactivity: A 16-Year Prospective Cohort Study. Int J Environ Res Public Health. 2019 Apr 26; 16(9). https://doi.org/10.3390/ijerph16091486 PMID: 31035527

22. Kwon S, Crowley G, Caraher EJ, Haider SH, Lam R, Veerappan A, et al. Validation of Predictive Metabolic Syndrome Biomarkers of World Trade Center Lung Injury: A 16-Year Longitudinal Study. Chest. 2019 Sep; 156(3):486–96. https://doi.org/10.1016/j.chest.2019.02.019 PMID: 30836056

23. Schenck EJ, Echevarria GC, Girvin FG, Kwon S, Comfort AL, Rom WN, et al. Enlarged pulmonary artery is predicted by vascular injury biomarkers and is associated with WTC-Lung Injury in exposed fire fighters: a case-control study. BMJ Open. 2014 Sep 29; 4(9):e005575. https://doi.org/10.1136/bmjopen-2014-005575 PMID: 25270856

24. Haider SH, Veerappan A, Crowley G, Caraher EJ, Ostrofsky D, Mikhail M, et al. Multicomics of World Trade Center Particulate Matter-induced Persistent Airway Hyperreactivity. Role of Receptor for Advanced Glycation End Products. Am J Respir Cell Mol Biol. 2020 Aug; 63(2):219–33. https://doi.org/10.1165/rcmb.2019-0064OC PMID: 32315541

25. Haider SH, Osksuei A, Crowley G, Kwon S, Lam R, Riggs J, et al. Receptor for advanced glycation end-products and environmental exposure related obstructive airways disease: a systematic review. Eur Respir Rev. 2019 Mar 31; 28(151). https://doi.org/10.1183/16000617.0096-2018 PMID: 30918021

26. Weiden MD, Kwon S, Caraher E, Berger KI, Reibman J, Rom WN, et al. Biomarkers of World Trade Center Particulate Matter Exposure: Physiology of Distal Airway and Blood Biomarkers that Predict FEV(1) Decline. Semin Respir Crit Care Med. 2015 Jun; 36(3):323–3. https://doi.org/10.1055/s-0035-1547349 PMID: 26024341

27. Crowley G, Kwon S, Haider SH, Caraher EJ, Lam R, St-Jules DE, et al. Metabolomics of World Trade Center-Lung Injury: a machine learning approach. BMJ Open Respir Res. 2018 September 4th, 2018; 5 (1):e000274. https://doi.org/10.1136/bmjresp-2017-000274 PMID: 30233801

28. Caraher EJ, Kwon S, Haider SH, Crowley G, Lee A, Ebrahim M, et al. Receptor for advanced glycation end-products and World Trade Center particulate induced lung function loss: A case-cohort study and murine model of acute particulate exposure. PLoS One. 2017 Sep 19; 12(9):e0184331. https://doi.org/10.1371/journal.pone.0184331 PMID: 28926576
29. Crowley G, Kwon S, Ostrofsky DF, Clementi EA, Haider SH, Caraher EJ, et al. Assessing the Protective Metabolome Using Machine Learning in World Trade Center Particulate Exposed Firefighters at Risk for Lung Injury. Sci Rep. 2019 Sep 3; 9(1):11939. https://doi.org/10.1038/s41598-019-48458-w PMID: 31481674

30. Li X, Wang T, Yang T, Shen Y, An J, Liu L, et al. Elevated plasma levels of pigment epithelium-derived factor correlated with inflammation and lung function in COPD patients. Int J Chron Obstruct Pulmon Dis. 2015; 10:587–94. https://doi.org/10.2147/COPD.S78546 PMID: 2584034

31. Nolan A, Naveed B, Comfort AL, Ferrier N, Hall CB, Kwon S, et al. Inflammatory biomarkers predict airflow obstruction after exposure to World Trade Center dust. Chest. 2012 Aug; 142(2):412–8. https://doi.org/10.1378/chest.11-1202 PMID: 21998260

32. Cho SJ, Nolan A, Echevarria GC, Kwon S, Naveed B, Schenck E, et al. Chitotriosidase is a biomarker for the resistance to World Trade Center lung injury in New York City firefighters. J Clin Immunol. 2013 Aug; 33(6):1134–42. https://doi.org/10.1007/s10875-013-9913-2 PMID: 23744081

33. Banauch GI, Dhala A, Prezant DJ. Pulmonary disease in rescue workers at the World Trade Center site. Curr Opin Pulm Med. 2005 Mar; 11(2):160–8. https://doi.org/10.1097/01.mcp.0000151716.96241.0a PMID: 15699790

34. Weiden MD, Ferrier N, Nolan A, Rom WN, Comfort A, Gustave J, et al. Obstructive airways disease with air trapping among firefighters exposed to World Trade Center dust. Chest. 2010 Mar; 137(3):566–74. https://doi.org/10.1378/chest.09-1580 PMID: 19820077

35. Tsukiji J, Cho SJ, Echevarria GC, Kwon S, Joseph P, Schenck EJ, et al. Lysophosphatidic acid and apolipoprotein A1 predict increased risk of developing World Trade Center-lung injury: a case-control study. Biomarkers. 2014 Mar; 19(2):159–65. https://doi.org/10.3109/1354750X.2014.891047 PMID: 24548082

36. Nolan A, Kwon S, Cho SJ, Naveed B, Comfort AL, Prezant DJ, et al. MMP-2 and TIMP-1 predict healing of WTC-lung injury in New York City firefighters. Respir Res. 2014 Jan 21; 15:5. https://doi.org/10.1186/1465-9921-15-5 PMID: 24447332

37. Boudonck KJ, Rose DJ, Karoly ED, Lee DP, Lawton KA, Lapinskas PJ. Metabolomics for early detection of drug-induced kidney injury: review of the current status. Bioanalysis. 2009 Dec; 1(9):1645–63. https://doi.org/10.4155/bio.09.142 PMID: 21083109

38. Boudonck KJ, Mitchell MW, Nemet L, Keresztes L, Nyska A, Shinar D, et al. Discovery of metabolomics biomarkers for early detection of nephrotoxicity. Toxicologic pathology. 2009 Apr; 37(3):280–92. https://doi.org/10.1177/0197305X.2009.031439 PMID: 19369039

39. Nielsen KA, Tattersall DB, Jones PR, Moller BL. Metabolon formation in dhurrin biosynthesis. Phytochemistry. 2008 Jan; 69(1):88–98. https://doi.org/10.1016/j.phytochem.2007.06.033 PMID: 17706731

40. Ryals J, Lawton K, Stevens D, Milburn M. Metabolon, Inc. Pharmacogenomics. 2007 Jul; 8(7):863–6. https://doi.org/10.2217/14622416.8.7.863 PMID: 17638516

41. Hackstadt AJ, Hess AM. Filtering for increased power for microarray data analysis. BMC Bioinformatics. 2009 Jan 8; 10:11. https://doi.org/10.1186/1471-2105-10-11 PMID: 19133141

42. Genuer R, Poggi JM, Tuleau-Malot C. Variable selection using random forests. Pattern Recogn Lett. 2010 Oct 15; 31(14):2225–36.

43. Tolosi L, Lengauer T. Classification with correlated features: unreliability of feature ranking and solutions. Bioinformatics. 2011 Jul 15; 27(14):1986–94. https://doi.org/10.1093/bioinformatics/btr300 PMID: 21576180

44. Strobl C, Boulesteix AL, Kneib T, Augustin T, Zeileis A. Conditional variable importance for random forests. BMC Bioinformatics. 2008 Jul 11; 9:307. https://doi.org/10.1186/1471-2105-9-307 PMID: 18620558

45. Debeer D, Strobl C. Conditional permutation importance revisited. BMC Bioinformatics. 2020 Jul 14; 21(1):307. https://doi.org/10.1186/s12859-020-03622-2 PMID: 32664864

46. Breiman L. Random Forests. Machine Learning. 2001; 45(1):5–32.

47. Tibshirani R. Regression Shrinkage and Selection Via the Lasso. Journal of the Royal Statistical Society: Series B (Methodological). 1996; 58(1):267–88.

48. Hastie T, Tibshirani R., and Friedman J. The elements of statistical learning: data mining, inference, and prediction.2009.

49. Xia J, Broadhurst DI, Wilson M, Wishart DS. Translational biomarker discovery in clinical metabolomics: an introductory tutorial. Metabolomics. 2013 Apr; 9(2):280–99. https://doi.org/10.1007/s11306-012-0482-9 PMID: 23543913

50. Zeig-Owens R, Singh A, Aldrich TK, Hall CB, Schwartz T, Webber MP, et al. Blood Leukocyte Concentrations, FEV1 Decline, and Airlflow Limitation. A 15-Year Longitudinal Study of World Trade Center-
exposed Firefighters. Ann Am Thorac Soc. 2018 Feb; 15(2):173–83. https://doi.org/10.1513/AnnalsATS.201703-276OC PMID: 29099614

51. Zeig-Owens R, Nolan A, Putman B, Singh A, Prezant DJ, Weiden MD. Biomarkers of patient intrinsic risk for upper and lower airway injury after exposure to the World Trade Center acroty. Am J Ind Med. 2016 Sep; 59(9):788–94. https://doi.org/10.1002/ajim.22643 PMID: 27582481

52. Kwon S, Weiden MD, Echevarria GC, Comfort AL, Naveed B, Prezant DJ, et al. Early elevation of serum MMP-3 and MMP-12 predicts protection from World Trade Center-lung injury in New York City Firefighters: a nested case-control study. PLoS One. 2013; 8(10):e76099. https://doi.org/10.1371/journal.pone.0076099 PMID: 24468280

53. Kwon S, Putman B, Weakley J, Hall CB, Zeig-Owens R, Schwartz T, et al. Blood Eosinophils and World Trade Center Exposure Predict Surgery in Chronic Rhinosinusitis. A 13.5-Year Longitudinal Study. Ann Am Thorac Soc. 2016 Aug; 13(8):1253–61. https://doi.org/10.1513/AnnalsATS.201511-742OC PMID: 27096198

54. Cho SJ, Echevarria GC, Lee YI, Kwon S, Park KY, Tsukiji J, et al. YKL-40 is a Protective Biomarker for Fatty Liver in World Trade Center-Exposed Patients. J Mol Biomark Diagn. 2014; 5. https://doi.org/10.4172/2155-9929.1000174 PMID: 25717419

55. Filleur S, Nelius T, de Riese W, Kennedy RC. Characterization of PEDF: a multi-functional serpin family protein. Journal of cellular biochemistry, 2009 Apr 1; 106(5):769–75. https://doi.org/10.1002/jcb.22072 PMID: 19180572

56. Cosgrove GP, Brown KK, Schieman WP, Serls AE, Parr JE, Geraci MW, et al. Pigment epithelium-derived factor in idiopathic pulmonary fibrosis: a role in aberrant angiogenesis. Am J Respir Crit Care Med. 2004 Aug 1; 170(3):242–51. https://doi.org/10.1164/rccm.200308-1151OC PMID: 15117744

57. Shin ES, Sorenson CM, Sheibani N. PEDF expression regulates the proangiogenic and proinflammatory phenotype of the lung endothelium. Am J Physiol Lung Cell Mol Physiol. 2014 Apr 1; 306(7):L620–34. https://doi.org/10.1152/ajplung.00166.2013 PMID: 24318110

58. He X, Cheng R, Benyajati S, Ma JX. PEDF and its roles in physiological and pathological conditions: implication in diabetic and hypoxia-induced angiogenic diseases. Clin Sci (Lond). 2015 Jun; 128(11):805–23. https://doi.org/10.1042/CS20130463 PMID: 25881671

59. Crawford SE, Fitchev P, Veliceasa D, Volpert OV. The many facets of PEDF in drug discovery and disease. Science. 2013 Jul; 8(7):769–92. https://doi.org/10.1126/science.1236405 PMID: 23642051

60. Chen J, Ye L, Zhang L, Jiang WG. The molecular impact of pigment epithelium-derived factor, PEDF, on lung cancer cells and the clinical significance. International journal of oncology. 2009 Jul; 35(1):159–66. https://doi.org/10.3892/ijo.2009.1153 PMID: 19513563

61. Chavan SS, Hudson LK, Li JH, Ochani M, Harris Y, Patel NB, et al. Identification of pigment epithelium-derived factor as an adipocyte-derived inflammatory factor. Molecular medicine. 2012 Oct 24; 18:1161–8. https://doi.org/10.2119/molmed.2012.00156 PMID: 22714715

62. Famulla S, Lamers D, Hartwig S, Passlack W, Horricks A, Cramer A, et al. Pigment epithelium-derived factor (PEDF) is one of the most abundant proteins secreted by human adipocytes and induces insulin resistance and inflammatory signaling in muscle and fat cells. International journal of obesity. 2011 Jun; 35(6):762–72. https://doi.org/10.1038/ijo.2011.60 PMID: 21631840

63. Nakamura K, Yamagishi S, Adachi H, Matsu T, Kurita Y, Imaizumi T. Serum levels of pigment epithelium-derived factor (PEDF) are an independent determinant of insulin resistance in patients with essential hypertension. International journal of cardiology. 2010 Aug 6; 143(1):96–8. https://doi.org/10.1016/j.ijcard.2008.11.169 PMID: 20573347

64. Huang M, Qi W, Fang S, Jiang P, Yang C, Mo Y, et al. Pigment Epithelium-Derived Factor Plays a Role in Alzheimer’s Disease by Negatively Regulating Abeta42. Neurotherapeutics. 2016; 15(3):728–41. https://doi.org/10.1007/s13311-016-0623-1 PMID: 29736859

65. Sauter W, Rosenberger A, Beckmann L, Kropp S, Mittelstrass K, Timofeeva M, et al. Matrix metalloproteinase 1 (MMP1) is associated with early-onset lung cancer. Cancer Epidemiol Biomarkers Prev. 2008 May; 17(5):1127–35. https://doi.org/10.1158/1055-9965.EPI-07-2840 PMID: 18483334

66. Morais A, Beltrao M, Sokhatska O, Costa D, Melo N, Mota P, et al. Serum metalloproteinases 1 and 7 in the diagnosis of idiopathic pulmonary fibrosis and other interstitial pneumonias. Respir Med. 2015 Aug; 109(8):1063–8. https://doi.org/10.1016/j.rmed.2015.06.003 PMID: 26174192

67. Hunningshake GM, Cho MH, Tesfaigzi Y, Soto-Quiros ME, Avila L, Lasky-Su J, et al. MMP12, lung function, and COPD in high-risk populations. N Engl J Med. 2009 Dec 31; 361(27):2599–608. https://doi.org/10.1056/NEJMoa0904006 PMID: 20018959

68. Rosas IO, Richards TJ, Konishi K, Zhang Y, Gibson K, Lokshin AE, et al. MMP1 and MMP7 as potential peripheral blood biomarkers in idiopathic pulmonary fibrosis. PLoS Med. 2008 Apr 29; 5(4):e93. https://doi.org/10.1371/journal.pmed.0050093 PMID: 18447576
69. Jones CB, Sane DC, Herrington DM. Matrix metalloproteinases: a review of their structure and role in acute coronary syndrome. Cardiovasc Res. 2003 Oct 1; 59(4):812–23. https://doi.org/10.1016/s0008-6363(03)00516-9 PMID: 15435821

70. Death AK, Nakha S, McGrath KC, Martell S, Yue DK, Jessup W, et al. Nitroglycerin upregulates matrix metalloproteinase expression by human macrophages. J Am Coll Cardiol. 2002 Jun 19; 39(12):1943–50. https://doi.org/10.1016/s0735-1097(02)01907-1 PMID: 12084592

71. Lemaitre V, D’Armiento J. Matrix metalloproteinases in development and disease. Birth defects research Part C, Embryo today: reviews. 2006 Mar; 78(1):1–10. https://doi.org/10.1002/bdrc.20065 PMID: 16622845

72. D’Armiento JM, Goldklang MP, Hardigan AA, Geraghty P, Roth MD, Connell JE, et al. Increased matrix metalloproteinase (MMPs) levels do not predict disease severity or progression in emphysema. PLoS One. 2013; 8(2):e56352. https://doi.org/10.1371/journal.pone.0056352 PMID: 23441181

73. Mercer BA, Wallace AM, Brinckerhoff CE, D’Armiento JM. Identification of a cigarette smoke-responsive region in the distal MMP-1 promoter. Am J Respir Cell Mol Biol. 2009 Jan; 40(1):4–12. https://doi.org/10.1165/rcmb.2007-0310OC PMID: 18617682

74. Foronjy R, Nkyimeng T, Wallace A, Thankachen J, Okada Y, Lemaitre V, et al. Transgenic expression of matrix metalloproteinase-9 causes adult-onset emphysema in mice associated with the loss of alveolar elastin. Am J Physiol Lung Cell Mol Physiol. 2008 Jun; 294(6):L1149–57. https://doi.org/10.1152/ajplung.00481.2007 PMID: 18408070

75. Hammars tedt A, Gogg S, Hedjazifar S, Nerstedt A, Smith U. Impaired Adipogenesis and Dysfunction al Adipose Tissue in Human Hypertrop hic Obesity. Physiol Rev. 2018 Oct 1; 98(4):1911 –41. https://doi.org/10.1152/physrev.00034.2017 PMID: 30067159

76. Xiao LI, Cao Y, Wang Y, Lai X, Gao KQ, Du P, et al. Aberrant histone modifications of global histone and MCP-1 promoter in CD14(+) monocytes from patients with coronary artery disease. PloS One. 2013; 8(2):e56352. https://doi.org/10.1371/journal.pone.0056352 PMID: 23441181

77. Richter JR, Sutton JM, Belizaire RM, Friend LA, Schuster RM, Johannigman TA, et al. Macrophage-derived chemokine (CCL22) is a novel mediator of lung inflammation following hemorrhage and resuscitation. Shock. 2014 Dec; 42(6):525–31. https://doi.org/10.1097/SHK.0000000000000253 PMID: 25136780

78. Vulcano M, Struyf S, Scapini P, Cassatella M, Bernasconi S, Bonecchi R, et al. Unique regulation of CCL18 production by maturing dendritic cells. Journal of immunology. 2003 Apr 1; 170(7):3843–9. https://doi.org/10.4049/jimmunol.170.7.3843 PMID: 12646652

79. Azzaoui I, Yahia SA, Chang Y, Vorng H, Morales O, Fan Y, et al. CCL18 differentiates dendritic cells in tolerogenic cells able to prime regulatory T cells in healthy subjects. Blood. 2018 Apr 2; 73(4):202–6. https://doi.org/10.16198/2018.73.4 PMID: 29960866

80. Grommes J, Soehnlein O. Contribution of neutrophils to acute lung injury. Molecular medicine. 2011 Mar-Apr; 17(3–4):293–307. https://doi.org/10.2119/molmed.2010.00138 PMID: 21046059

81. Konrad FM, Reutershan J. CXCR2 in acute lung injury. Mediators Inflamm. 2012; 2012:740987. https://doi.org/10.1155/2012/740987 PMID: 22719179

82. Russo RC, Guabiraba R, Garcia CC, Barcelos LS, Rolfe E, Souza AL, et al. Role of the chemokine receptor CXCR2 in bleomycin-induced pulmonary inflammation and fibrosis. Am J Respir Cell Mol Biol. 2009 Apr; 40(4):410–21. https://doi.org/10.1165/rcmb.2007-0364OC PMID: 18836137

83. Holz O, Khalilieh S, Ludwig-Sengpiel A, Watz H, Strozsak P, Soni P, et al. SCH527123, a novel CXCR2 antagonist, inhibits ozone-induced neutrophilia in healthy subjects. Eur Respir J. 2010 Mar; 35(3):564–70. https://doi.org/10.1183/09031936.00048509 PMID: 19643947

84. Chapman RW, Phillips JE, Hipkyn RW, Curran AK, Lundell D, Fine JS. CXCR2 antagonists for the treatment of pulmonary disease. Pharmacol Ther. 2009 Jan; 121(1):55–68. https://doi.org/10.1016/j. pharma.2008.10.005 PMID: 19026683

85. Altman DG, Royston P. The cost of dichotomising continuous variables. BMJ. 2006 May 6; 332 (7549):1080. https://doi.org/10.1136/bmj.332.7549.1080 PMID: 16675816