Enlarged Pulmonary Artery is Predicted by Vascular Injury Biomarkers and is Associated with an Abnormal FEV1 in WTC Exposed Fire Fighters: A Case-Control Study

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<th>BMJ Open</th>
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<td>bmjopen-2014-005575</td>
</tr>
<tr>
<td>Article Type:</td>
<td>Research</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>27-Apr-2014</td>
</tr>
<tr>
<td>Complete List of Authors:</td>
<td>Schenck, Edward; New York University School of Medicine, Medicine Echevaria, Ghislaine; New York University School of Medicine, Medicine Girvin, Francis; New York University School of Medicine, Radiology Kwon, Sophia; New York University School of Medicine, Medicine Comfort, Ashley; New York University School of Medicine, Medicine Rom, William; New York University School of Medicine, Medicine Prezant, David; Fire Department of the City of New York, Bureau of Health Services Weiden, Michael; New York University School of Medicine, Medicine Nolan, Anna; New York University School of Medicine, Medicine</td>
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<td>Primary Subject Heading:</td>
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<tr>
<td>Secondary Subject Heading:</td>
<td>Occupational and environmental medicine, Respiratory medicine</td>
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<tr>
<td>Keywords:</td>
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Enlarged Pulmonary Artery is Predicted by Vascular Injury Biomarkers and is Associated with an Abnormal FEV\textsubscript{1} in WTC Exposed Fire Fighters: A Case-Control Study

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Running Title: CT and Serum Biomarkers of Vascular Injury in Obstruction

Main Text Word Count: 2705

Key Words: Particulate Matter, Vascular Disease, COPD, World Trade Center
ABSTRACT

RATIONALE: Vascular injury is an early manifestation of Obstructive lung disease. Increased pulmonary artery to aorta (PA/A) on computed tomography (CT) predicts exacerbations of COPD.

Objectives: We hypothesize that there is an association between an elevated PA/A and an FEV$_1$ less than the lower limit of normal (LLN) in WTC (World Trade Center)-exposed firefighters. We also assessed if serum biomarkers of vascular disease drawn within 6 months of exposure were predictive of an elevated PA/A.

METHODS: Cases (FEV$_1$ <LLN) and controls (FEV$_1$ ≥LLN) were selected based on post-9/11/2001 FEV$_1$ measured at symptomatic presentation. Diameter of the main PA at its bifurcation and the ascending aorta at the same level on inspiration were measured. Serum sampled with 6 months of 9/11/2001 was assayed for biomarkers.

Measurements and Main Results: The odds ratio (OR) of FEV$_1$<LLN if the PA/A was ≥0.92 was 4.02 (95%CI 1.21-13.41; p=0.023) when adjusted for exposure, BMI, age at CT and baseline FEV$_1$. Using a PA/A of ≥0.92 as a dichotomous outcome in logistic regression, elevated MDC and sE-selectin, were associated with an increased OR (2.08, 1.05-4.11, p=0.036; 1.33, 1.06-1.68, p=0.016; respectively) adjusted for age and exposure, while, increased tPAI-1 was associated with decreased odds of an elevated PA/A (OR 0.88, 0.79-0.98; p=0.024).

CONCLUSIONS: Elevated PA/A was associated with WTC-related decline in FEV$_1$. Development of an elevated PA/A was predicted by biomarkers of vascular disease found in serum drawn within 6 months of WTC exposure. Increased PA/A is a potentially useful non-invasive biomarker of obstructive lung dysfunction and warrants further study.

Article Summary Statement

Strengths and Limitations of this Study
Particulate matter inhalation such as what occurred as a result of the World Trade Center (WTC) disaster and in biomass exposure is one of the main causes of obstructive lung dysfunction. Vascular injury is seen in very early obstructive lung disease. An elevated pulmonary artery/aorta ratio (PA/A) was associated with developing an FEV1 less than the lower limit of normal in symptomatic WTC-exposed firefighters. This suggests that vascular injury may occur early in WTC-Lung Injury. To further investigate this relationship we demonstrated that serum biomarkers of vascular injury drawn soon after WTC exposure predicted a future abnormal PA/A. Replication of these findings in other populations at risk for COPD with and without particulate matter exposure is needed. This study provides insight into potential vasculopathic mechanisms of particulate matter related obstructive lung disease.
BACKGROUND

Development of abnormal spirometry after World Trade Center (WTC) exposure has been a common finding among exposed workers, volunteers, and lower Manhattan residents. In rescue/recovery workers from the Fire Department of the City of New York (FDNY), WTC exposure led to WTC lung injury (WTC-LI) as evidenced by substantial declines in pulmonary function in the first six months after 9/11 that persisted over the next 6.5 years. Our group has previously shown that systemic biomarkers of inflammation, metabolic derangement and cardiovascular disease predict this decline.[1-3]

One of the hallmarks of particulate matter exposure is systemic inflammation, endothelial dysfunction, and subsequent end-organ damage. High ambient particulate matter exposures significantly decrease FEV$_1$, in as soon as five to seven days. Epidemiologic investigation has documented associations between increased ambient particulates, lung disease, and cardiovascular disease (CVD). Systemic inflammation produces vascular endothelial injury and subsequent vascular disease. Recent studies associate systemic vascular involvement with lung disease and prospective studies have demonstrated an association between impaired lung function and central arterial stiffness even before the development of frank vascular disease, with systemic inflammation contributing to this association.[4-6]

Pulmonary vascular injury occurs early in smoking related chronic obstructive lung disease (COPD) with pulmonary perfusion abnormalities and reduced blood return to the heart is observed prior to development of abnormal FEV$_1$.[7-9] A similar pathophysiology likely occurs in irritant induced lung disease. Pulmonary arteriopathy was present in 58% of lung biopsies from non-FDNY WTC exposed individuals and in 74% with constrictive bronchiolitis after inhalational exposures during military service in Iraq and Afghanistan.[10,11] An increased ratio of the
pulmonary artery to aorta (PA/A) diameter measured by computed tomography (CT), has been
associated with pulmonary hypertension and poor outcomes in various disease states.
Additionally, an elevated PA/A has been associated with past and future exacerbations in
patients with severe COPD.[12] The PA/A has been associated with a decreased FEV₁ in the
same population. Multiple serum biomarkers have been identified that predict vascular disease
and several have been incorporated into clinical practice. To date, there have been no serum
biomarkers identified that predict an enlarged PA/A in obstructive lung disease.

Utilizing a nested case-control design, we investigated if an elevated PA/A was associated with
WTC lung injury in a population that also had serum biomarkers. We then determined the
relationship between vasoactive serum biomarkers drawn within six months of 9/11/01 and the
eventual development of an elevated PA/A.
METHODS

Study Design

WTC exposed FDNY firefighters entered the FDNY-WTC Health Program and had spirometry at entry into medical monitoring as previously described. Symptomatic subjects referred for subspecialty pulmonary evaluation (Pulm-Eval) between 10/1/2001 and 3/10/2008 underwent specialized pulmonary function testing as previously described.[13] The baseline cohort N=801 consisted of: never-smokers, male, had reliable NHANES normative data for FEV₁ %predicted, entered FDNY-WTC Health Program within 200 days of 9/11/01 and had pre-9/11 FEV₁ >75% predicted (801/1720, 47%). All subjects signed informed Institutional Review Board approved consent at the time of enrollment allowing analysis of their information and samples for research (Montefiore Medical Center; #07-09-320 and New York University; #11-00439).

Cases and controls were defined by their post-9/11 FEV₁ % Predicted measured at Pulm-Eval using NHANES III criteria. Cases, those susceptible to WTC lung injury, were defined as being within one SD of the lowest FEV₁ % predicted of the cohort (N=100), an FEV₁ <77%. Controls (N=153) had an FEV₁ ≥77% predicted and were randomly selected from the study cohort after stratification based on body mass index (BMI) and FEV₁ as previously defined. Serum biomarkers and CT scans were available for N=34/100 cases and N=63/153 controls, Figure 1.

Demographics

Age, gender, and years of service at FDNY were obtained from the FDNY-WTC Health Program database. Degree of exposure was self-reported at the first FDNY-WTC monitoring exam and was categorized using the FDNY-WTC Exposure Intensity Index (Arrival Time): Present on the morning of 9/11/2001 or Arrived after noon on 9/11/2001.[13] Those arriving after day three
were excluded from analysis as a result of their low numbers in this sample. Height and weight was measured at the Pulm-Eval.

**Lung Function Measures and Computed Tomography**

Pulmonary function testing was performed according to ATS/ERS guidelines as described. The first available post-9/11 CT was retrieved for 97 individuals. Contrast studies and CT scans obtained after 2009 were not included. Bronchial wall thickening (BWT) and air trapping (AT) were previously assessed.[13] Inspiratory images, collected with a BF40 algorithm and viewed on standard mediastinal windows were reviewed using iSite PACS (Philips iSite Enterprise, Version 3.6.114; www.healthcare.philips.com). The diameter of the main pulmonary artery (PA) at the level of its bifurcation and the diameter of the ascending aorta (A) in its maximum dimension were recorded using the same image. The reader, trained by a board certified radiologist in this method, was blinded to case status.[12,14]

**Serum Sampling and Analysis**

Fasting blood was drawn at the first post-9/11 FDNY-WTC monitoring exam, processed and stored (Bio-Reference Laboratories, Inc. Elmwood Park, NJ) as previously described. Serum was analyzed using a CVD-1(HCVD1-67AK) panel and a previous analyte macrophage derived chemokine (MDC) found associated with an abnormal FEV$_1$ from a 39-Plex Human Pro-inflammatory Panel according to manufacturer’s instructions (Millipore, Billerica, MA) on a Luminex 200IS (Luminex Corporation, Austin, TX). Data analyzed with MasterPlex QT software (Version 1·2; MiraiBio, Inc.). Each batch of samples processed contained controls and cases in an approximate 12/7 ratio as previously described.[2]

**Statistical Analysis**

SPSS 20 (IBM, Armonk, NY) and STATA 12.1 (StataCorp LP, College Station, Texas) were
used for database management and statistics. Demographics, CT data and analyte levels were compared by Student’s T-test, Mann-Whitney U and Chi-squared test where appropriate. The cutoff point of the PA/A ratio that maximized the value of the Youden index (Sensitivity + Specificity -1) was used for predicting the development of WTC lung injury.[15] Logistic regression was used to analyze the relationships between the CT derived measurements, abnormal FEV₁, and serum biomarkers. Variables identified as potential confounding factors in previous studies and those with a P value <0.20 in the univariable analysis were included in the multivariable logistic regression model. A backward stepwise approach was used to determine the most parsimonious model for the serum biomarkers, with a pre-specified p-value <0.10. The Hosmer-Lemeshow goodness-of-fit test was used to assess calibration of the model. The model discrimination was evaluated through the receiver operating characteristic area under the curve (AUC). To test the robustness of the serum biomarker models, internal validation was performed using bootstrapping (10,000 bootstrap samples). Data are expressed as mean (standard deviation, SD), median (interquartile range, IQR) or Odds Ratio (95% confidence interval), unless otherwise stated. A two-sided p value less than 0.05 was considered significant.
RESULTS

Demographics of Case-control Study

This case control study was drawn from a population of 801 never-smokers with normal pre-9/11 lung function. 253/801 individuals had serum available from their first post-9/11 monitoring exam. Non-contrast chest CT was available for review from 97 subjects (34/100 cases and 63/153 controls). The demographics of individuals with available serum and CT’s are summarized in Table 1. The control and case groups had similar WTC exposure; time from 9/11 to first post-9/11 monitoring exam, time from 9/11 to Pulm-Eval, years of service, and age at 9/11. BMI, body surface area (BSA) and height of cases were higher than controls at Pulm-Eval.

Lung Function

Cases had a lower FEV\(_1\) than controls at pre-9/11, and at Pulm-Eval; Table 1. Cases also had a lower FEV\(_1\)/FVC ratio, TLC, FRC and DLCO at Pulm-Eval. Cases had an increased bronchodilator response. At pre-9/11 testing the FEV\(_1\)/FVC ratio was similar. FEV\(_1\) in cases and controls declined from pre-9/11 to Pulm-Eval (104% to 96% and 86% to 72 % respectively; p<0.001 all comparisons). FVC demonstrated a similar pattern. To confirm that the median FEV\(_1\) difference represented individual changes we used patients as their own controls. The mean ratio of FEV\(_1\) was 0.92 vs 0.77 in controls vs cases respectively between pre-9/11 and Pulm-Eval testing, p <0.001, demonstrating a significantly greater loss of lung function in cases as compared to controls, even when analyzed individually.

CT Scan Measurements

PA and A measurements on available CT’s from cases and controls are outlined in Table 1. The PA diameter was modestly correlated with BSA and BMI (r = 0.219, 0.265 p = 0.031, 0.009 respectively), but did not vary with height or age. The A diameter demonstrated modest
correlation with age and BMI ($r = 0.376, 0.284 \ p = <0.001, 0.005$ respectively). The measured PA/A declined with increased age ($r = -0.256, p = 0.011$) but was not significantly associated with height, BSA or BMI, which is similar to reported associations in a larger cohort study.[16] The mean PA/A in cases was 0.92, similar to the 90$^{th}$% upper limit of normal in the Framingham Heart Study.[16] Cases had similar proportions of AT and BWT to controls.

**PA/A as a biomarker of Abnormal FEV$_1$**

After calculating the Youden index, a PA/A value of 0.92 was selected as the cutoff for predicting the development of WTC lung injury in a logistic regression analysis. After adjusting for age at CT, pre-9/11 FEV$_1$, BMI at Pulm-Eval and exposure, the odds of having a low FEV$_1$ at Pulm-Eval in patients with a value of PA/A $\geq 0.92$ was 4.02 (95% CI: 1.21-13.41, $p = 0.023$) times larger than the odds of those patients with a value $<0.92$ (AUC = 0.854 (95% CI: 0.773-0.934), Hosmer-Lemeshow’s $p=0.55$).

**Vascular Disease Biomarkers and PA/A Association**

CT measurements across the PA/A of 0.92 are displayed in Supplemental Table 1. There were 38 individuals with a ratio $\geq 0.92$ and 59 with a ratio $<0.92$. There was a higher proportion of measured BWT in the low ratio group. There was a similar amount of AT in both groups. Age and time since 9/11 were similar in both groups. Height, age, BSA, BMI and exposure intensity were similar across the PA/A ratio of 0.92 (data not shown).

Levels of analytes across the ratio of 0.92 are displayed in Table 2. The results of multivariable logistic analysis using backward stepwise approach are shown in Table 3. For each 1 ng/ml increased of serum MDC, the odds of have a subsequent PA/A ratio $\geq 0.92$ increased by 2.08 fold (95%CI: 1.05, 4.11). For each 10ng/ml increase in soluble endothelial selectin (sE-selectin)
and total plasminogen activator inhibitor 1 (tPAI-1), the odds of having a PA/A ratio ≥0.92 increased by 33% (95%CI: 6%, 68%) and decreased by 12% (95%CI: 2%, 21%) respectively.

The probability of having PA/A ratio ≥0.92 was determined for each of our three analytes when holding all other variables in the model constant, Figure 2. The probability increased from 0.21 to 0.79 as the concentration of MDC increased from 0.20 ng/ml to 4.41 ng/ml. (Figure 2A) The probability of an increased PA/A increased from 0.14 to 0.75 as the concentration of sE-selectin increased from 12.7 ng/ml to 125.4 ng/ml (Figure 2B). Increase in the concentration of tPAI-1 from 41.4 ng/ml to 288.9 ng/ml decreased the probability of having an increased PA/A ratio from 0.63 to 0.11 (Figure 2C).
DISCUSSION

We have identified biomarkers of cardiovascular risk, inflammation and metabolic syndrome expressed within 6 months of 9/11/2001 predict the development of WTC-LI. Vascular changes occur early in COPD, prior to development of an abnormal FEV₁.[9] In a recently published analysis of the Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE)/COPDGene cohorts, in patients with moderate to severe disease, a PA/A > 1 was significantly associated with COPD exacerbations.[12] In this nested case control study of our well-phenotyped WTC exposed FDNY cohort, we found that levels of MDC, sE-selectin and tPAI-1 predicted an abnormal PA/A ratio measured years later. This elevated PA/A ratio was also associated with a contemporaneously observed decline in FEV₁. Combining these three biomarkers together in a multivariable model adjusted for confounders strengthened the former results. A unique advantage to observing the PA/A ratio by non-contrast CT is that it is a non-invasive mechanism that provides insight into the pulmonary and systemic circulation.[17,18]

Recently, CT measurements of the PA/A have been associated with outcomes other than pulmonary hypertension. The ECLIPSE/COPDGene cohort examined patients with advanced disease, many requiring supplemental O₂.[12] Our cohort's mean PA and A were similar to those measured in ECLIPSE/COPDGene. Since our population had less advanced COPD, mostly GOLD 0, it was expected that the PA/A would be less abnormal.[12] When comparing our cohort to the Framingham Heart Study, our case mean PA/A and PA values are similar to their 90th% upper limit of normal. Although we found a similar inverse relationship with PA/A and age, there were only weak associations with height and BSA.[16] In a very recent study, an abnormal PA/A has been associated with increased mortality in patients with coronary artery disease.[19] Our study would indicate that abnormalities in the PA/A may represent a marker of
early vascular injury in particulate matter related lung disease and is in line with these recent publications.

Our group and others have linked biomarkers of systemic inflammation and vascular disease with COPD. The proposed pathobiologic mechanism of this relationship is related to inflammatory loss of lung parenchyma and vascular beds. COPD is systemic process affecting not only the airways but also affecting lung parenchyma, vascular structures and other organ systems such as skeletal muscle and adipose tissue.[20] Non-invasive biomarkers guiding management and prognostication in COPD are needed.[21,22]

We chose to study a limited number of biologically plausible vasoactive biomarkers, to examine the link between their expression in a serum soon after massive particulate matter exposure and PA/A. MDC, also known as CCL22, is an inflammatory mediator that has been linked to obstructive lung dysfunction by our lab and others.[23] It is important in platelet activation and has been associated with systemic vascular phenomenon.[24,25] E-selectin is a member of the selectin family of carbohydrate binding lectins, and is specifically produced by activated endothelial cells. [26] It is one of the main endothelial neutrophil adhesion molecules and has been linked to poor CVD outcomes. Additionally, well-known metabolic risk factors for CVD are associated with increase in the soluble form sE-selectin.[27,28] TPAI-1, the main inhibitor of plasminogen activator, has been associated with lung diseases,[29] atherosclerosis, thrombosis, and vascular remodeling.[30-32] It has been analyzed in pulmonary hypertension and right heart failure, although whether it is inhibitory or stimulatory in these disease states has yet to be elucidated and is under investigation.[33,34]

In our population, we found that elevated levels of MDC, sE-selectin were significantly associated with an increased ratio PA/A ratio. TPAI-1 was inversely related to this ratio. These
associations correlate with previous reports on cardiovascular disease. This association is novel because of it is the first time these biomarkers have been associated with a non-invasive marker of vascular injury in lung disease.

There are several limitations to this study. Understandably, this cohort of FDNY firefighters did not have pre-exposure serum banked for future analyses. We did achieve the next best option by obtaining serum samples within a few months post-exposure. There were no unexposed or asymptomatic exposed controls in our study. Replication of these findings in other populations with and without particulate matter exposure will be important. We were only able to obtain CT images on 97 subjects with available serum. We do not have longitudinal follow-up data (FEV₁, CTs, serum biomarkers) for additional correlation. Finally, we have no data on the prevalence of important co-morbidities such as sleep apnea, left heart failure and thromboembolic disease in this population. Thus, the finding linking an elevated PA/A to a decreased FEV₁ may be influenced by the presence of these unaccounted for confounders.

In this nested case-cohort study, we were able to identify cardiovascular related serum biomarkers drawn within six months of 9/11/01 that predicted an abnormal PA/A ratio. The observation that biomarkers predict changes in PA/A and that PA/A was a non-invasive marker lung injury (FEV₁ loss) in this post-exposure population, provides a novel association to well characterized processes in vascular biology and inflammation secondary to particulate matter exposure. Importantly, these biomarkers were expressed during the early stages of WTC lung injury and reflect potential processes leading to disease susceptibility. This insight on protein expression and its relationship to FEV₁ loss and vascular injury may guide future mechanistic and therapeutic studies in the field.
Acknowledgments
The authors would like to thank the firefighters and rescue workers for their participation in this study and for their selfless contributions.

Competing interests: The authors report no financial or competing interests.

Funding Sources:
This work was supported by NIH-NHLBI K23HL084191(AN), NIAID K24A1080298(MDW), NIH-R01HL057879 (MDW), and NIOSH (U10-OH008243, U10-OH008242) and UL1RR029893 (DJP). This work was also partially funded by the NYU-HHC CTSI UL1TR000038 from the National Center for Advancing Translational Sciences of the National Institutes of Health. The funding agencies did not participate in the study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

Contributorship: AN, MDW, EJS, DJP participated in study conception and design; AN, EJS were the primary investigators; EJS, AN, SK, AC were responsible for data collection; AN, EJS, FGG, SK were responsible for data validation; AN, EJS, SK participated in data analysis; AN, GCE, EJS undertook the statistical analysis. All authors participated in data interpretation, writing and revision of the report and approval of the final version

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Data Sharing Statement: No Additional Data is Available
Figure Legends

Figure 1. Study Design. Subjects in the FDNY-WTC health program presented for pulmonary evaluation (Pulm-Eval). Baseline Cohort met the listed inclusion criteria. Cases (N=34) and Controls (N=63) had CT and biomarkers available.

Figure 2. Probability Plots. Probability of developing WTC lung injury over the range of MDC (A), sE-Selectin (B) and tPAI-1 (C) are represented when adjusting for the covariates of exposure, and age. Plots express probability isopleths for the development of WTC lung injury (FEV₁ loss) with all other covariates held constant.

Supplemental Figure 1. PA/A Measurement. Inspiratory series were evaluated at the level of the main pulmonary artery bifurcation. The main pulmonary artery trunk was measured perpendicular to its axial longitudinal axis (red) and the ascending thoracic aorta was measured in its widest dimension (yellow).
REFERENCES


Table 1: Demographics, CT and Pulmonary Function Test Data

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<td>84 (80-94)</td>
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<td>97 (89-111)</td>
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<td>FEV₁, %</td>
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<td>FEV₁/FVC</td>
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<td>18 (29)</td>
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<td>Arrived Later*</td>
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<td>9/11 to Pulm-Eval, Months</td>
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<td>34</td>
<td>33 (24-57)</td>
<td>63</td>
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<td>Pulm-Eval</td>
<td>FVC ,%</td>
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<td>34</td>
<td>96 (90-104)</td>
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<td>34</td>
<td>28.19 (3.23)</td>
<td>63</td>
<td>0.138</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A†</td>
<td>32.09 (3.68)</td>
<td>34</td>
<td>31.95 (3.19)</td>
<td>63</td>
<td>0.850</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PA/A†</td>
<td>0.92 (0.11)</td>
<td>34</td>
<td>0.89 (0.10)</td>
<td>63</td>
<td>0.151</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BWT*</td>
<td>12 (36)</td>
<td>33</td>
<td>21(34)</td>
<td>61</td>
<td>0.851</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Air Trapping*</td>
<td>19 (58)</td>
<td>33</td>
<td>25 (41)</td>
<td>61</td>
<td>0.124</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>47 (41-51)</td>
<td>34</td>
<td>46 (42-51)</td>
<td>63</td>
<td>0.791</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Height, cm</td>
<td>182 (178-183)</td>
<td>34</td>
<td>178 (173-183)</td>
<td>63</td>
<td>0.041</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BSA, m²</td>
<td>2.24 (2.17-2.43)</td>
<td>34</td>
<td>2.09 (2.01-2.28)</td>
<td>63</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: Pulm-Eval Pulmonary Evaluation; BSA Body Surface Area; BD Bronchodilator; DL₁CO: Diffusion Capacity of the Lung for Carbon Monoxide. PA = Pulmonary Artery, A = aorta, BWT = Bronchial Wall Thickening
Expressed as Median (IQR) except *Expressed as N (%), †Expressed as Mean (SD)
Table 2: Biomarker PA/A relationship

<table>
<thead>
<tr>
<th>Analyte</th>
<th>PA/A</th>
<th></th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≥0.92</td>
<td>&lt;0.92</td>
<td></td>
</tr>
<tr>
<td>ng/mL</td>
<td>N=37</td>
<td>N=59</td>
<td></td>
</tr>
<tr>
<td>MDC</td>
<td>1.51 (1.21-2.01)</td>
<td>1.41 (0.99-1.77)</td>
<td>0.101</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>12770 (9510-20941)</td>
<td>13218 (10037-18537)</td>
<td>0.789</td>
</tr>
<tr>
<td>sE-Selectin</td>
<td>49.7 (36.5-63.2)</td>
<td>42.8 (35.3-56.1)</td>
<td>0.153</td>
</tr>
<tr>
<td>tPAI-1</td>
<td>119.8 (84.5-152.4)</td>
<td>139.7 (119.5-173.3)</td>
<td>0.057</td>
</tr>
<tr>
<td>MMP-9</td>
<td>345.7 (265.8-465.3)</td>
<td>352.4 (267.9-517.9)</td>
<td>0.949</td>
</tr>
<tr>
<td>MPO</td>
<td>141.0 (104.3-226.1)</td>
<td>135.3 (95.1-289.0)</td>
<td>0.865</td>
</tr>
<tr>
<td>sICAM-1</td>
<td>165.2 (130.4-197.4)</td>
<td>157.8 (136.1-201.8)</td>
<td>0.952</td>
</tr>
<tr>
<td>sVCAM-1</td>
<td>1355 (1119-1679)</td>
<td>1335 (1108-1602)</td>
<td>0.617</td>
</tr>
</tbody>
</table>

Abbreviations: MDC Macrophage Derived Chemokine; sE-Selectin Soluble Endothelial Selectin; tPAI Total Plasminogen Activator Inhibitor; MMP Matrix Metaloproteinase; MPO Myeloperoxidase; sICAM Soluble Intercellular Adhesion Molecule; sVCAM Soluble Vascular Cell Adhesion Molecule

Values expressed as median (IQR)

MDC Ratio ≥0.92 N=38 Ratio <0.92 N=59
Table 3. Serum Biomarker Models Predicting PA/A ≥0.92

<table>
<thead>
<tr>
<th>Model</th>
<th>Analytes</th>
<th>Crude OR(95%CI)</th>
<th>p</th>
<th>Adjusted OR(95%CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uni-Variable</td>
<td>MDC</td>
<td>1.70 (0.90-3.19)</td>
<td>0.100</td>
<td>1.78 (0.93-3.37)</td>
<td>0.080</td>
</tr>
<tr>
<td></td>
<td>sE-Selectin</td>
<td>1.18 (0.97-1.44)</td>
<td>0.093</td>
<td>1.20 (0.98-1.47)</td>
<td>0.075</td>
</tr>
<tr>
<td></td>
<td>tPAI-1</td>
<td>0.92 (0.84-1.01)</td>
<td>0.093</td>
<td>0.93 (0.85-1.03)</td>
<td>0.148</td>
</tr>
<tr>
<td>Multi-Variable</td>
<td>MDC</td>
<td>1.99 (1.02-3.88)</td>
<td>0.043</td>
<td>2.08 (1.05-4.11)</td>
<td>0.036</td>
</tr>
<tr>
<td></td>
<td>sE-Selectin</td>
<td>1.32 (1.05-1.65)</td>
<td>0.018</td>
<td>1.33 (1.06-1.68)</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>tPAI-1</td>
<td>0.87 (0.78-0.97)</td>
<td>0.013</td>
<td>0.88 (0.79-0.98)</td>
<td>0.024</td>
</tr>
</tbody>
</table>

Abbreviations: MDC Macrophage Derived Chemokine; sE-Selectin Soluable Endothelial Selectin; tPAI Total Plasminogen Activator Inhibitor

*Adjusted for age at CT and Exposure Group

Per 1 ng/ml MDC, Per 10 ng/ml sE-Selectin, tPAI-1

X2 (5) = 15.69, p = 0.008. Hosmer and Lemeshow’s goodness-of-fit test P=0.25.

Area Under ROC curve=0.728 (0.623-0.834)
FDNY MMTP

12,781 of 13,934 (92%)

Pulmonary Evaluation

1,720

Inclusion Criteria for Baseline Cohort
i. Pre 9/11 FEV1%Pred > 75%
ii. Post 9/11 - FDNY PFT ≤ 200 days from 9/11
iii. Pulmonary function study at NYU
iv. Arrived at the WTC site before 9/12/01
v. Never Smoking Male FDNY rescue workers
vi. Accurate NHANES PFT normative data

Baseline Cohort

801 (47%)

Case-Control

Cases
N=100 (12.5%)

Controls
N=153 (19%)

Case-Control w/ CT

Cases w/ CT
N=34 (34%)

Controls w/ CT
N=63 (41%)
### Supplemental Table 1: CT Data Across 0.92

<table>
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<th>PA/A</th>
<th></th>
<th>P</th>
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<td></td>
<td>≥0.92</td>
<td>&lt;0.92</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>38</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td><strong>PA mm</strong></td>
<td>30.2</td>
<td>27.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(SD)</td>
<td>(2.46)</td>
<td>(3.29)</td>
<td></td>
</tr>
<tr>
<td><strong>A mm</strong></td>
<td>30.3</td>
<td>33.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(SD)</td>
<td>(2.41)</td>
<td>(3.46)</td>
<td></td>
</tr>
<tr>
<td><strong>Ratio</strong></td>
<td>1</td>
<td>0.83</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(SD)</td>
<td>(0.06)</td>
<td>(0.07)</td>
<td></td>
</tr>
<tr>
<td><strong>BWT; N(%)</strong></td>
<td>8</td>
<td>25</td>
<td>0.019</td>
</tr>
<tr>
<td>(21)</td>
<td>(45)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Air Trapping; N(%)</strong></td>
<td>16</td>
<td>28</td>
<td>0.452</td>
</tr>
<tr>
<td>(42)</td>
<td>(50)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age CT Scan</strong></td>
<td>45</td>
<td>47</td>
<td>0.119</td>
</tr>
<tr>
<td>(40-50)</td>
<td>(42-51)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>9/11 to CT Scan, Months</strong></td>
<td>52</td>
<td>58</td>
<td>0.286</td>
</tr>
<tr>
<td>(33-67)</td>
<td>(32-73)</td>
<td></td>
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Abbreviations: PA = Pulmonary Artery, A = aorta, BWT = Bronchial Wall Thickening, 
*Expressed as Mean (SD) †Expressed as Median (IQR)
Air Trapping/Bronchial Wall Thickening PA/A <0.92
N=56, PA/A ≥0.92 N=38
**Enlarged Pulmonary Artery is Predicted by Vascular Injury Biomarkers and is Associated with WTC-Lung Injury in Exposed Fire Fighters: A Case-Control Study**

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<td>10-Jul-2014</td>
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Enlarged Pulmonary Artery is Predicted by Vascular Injury Biomarkers and is Associated with WTC-Lung Injury in Exposed Fire Fighters: A Case-Control Study

Edward J. Schenck, MD¹, Ghislaine C. Echevaria, MD², Francis G. Girvin, MD⁷, Sophia Kwon, DO, MPH¹, Ashley L. Comfort, BS¹, William N. Rom, MD¹,³, David J. Prezant, MD⁴,⁶, Michael D. Weiden, MD¹,³,⁴, Anna Nolan, MD¹,³,⁴

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Author Contributions: AN, MDW, EJS, DJP participated in study conception and design; AN, EJS were the primary investigators; EJS, AN, SK, AC were responsible for data collection; AN, EJS, FGG, SK were responsible for data validation; AN, EJS, SK participated in data analysis; AN, GCE, EJS undertook the statistical analysis. All authors participated in data interpretation, writing and revision of the report and approval of the final version.

Running Title: CT and Serum Biomarkers of Vascular Injury in Obstruction

Main Text Word Count: 2942

Key Words: Particulate Matter, Vascular Disease, COPD, World Trade Center
ABSTRACT

Objectives: We hypothesize that there is an association between an elevated PA/A and World Trade Center-Lung Injury (WTC-LI). We assessed if serum vascular disease biomarkers were predictive of an elevated PA/A.

Design: Retrospective case-cohort analysis of thoracic CT-scans of WTC-exposed firefighters who were symptomatic between 9/12/2001 and 3/10/2008. Quantification of vascular-associated biomarkers from serum collected within 200 days of exposure.

Setting: Urban tertiary care center and occupational health care center.

Participants: Male never-smoking firefighters with accurate pre-9/11 FEV₁ ≥75%, serum sampled ≤200 days of exposure was the baseline cohort (n=801). Subcohort (n=97) with available CT scan and serum biomarkers were identified. WTC-LI was defined as FEV₁ ≤77% at subspecialty pulmonary evaluation (n=34) and compared to controls (n=63) to determine associated PA/A ratio. The subcohort was restratified based on PA/A ≥0.92 (n=38) and PA/A <0.92 (n=59) to determine serum vascular biomarkers that were predictive of this vasculopathy.

Outcome Measures: Primary outcome of this study was to identify a PA/A ratio in a cohort of individuals exposed to WTC dust that was associated with WTC-LI. Secondary outcome was to identify serum biomarkers predictive of the PA/A ratio using logistic regression.

Results: PA/A ≥0.92 was associated with WTC-LI, odds ratio (OR) of 4.02 (95%CI 1.21-13.41; p=0.023) when adjusted for exposure, BMI, and age at CT. Elevated MDC and sE-selectin, were predictive of PA/A ≥0.92, OR,95%CI: 2.08, 1.05-4.11, p=0.036; 1.33, 1.06-1.68, p=0.016; respectively) while increased tPAI-1 was predictive of not having PA/A ≥0.92 (OR 0.88,0.79-0.98; p=0.024).

Conclusions: Elevated PA/A was associated with WTC-LI. Development of an elevated PA/A was predicted by biomarkers of vascular disease found in serum drawn within 6 months of WTC exposure. Increased PA/A is a potentially useful non-invasive biomarker of WTC-LI and warrants further study.
Article Summary

Strengths and Limitations of this Study

- Well-phenotyped cohort with lung function tests prior to exposure available
- Identification of a viable PA/A ratio (0.92) as a biomarker associated with WTC-LI
- Identification of biomarkers predictive of PA/A vasculopathy
- Limited generalizability because of unique WTC-related exposure
- Retrospective study design and logistic regression implies only associated findings, and does not reflect causality.
BACKGROUND

Development of lung disease after World Trade Center (WTC) exposure has been a common finding among exposed workers, volunteers, and lower Manhattan residents. In rescue/recovery workers from the Fire Department of the City of New York (FDNY), WTC exposure led to WTC lung injury (WTC-LI). Our group has previously defined WTC-LI as the chronic inflammatory lung dysfunction experienced by a subcohort of firefighters with intense exposure to WTC dust. It is characterized by primarily obstructive respiratory dysfunction with substantial and persistent losses in FEV₁% predicted to ≤77% in the subsequent 6.5 years post-exposure. In addition, systemic biomarkers of inflammation, metabolic derangement and cardiovascular disease predict WTC-LI.

One of the hallmarks of particulate matter exposure is systemic inflammation, endothelial dysfunction, and subsequent end-organ damage. High ambient particulate matter exposures significantly decrease FEV₁, in as soon as five to seven days. Epidemiologic investigation has documented associations between increased ambient particulates, lung disease, and cardiovascular disease (CVD). Systemic inflammation produces vascular endothelial injury and subsequent vascular disease. Recent studies associate systemic vascular involvement with lung disease and prospective studies have demonstrated an association between impaired lung function and central arterial stiffness even before the development of frank vascular disease, with systemic inflammation contributing to this association.

Pulmonary vascular injury occurs early in smoking related chronic obstructive lung disease (COPD) with pulmonary perfusion abnormalities and reduced blood return to the heart is observed prior to development of WTC-LI. A similar pathophysiology likely occurs in irritant induced lung disease. Pulmonary arteriopathy was present in 58% of lung biopsies from non-
FDNY WTC exposed individuals and in 74% with constrictive bronchiolitis after inhalational exposures during military service in Iraq and Afghanistan.\textsuperscript{11, 12} An increased ratio of the pulmonary artery to aorta (PA/A) diameter measured by computed tomography (CT), has been associated with pulmonary hypertension and poor outcomes in various disease states. Additionally, an elevated PA/A has been associated with past and future exacerbations in patients with moderate-to-severe COPD.\textsuperscript{13} The PA/A has been associated with a decreased FEV\textsubscript{1} in the same population. Multiple serum biomarkers have been identified that predict vascular disease and several have been incorporated into clinical practice. To date, there have been no serum biomarkers identified that predict an enlarged PA/A in obstructive lung disease.

Utilizing a nested case-control design, we investigated if an elevated PA/A was associated with WTC-LI in a population that also had serum biomarkers. We then determined the ability of vasoactive serum biomarkers drawn within six months of 9/11/01 to predict the eventual development of an elevated PA/A.
METHODS

Study Design

Annual physicals occurred prior to 9/11/2001, and active-duty firefighters had normal lung function testing, EKG assessment and measures of exercise capacity. Those with abnormal cardiopulmonary testing were placed on medical leave and were not part of the rescue and recovery efforts.

WTC exposed FDNY firefighters (N=1720) entered the FDNY-WTC Health Program and had spirometry at entry into medical monitoring as previously described. Symptomatic subjects referred for subspecialty pulmonary evaluation (SPE) between 10/1/2001 and 3/10/2008, underwent specialized pulmonary function testing as previously described. Inclusion criteria were applied to the symptomatic cohort. N=801/1720 (47%) were never-smokers, male, had reliable NHANES normative data for FEV$_1$%predicted, entered FDNY-WTC Health Program within 200 days of 9/11/01 and had pre-9/11 FEV$_1$$>75\%$ predicted. All subjects signed informed Institutional Review Board approved consent at the time of enrollment allowing analysis of their information and samples for research (Montefiore Medical Center; #07-09-320 and New York University; #11-00439).

FEV$_1$% predicted at SPE was used as a measurable, phenotypic marker of WTC-LI. Cases represented those who had continued lung dysfunction that we termed WTC-LI, whereas controls represented those who did not have WTC-LI. Cases (N=100) were defined as being within one SD of the lowest FEV$_1$% predicted of the cohort at SPE (FEV$_1$$\leq77\%$). Controls (N=153) were those who had FEV$_1$$>77\%$ and were randomly selected after stratification based on body mass index (BMI) and FEV$_1$ as previously defined.
CT scans were administered as standard of care measures to the population, but only a subset was available at the single center where this research was conducted. Serum biomarkers and CT scans were available for N=34/100 cases and N=63/153 controls, Figure 1. The population was then re-stratified by PA/A ratio using a cutoff of 0.92 for analysis of biomarkers predictive of PA/A.

Demographics

Age, gender, and years of service at FDNY were obtained from the FDNY-WTC Health Program database. Degree of exposure was self-reported at the first FDNY-WTC monitoring exam and was categorized using the FDNY-WTC Exposure Intensity Index (Arrival Time): Present on the morning of 9/11/2001 or Arrived after noon on 9/11/2001. Those arriving after day three were excluded from analysis as a result of their low numbers in this sample. Height and weight was measured at the SPE.

Lung Function Measures and Computed Tomography

Pulmonary function testing was performed according to ATS/ERS guidelines as described. The first available post-9/11 CT was retrieved for the 34 cases and 63 controls. Contrast studies and CT scans obtained after 2009 were not included. Bronchial wall thickening (BWT) and air trapping (AT) were previously assessed. Inspiratory images, collected with a BF40 algorithm and viewed on standard mediastinal windows were reviewed using iSite PACS (Philips iSite Enterprise, Version 3.6.114; www.healthcare.philips.com). The diameter of the main pulmonary artery (PA) at the level of its bifurcation and the diameter of the ascending aorta (A) in its maximum dimension were recorded using the same image, Supplementary Figure 1. The reader, trained by a board certified radiologist in this method, was blinded to case status.

Serum Sampling and Analysis
Fasting blood was drawn at the first post-9/11 FDNY-WTC monitoring exam, processed and stored (Bio-Reference Laboratories, Inc. Elmwood Park, NJ) as previously described. Serum was analyzed using a CVD-1(HCVD1-67AK) and a 39-Plex Human Pro-inflammatory Panel according to manufacturer’s instructions (Millipore, Billerica, MA) on a Luminex 200IS (Luminex Corporation, Austin, TX). Data analyzed with MasterPlex QT software (Version 1.2; MiraiBio, Inc.). Each batch of samples processed contained controls and cases in an approximate 12/7 ratio as previously described.

**Statistical Analysis**

SPSS 20 (IBM, Armonk, NY) and STATA 12.1 (StataCorp LP, College Station, Texas) were used for database management and statistics. Demographics, CT data and analyte levels were compared by Student’s T-test, Mann-Whitney U and Chi-squared test where appropriate. The cutoff point of the PA/A ratio that maximized the value of the Youden index (Sensitivity + Specificity -1) was used for predicting the development of WTC-LI. Logistic regression was used to first see if the PA/A ratio was a marker of WTC-LI. Then, a separate logistic regression was used to analyze if serum biomarkers could predict PA/A ratio. Variables identified as potential confounding factors in previous studies and those with a P value <0.20 in the univariable analysis were included in the multivariable logistic regression model. A backward stepwise approach was used to determine the most parsimonious model for the serum biomarkers, with a pre-specified p-value <0.10. The Hosmer-Lemeshow goodness-of-fit test was used to assess calibration of the model. The model discrimination was evaluated through the receiver operating characteristic area under the curve (AUC). To test the robustness of the serum biomarker models, internal validation was performed using bootstrapping (10,000 bootstrap samples). Data are expressed as mean (standard deviation, SD), median
(interquartile range, IQR) or Odds Ratio (95% confidence interval), unless otherwise stated. A

 **two-sided** p value less than 0.05 was considered significant.
RESULTS

Demographics of Case-Control Study

This case control study was drawn from a population of 801 never-smokers with normal pre-9/11 lung function. 253/801 individuals had serum available from their first post-9/11 monitoring exam. Non-contrast chest CT was available for review from 97 subjects (34/100 cases and 63/153 controls). The demographics of individuals with available serum and CT’s are summarized in Table 1. The control and case groups had similar WTC exposure; time from 9/11 to first post-9/11 monitoring exam, time from 9/11 to SPE, years of service, and age at 9/11. BMI, body surface area (BSA) and height of cases were higher than controls at SPE.

Lung Function

Cases had a lower FEV\textsubscript{1} than controls at pre-9/11, and at SPE; Table 1. Cases also had a lower FEV\textsubscript{1}/FVC ratio, TLC, FRC and DLCO at SPE. Cases had an increased bronchodilator response. At pre-9/11 testing the FEV\textsubscript{1}/FVC ratio was similar. FEV\textsubscript{1} in cases and controls declined from pre-9/11 to SPE (104% to 96% and 86% to 72% respectively; p<0.001 all comparisons). FVC demonstrated a similar pattern. To confirm that the median FEV\textsubscript{1} difference represented individual changes we used patients as their own controls. The mean ratio of FEV\textsubscript{1} was 0.92 vs 0.77 in controls vs cases respectively between pre-9/11 and SPE testing, p<0.001, demonstrating a significantly greater loss of lung function in cases as compared to controls, even when analyzed individually.

CT Scan Measurements

PA and A measurements on available CT’s from cases and controls are outlined in Table 1. The PA diameter was modestly correlated with BSA and BMI (r = 0.219, 0.265 p = 0.031, 0.009 respectively), but did not vary with height or age. The A diameter demonstrated modest
correlation with age and BMI ($r = 0.376, 0.284 \ p = \ <0.001, 0.005$ respectively). The measured PA/A declined with increased age ($r = -0.256, \ p = 0.011$) but was not significantly associated with height, BSA or BMI, which is similar to reported associations in a larger cohort study. The mean PA/A in cases was 0.92, similar to the 90th% upper limit of normal in the Framingham Heart Study. Cases had similar proportions of AT and BWT to controls.

**PA/A as a biomarker of WTC-LI**

After calculating the Youden index, a PA/A value of 0.92 was selected as the cutoff for predicting the development of WTC lung injury in a logistic regression analysis. After adjusting for age at CT, pre-9/11 FEV$_1$, BMI at SPE and exposure, the odds of having a low FEV$_1$ at SPE in patients with a value of PA/A $\geq 0.92$ was 4.02 (95% CI: 1.21-13.41, $p = 0.023$) times larger than the odds of those patients with a value $<0.92$ (AUC = 0.854 (95% CI: 0.773-0.934), Hosmer-Lemeshow’s $p=0.55$).

**Vascular Disease Biomarkers and PA/A Association**

CT measurements across the PA/A of 0.92 are displayed in Supplemental Table 1. There were 38 individuals with a ratio $\geq 0.92$ and 59 with a ratio $<0.92$. There was a higher proportion of measured BWT in the low ratio group. There was a similar amount of AT in both groups. Age and time since 9/11 were similar in both groups. Height, age, BSA, BMI and exposure intensity were similar across the PA/A ratio of 0.92 (data not shown).

Levels of analytes across the ratio of 0.92 are displayed in Table 2. The results of multivariable logistic analysis using backward stepwise approach are shown in Table 3. For each 1 ng/ml increased of serum MDC, the odds of have a subsequent PA/A ratio $\geq 0.92$ increased by 2.08 fold (95%CI: 1.05, 4.11). For each 10ng/ml increase in soluble endothelial selectin (sE-selectin)
and total plasminogen activator inhibitor 1 (tPAI-1), the odds of having a PA/A ratio \( \geq 0.92 \) increased by 33% (95%CI: 6%, 68%) and decreased by 12% (95%CI: 2%, 21%) respectively.

The probability of having PA/A ratio \( \geq 0.92 \) was determined for each of our three analytes when holding all other variables in the model constant, Figure 2. The probability increased from 0.21 to 0.79 as the concentration of MDC increased from 0.20 ng/ml to 4.41 ng/ml. (Figure 2A) The probability of an increased PA/A increased from 0.14 to 0.75 as the concentration of sE-selectin increased from 12.7 ng/ml to 125.4 ng/ml (Figure 2B). Increase in the concentration of tPAI-1 from 41.4 ng/ml to 288.9 ng/ml decreased the probability of having an increased PA/A ratio from 0.63 to 0.11 (Figure 2C). When using biomarkers to predict WTC-LI, MDC remains significant with an OR of 2.1, data not shown. However, sE-selectin and TPAI-I were not significant predictors of WTC-LI.
DISCUSSION

We have identified biomarkers of cardiovascular risk, inflammation and metabolic syndrome expressed within 6 months of 9/11/2001 predict the development of WTC-LI. Vascular changes occur early in COPD, prior to development of WTC-LI. In a recently published analysis of the Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE)/COPDGene cohorts, in patients with moderate to severe disease, a PA/A > 1 was significantly associated with COPD exacerbations. A unique advantage to observing the PA/A ratio by non-contrast CT is that it is a non-invasive mechanism that provides insight into the pulmonary and systemic circulation.

In this nested case control study of our well-phenotyped WTC exposed FDNY cohort, we found that an elevated PA/A ≥ 0.92 was associated with an observed development of WTC-LI, with odds of 4.02. Early levels of MDC, sE-selectin and tPAI-1 showed ability to predict PA/A ≥ 0.92.

Recently, CT measurements of the PA/A have been associated with outcomes other than pulmonary hypertension. The ECLIPSE/COPDGene cohort examined patients with advanced disease, many requiring supplemental O₂. Although our cohort’s mean PA and A were similar to those measured in ECLIPSE/COPDGene, 81% of the cohort did not meet GOLD COPD criteria. Therefore, it was expected that the PA/A would be less than previously reported ratios of 1. When comparing our cohort to the Framingham Heart Study, our case mean PA/A and PA values are similar to their 90th% upper limit of normal. Although we found a similar inverse relationship with PA/A and age, there were only weak associations with height and BSA.

In a very recent study, an abnormal PA/A has been associated with increased mortality in patients with coronary artery disease. Our study would indicate that abnormalities in the PA/A may
represent a marker of early vascular injury in particulate matter related lung disease and is in line with these recent publications.

Bronchial wall thickening was also found to be more prevalent in those with PA/A ≥ 0.92. Our previous work has linked this indicator of proximal airway inflammation and/or remodeling, with WTC-LI. Our new finding may show that bronchial wall thickening is related to early vascular changes. However, it is unclear what the temporal relationship between bronchial wall thickening and the vascular changes is.

Our group and others have linked biomarkers of systemic inflammation and vascular disease with COPD. The proposed pathobiologic mechanism of this relationship is related to inflammatory loss of lung parenchyma and vascular beds. COPD is systemic process affecting not only the airways but also affecting lung parenchyma, vascular structures and other organ systems such as skeletal muscle and adipose tissue. Non-invasive biomarkers guiding management and prognostication in COPD are needed.

We chose to study a limited number of biologically plausible vasoactive biomarkers, to examine the link between their expression in a serum soon after massive particulate matter exposure and PA/A. MDC, also known as CCL22, is an inflammatory mediator that has been linked to obstructive lung dysfunction by our lab and others. It is important in platelet activation and has been associated with systemic vascular phenomenon. sE-selectin is a member of the selectin family of carbohydrate binding lectins, and is specifically produced by activated endothelial cells. It is one of the main endothelial neutrophil adhesion molecules and has been linked to poor CVD outcomes. Additionally, well-known metabolic risk factors for CVD are associated with increase in the soluble form sE-selectin. TPAI-1, the main inhibitor of plasminogen activator, has been associated with lung diseases, atherosclerosis, thrombosis, and vascular remodeling. It has been analyzed in pulmonary hypertension and right heart
failure, although whether it is inhibitory or stimulatory in these disease states has yet to be elucidated and is under investigation.\textsuperscript{33, 34}

In our population, we found that elevated levels of MDC, sE-selectin were significantly associated with an increased ratio PA/A ratio. TPAI-1 was inversely related to this ratio. These associations correlate with previous reports on cardiovascular disease. This association is novel because of it is the first time these biomarkers have been associated with a non-invasive marker of vascular injury in lung disease. However, in using the biomarkers to predict WTC-LI, only MDC remained a predictive biomarker. This may indicate that sE-Selectin and TPA-I may be involved with the vascular remodeling after exposure, but may not be directly related to the lung damage seen in those with WTC-LI. The population size may also have limited the ability to find significant differences in the two groups.

There are several limitations to this study. Understandably, this cohort of FDNY firefighters did not have pre-exposure serum banked for future analyses. We did achieve the next best option by obtaining serum samples within a few months post-exposure. There were no unexposed or asymptomatic exposed controls in our study. Replication of these findings in other populations with and without particulate matter exposure will be important. We were only able to obtain CT images on 97 subjects with available serum. We do not have longitudinal follow-up data (FEV\textsubscript{1}, CTs, serum biomarkers) for additional correlation. Finally, we have no data on the prevalence of important co-morbidities such as sleep apnea, left heart failure and thromboembolic disease in this population. Thus, the finding linking an elevated PA/A to a decreased FEV\textsubscript{1} may be influenced by the presence of these unaccounted for confounders.

In this nested case-cohort study, we were able to identify cardiovascular related serum biomarkers drawn within six months of 9/11/01 that predicted an abnormal PA/A ratio. The observation that biomarkers predict changes in PA/A and that PA/A was a non-invasive marker
lung injury (FEV\textsubscript{1} loss) in this post-exposure population, provides a novel association to well-characterized processes in vascular biology and inflammation secondary to particulate matter exposure. Importantly, these biomarkers were expressed during the early stages of WTC lung injury and reflect potential processes leading to disease susceptibility. This insight on protein expression and its relationship to FEV\textsubscript{1} loss and vascular injury may guide future mechanistic and therapeutic studies in the field.
Acknowledgments

The authors would like to thank the firefighters and rescue workers for their participation in this study and for their selfless contributions.

Funding Sources:

This work was supported by NIH-NHLBI K23HL084191 (AN), NIAID K24A1080298 (MDW), NIH-R01HL057879 (MDW), and NIOSH (U10-OH008243, U10-OH008242) and UL1RR029893 (DJP). This work was also partially funded by the NYU-HHC CTSI UL1TR000038 from the National Center for Advancing Translational Sciences of the National Institutes of Health. The funding agencies did not participate in the study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

Contributorship: AN, MDW, EJS, DJP participated in study conception and design; AN, EJS were the primary investigators; EJS, AN, SK, AC were responsible for data collection; AN, EJS, FGG, SK were responsible for data validation; AN, EJS, SK participated in data analysis; AN, GCE, EJS undertook the statistical analysis. All authors participated in data interpretation, writing and revision of the report and approval of the final version.

Competing interests: The authors report no financial or competing interests.

Data Sharing Statement: No additional data available.

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REFERENCES


Table 1: Demographics, CT and Pulmonary Function Test Data

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<tr>
<th>Event</th>
<th>Measure</th>
<th>Cases</th>
<th>Controls</th>
<th>N</th>
<th>Controls</th>
<th>N</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td>Pre 9/11</td>
<td>FVC, %</td>
<td>84 (80-94)</td>
<td>34</td>
<td>97 (89-111)</td>
<td>63</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FEV₁, %</td>
<td>86 (82-95)</td>
<td>34</td>
<td>104 (93-114)</td>
<td>63</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FEV₁/FVC</td>
<td>84 (79-87)</td>
<td>34</td>
<td>85 (82-89)</td>
<td>63</td>
<td>0.960</td>
<td></td>
</tr>
<tr>
<td>At 9/11</td>
<td>Present atCollapse*</td>
<td>12 (35)</td>
<td>34</td>
<td>18 (29)</td>
<td>63</td>
<td>0.494</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Arrived Later*</td>
<td>22 (65)</td>
<td>34</td>
<td>45 (71)</td>
<td>63</td>
<td>0.494</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Years of Service</td>
<td>15 (7-20)</td>
<td>34</td>
<td>13 (6-18)</td>
<td>63</td>
<td>0.317</td>
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<td>SPE</td>
<td>9/11 to SPE, Months</td>
<td>34 (25-52)</td>
<td>34</td>
<td>33 (24-57)</td>
<td>63</td>
<td>0.928</td>
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<tr>
<td></td>
<td>FVC, %</td>
<td>76 (72-86)</td>
<td>34</td>
<td>96 (90-104)</td>
<td>63</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FEV₁, %</td>
<td>72 (64-74)</td>
<td>34</td>
<td>96 (90-102)</td>
<td>63</td>
<td>&lt;0.001</td>
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<tr>
<td></td>
<td>FEV₁/FVC</td>
<td>74 (65-78)</td>
<td>34</td>
<td>78 (75-82)</td>
<td>63</td>
<td>0.004</td>
<td></td>
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<tr>
<td></td>
<td>BD Response</td>
<td>15 (6-25)</td>
<td>30</td>
<td>4 (1-9)</td>
<td>33</td>
<td>0.001</td>
<td></td>
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<tr>
<td></td>
<td>TLC, %</td>
<td>86 (80-101)</td>
<td>28</td>
<td>105 (98-112)</td>
<td>32</td>
<td>&lt;0.001</td>
<td></td>
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<tr>
<td></td>
<td>FRC , %</td>
<td>84 (76-100)</td>
<td>28</td>
<td>102 (91-109)</td>
<td>32</td>
<td>0.002</td>
<td></td>
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<tr>
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<td>RV, %</td>
<td>130 (107-145)</td>
<td>28</td>
<td>129 (115-141)</td>
<td>32</td>
<td>0.859</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DLco, %</td>
<td>95 (85-106)</td>
<td>27</td>
<td>106 (99-113)</td>
<td>31</td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BMI, kg/m²</td>
<td>31 (29-34)</td>
<td>34</td>
<td>29 (27-31)</td>
<td>63</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>PA †</td>
<td>29.22 (3.24)</td>
<td>34</td>
<td>28.19 (3.23)</td>
<td>63</td>
<td>0.138</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A †</td>
<td>32.09 (3.68)</td>
<td>34</td>
<td>31.95 (3.19)</td>
<td>63</td>
<td>0.850</td>
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<tr>
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<td>PA/A †</td>
<td>0.92 (0.11)</td>
<td>34</td>
<td>0.89 (0.10)</td>
<td>63</td>
<td>0.151</td>
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<td>PA/A≥0.92 †</td>
<td>18 (53)</td>
<td>34</td>
<td>20 (32)</td>
<td>63</td>
<td>0.042</td>
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<tr>
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<td>BWT*</td>
<td>12 (36)</td>
<td>33</td>
<td>21 (34)</td>
<td>61</td>
<td>0.851</td>
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</tr>
<tr>
<td></td>
<td>Air Trapping*</td>
<td>19 (58)</td>
<td>33</td>
<td>25 (41)</td>
<td>61</td>
<td>0.124</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>47 (41-51)</td>
<td>34</td>
<td>46 (42-51)</td>
<td>63</td>
<td>0.791</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Height, cm</td>
<td>182 (178-183)</td>
<td>34</td>
<td>178 (173-183)</td>
<td>63</td>
<td>0.041</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BSA, m²</td>
<td>2.24 (2.17-2.43)</td>
<td>34</td>
<td>2.09 (2.01-2.28)</td>
<td>63</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: SPE Pulmonary Evaluation; BSA: Body Surface Area; BD Bronchodilator; DLco: Diffusion Capacity of the Lung for Carbon Monoxide. PA = Pulmonary Artery, A = aorta, BWT = Bronchial Wall Thickening
Expressed as Median (IQR) except *Expressed as N (%), †Expressed as Mean (SD)
Table 2: Biomarker PA/A relationship

<table>
<thead>
<tr>
<th>Analyte</th>
<th>PA/A</th>
<th>p</th>
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<tbody>
<tr>
<td></td>
<td>≥0.92</td>
<td>&lt;0.92</td>
</tr>
<tr>
<td>ng/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDC</td>
<td>1.51 (1.21-2.01)</td>
<td>1.41 (0.99-1.77)</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>12770 (9510-20941)</td>
<td>13218 (10037-18537)</td>
</tr>
<tr>
<td>sE-Selectin</td>
<td>49.7 (36.5-63.2)</td>
<td>42.8 (35.3-56.1)</td>
</tr>
<tr>
<td>tPAI-1</td>
<td>119.8 (84.5-152.4)</td>
<td>139.7 (119.5-173.3)</td>
</tr>
<tr>
<td>MMP-9</td>
<td>345.7 (265.8-465.3)</td>
<td>352.4 (267.9-517.9)</td>
</tr>
<tr>
<td>MPO</td>
<td>141.0 (104.3-226.1)</td>
<td>135.3 (95.1-289.0)</td>
</tr>
<tr>
<td>sICAM-1</td>
<td>165.2 (130.4-197.4)</td>
<td>157.8 (136.1-201.8)</td>
</tr>
<tr>
<td>sVCAM-1</td>
<td>1355 (1119-1679)</td>
<td>1335 (1108-1602)</td>
</tr>
</tbody>
</table>

Abbreviations: MDC Macrophage Derived Chemokine; sE-Selectin Soluble Endothelial Selectin; tPAI Total Plasminogen Activator Inhibitor; MMP Matrix Metaloproteinase; MPO Myeloperoxidase; sICAM Soluble Intercellular Adhesion Molecule; sVCAM Soluble Vascular Cell Adhesion Molecule

Values expressed as median (IQR)
Figure Legends

**Figure 1. Study Design.** Subjects in the FDNY-WTC health program presented for pulmonary evaluation (SPE). Baseline Cohort met the listed inclusion criteria. Cases (N=34) and Controls (N=63) had CT and biomarkers available.

**Figure 2. Probability Plots.** Probability of having PA/A ratio ≥0.92 over the range of MDC (A), sE-Selectin (B) and tPAI-1 (C) are represented when adjusting for the covariates of exposure, and age. Plots express probability isopleths for the development of WTC lung injury (FEV₁ loss) with all other covariates held constant.

**Supplemental Figure 1. PA/A Measurement.** Inspiratory series were evaluated at the level of the main pulmonary artery bifurcation. The main pulmonary artery trunk was measured perpendicular to its axial longitudinal axis (red) and the ascending thoracic aorta was measured in its widest dimension (yellow).
Table 3. Serum Biomarker Models Predicting PA/A ≥ 0.92

<table>
<thead>
<tr>
<th>Model</th>
<th>Analytes</th>
<th>Crude OR(95%CI)</th>
<th>p</th>
<th>Adjusted* OR(95%CI)</th>
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<tbody>
<tr>
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<tr>
<td>Uni-Variable</td>
<td>MDC</td>
<td>1.70 (0.90-3.19)</td>
<td>0.100</td>
<td>1.78 (0.93-3.37)</td>
<td>0.080</td>
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<tr>
<td></td>
<td>sE-Selectin</td>
<td>1.18 (0.97-1.44)</td>
<td>0.093</td>
<td>1.20 (0.98-1.47)</td>
<td>0.075</td>
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<tr>
<td></td>
<td>tPAI-1</td>
<td>0.92 (0.84-1.01)</td>
<td>0.093</td>
<td>0.93 (0.85-1.03)</td>
<td>0.148</td>
</tr>
<tr>
<td>Multi-Variable</td>
<td>MDC</td>
<td>1.99 (1.02-3.88)</td>
<td>0.043</td>
<td>2.08 (1.05-4.11)</td>
<td>0.036</td>
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<tr>
<td></td>
<td>sE-Selectin</td>
<td>1.32 (1.05-1.65)</td>
<td>0.018</td>
<td>1.33 (1.06-1.68)</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>tPAI-1</td>
<td>0.87 (0.78-0.97)</td>
<td>0.013</td>
<td>0.88 (0.79-0.98)</td>
<td>0.024</td>
</tr>
</tbody>
</table>

Abbreviations: MDC Macrophage Derived Chemokine; sE-Selectin Soluable Endothelial Selectin; tPAI Total Plasminogen Activator Inhibitor
*Adjusted for age at CT and Exposure Group
Per 1 ng/ml MDC, Per 10 ng/ml sE-Selectin, tPAI-1
X2 (5) = 15.69, p = 0.008. Hosmer and Lemeshow’s goodness-of-fit test P=0.25.
Area Under ROC curve=0.728 (0.623-0.834)
Enlarged Pulmonary Artery is Predicted by Vascular Injury Biomarkers and is Associated with an Abnormal FEV\textsubscript{1}, WTC-Lung Injury in WTC-Exposed Fire Fighters:

A Case-Control Study

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Author Contributions: AN, MDW, EJS, DJP participated in study conception and design; AN, EJS were the primary investigators; EJS, AN, SK, AC were responsible for data collection; AN, EJS, FGG, SK were responsible for data validation; AN, EJS, SK participated in data analysis; AN, GCE, EJS undertook the statistical analysis. All authors participated in data interpretation, writing and revision of the report and approval of the final version.

Running Title: CT and Serum Biomarkers of Vascular Injury in Obstruction

Main Text Word Count: 27052942

Key Words: Particulate Matter, Vascular Disease, COPD, World Trade Center
ABSTRACT

RATIONALE: Vascular injury is an early manifestation of Obstructive lung disease. Increased pulmonary artery to aorta (PA/A) on computed tomography (CT) predicts exacerbations of COPD.

Objectives: We hypothesize that there is an association between an elevated PA/A and an FEV₁ less than the lower limit of normal (LLN) World Trade Center Lung Injury (WTC-LI) in WTC (World Trade Center)-exposed firefighters. We also assessed if serum vascular disease biomarkers of vascular disease drawn within 6 months of exposure were predictive of an elevated PA/A.

Design: Retrospective case-cohort analysis of thoracic CT-scans of WTC-exposed firefighters who were symptomatic between 9/12/2001 and 3/10/2008. Quantification of vascular-associated biomarkers from serum collected within 200 days of exposure.

Setting: Urban tertiary care center and occupational health care center.

Participants: Male never-smoking firefighters with accurate pre-9/11 FEV₁≥75%, serum sampled ≤200 days of exposure was the baseline cohort (n=801). Subcohort (n=97) with available CT scan and serum biomarkers were identified. WTC-LI was defined as FEV₁≤77% at subspecialty pulmonary evaluation (n=34) and compared to controls (n=63) to determine associated PA/A ratio. The subcohort was re-stratified based on PA/A≥0.92 (n=38) and PA/A<0.92 (n=59) to determine serum vascular biomarkers that were predictive of this vasculopathy.

Outcome Measures: Primary outcome of this study was to identify a PA/A ratio in a cohort of individuals exposed to WTC dust that was associated with WTC-LI. Secondary outcome was to identify serum biomarkers predictive of the PA/A ratio using logistic regression.

Results: PA/A≥0.92 was associated with WTC-LI, odds ratio (OR) of 4.02 (95%CI 1.21-13.41; p=0.023) when adjusted for exposure, BMI, and age at CT. Elevated MDC and sE-selectin were predictive of PA/A≥0.92, OR,95%CI: 2.08, 1.05-4.11, p=0.036; 1.33, 1.06-1.68, p=0.016; respectively while increased tPAI-1 was predictive of not having PA/A≥0.92 (OR 0.88, 0.79-0.98; p=0.024).

METHODS: Cases (FEV₁<LLN) and controls (FEV₁≥LLN) were selected based on post-9/11/2001 FEV₁ measured at symptomatic presentation. Diameter of the main PA at its bifurcation and the ascending aorta at the same level on inspiration were measured. Serum sampled with 6 months of 9/11/2001 was assayed for biomarkers.

Measurements and Main Results: The odds ratio (OR) of FEV₁<LLN if the PA/A was ≥0.92 was 4.02 (95%CI 1.21-13.41; p=0.023) when adjusted for exposure, BMI, age at CT and baseline FEV₁. Using a PA/A of ≥0.92 as a dichotomous outcome in logistic regression, elevated MDC and sE-selectin were associated with an increased OR (2.08, 1.05-4.11; p=0.036; 1.33, 1.06-1.68, p=0.016; respectively) adjusted for age and exposure, while increased tPAI-1 was associated with decreased odds of an elevated PA/A (OR 0.88, 0.79-0.98; p=0.024).

CONCLUSIONS: Elevated PA/A was associated with WTC-related decline in FEV₁.
FEV₁/FVC Development of an elevated PA/A was predicted by biomarkers of vascular disease found in serum drawn within 6 months of WTC exposure. Increased PA/A is a potentially useful non-invasive biomarker of obstructive lung dysfunction and warrants further study.
Article Summary
Strengths and Limitations of this Study
- Well-phenotyped cohort with lung function tests prior to exposure available
- Identification of a viable PA/A ratio (0.92) as a biomarker associated with WTC-LI
- Identification of biomarkers predictive of PA/A vasculopathy
- Limited generalizability because of unique WTC-related exposure
- Retrospective study design and logistic regression implies only associated findings, and does not reflect causality.

What does this study adds
Particulate matter exposure such as what occurred as a result of the World Trade Center (WTC) Disaster and more commonly biomass exposure is one of the main causes of obstructive lung dysfunction. Vascular injury is seen in very early obstructive lung disease. The pulmonary artery to aorta ratio (PA/A) is a noninvasive marker of poor prognosis in advanced obstructive lung disease and may be a manifestation of the vascular injury. An elevated PA/A was associated with developing an FEV1 less than the lower limit of normal in symptomatic WTC-exposed firefighters. This suggests that vascular injury may occur early in WTC Lung Injury. To further investigate this relationship we demonstrated that serum biomarkers of vascular injury drawn soon after exposure predicted a future abnormal PA/A. This unique relationship provides insight into potential vasculopathic mechanisms of particulate matter related obstructive lung disease.
BACKGROUND

Development of abnormal spirometry due to lung disease after World Trade Center (WTC) exposure has been a common finding among exposed workers, volunteers, and lower Manhattan residents. In rescue/recovery workers from the Fire Department of the City of New York (FDNY), WTC exposure led to WTC lung injury (WTC-LI). Our group has previously defined WTC-LI as the chronic inflammatory lung dysfunction experienced by a subcohort of firefighters with intense exposure to WTC dust (Weidner & Al, 2013). It is characterized by primarily obstructive respiratory dysfunction with substantial and persistent losses in FEV₁% predicted to ≤77% in the subsequent 6.5 years post-exposure. In addition, systemic biomarkers of inflammation, metabolic derangement and cardiovascular disease predict WTC-LI.

WTC exposure led to WTC lung injury (WTC-LI) as evidenced by substantial declines in pulmonary function in the first six months after 9/11 that persisted over the next 6.5 years. Our group has previously shown that systemic biomarkers of inflammation, metabolic derangement and cardiovascular disease predict this decline.

One of the hallmarks of particulate matter exposure is systemic inflammation, endothelial dysfunction, and subsequent end-organ damage. High ambient particulate matter exposures significantly decrease FEV₁, in as soon as five to seven days. Epidemiologic investigation has documented associations between increased ambient particulates, lung disease, and cardiovascular disease (CVD). Systemic inflammation produces vascular endothelial injury and subsequent vascular disease. Recent studies associate systemic vascular involvement with lung disease and prospective studies have demonstrated an association between impaired lung function and central arterial stiffness even before the development of frank vascular disease, with systemic inflammation contributing to this association.
Pulmonary vascular injury occurs early in smoking related chronic obstructive lung disease (COPD) with pulmonary perfusion abnormalities and reduced blood return to the heart is observed prior to development of abnormal FeV1 WTC-LI. A similar pathophysiology likely occurs in irritant induced lung disease. Pulmonary arteriopathy was present in 58% of lung biopsies from non-FDNY WTC exposed individuals and in 74% with constrictive bronchiolitis after inhalational exposures during military service in Iraq and Afghanistan. An increased ratio of the pulmonary artery to aorta (PA/A) diameter measured by computed tomography (CT), has been associated with pulmonary hypertension and poor outcomes in various disease states. Additionally, an elevated PA/A has been associated with past and future exacerbations in patients with moderate-to-severe COPD. The PA/A has been associated with a decreased FEV1 in the same population. Multiple serum biomarkers have been identified that predict vascular disease and several have been incorporated into clinical practice. To date, there have been no serum biomarkers identified that predict an enlarged PA/A in obstructive lung disease.

Utilizing a nested case-control design, we investigated if an elevated PA/A was associated with WTC-LI lung injury in a population that also had serum biomarkers. We then determined the relationship between the ability of vasoactive serum biomarkers drawn within six months of 9/11/01 to predict and the eventual development of an elevated PA/A.
METHODS

Study Design

Annual physicals occurred prior to 9/11/2001, and active-duty firefighters had normal lung function testing, EKG assessment and measures of exercise capacity. Those with abnormal cardiopulmonary testing were placed on medical leave and were not part of the rescue and recovery efforts.

WTC exposed FDNY firefighters (N=1720) entered the FDNY-WTC Health Program and had spirometry at entry into medical monitoring as previously described. Symptomatic subjects referred for subspecialty pulmonary evaluation (Pulm-EvalSPE) between 10/1/2001 and 3/10/2008, underwent specialized pulmonary function testing as previously described. Inclusion criteria were applied to the symptomatic cohort. The baseline cohort N=801 consisted of: N=801/1720 (47%) were never-smokers, male, had reliable NHANES normative data for FEV₁% predicted, entered FDNY-WTC Health Program within 200 days of 9/11/01 and had pre-9/11 FEV₁>75% predicted. All subjects signed informed Institutional Review Board approved consent at the time of enrollment allowing analysis of their information and samples for research (Montefiore Medical Center; #07-09-320 and New York University; #11-00439).

FEV₁% predicted at SPE was used as a measurable, phenotypic marker of WTC-LI. Cases represented those who had continued lung dysfunction that we termed WTC-LI, whereas controls represented those who did not have WTC-LI. Cases (N=100) were defined as being within one SD of the lowest FEV₁% predicted of the cohort at SPE (FEV₁≤77%). Cases and controls were defined by their post-9/11 FEV₁% Predicted measured at Pulm-Eval using NHANES III criteria. Cases, those susceptible to WTC lung injury, were defined as being within
one SD of the lowest FEV₁ % predicted of the cohort (N=100), an FEV₁ <77%. Controls (N=153) were those who had FEV₁ >77% and were randomly selected after stratification based on body mass index (BMI) and FEV₁ as previously defined. ¹ had an FEV₁ >77% predicted and were randomly selected from the study cohort after stratification based on body mass index (BMI) and FEV₁ as previously defined.

CT scans were administered as standard of care measures to the population, but only a subset was available at the single center where this research was conducted. Serum biomarkers and CT scans were available for N=34/100 cases and N=63/153 controls, Figure 1. The population was then re-stratified by PA/A ratio using a cutoff of 0.92 for analysis of biomarkers predictive of PA/A.

Demographics
Age, gender, and years of service at FDNY were obtained from the FDNY-WTC Health Program database. Degree of exposure was self-reported at the first FDNY-WTC monitoring exam and was categorized using the FDNY-WTC Exposure Intensity Index (Arrival Time): Present on the morning of 9/11/2001 or Arrived after noon on 9/11/2001.¹ Those arriving after day three were excluded from analysis as a result of their low numbers in this sample. Height and weight was measured at the Pulm-EvalSPE.

Lung Function Measures and Computed Tomography
Pulmonary function testing was performed according to ATS/ERS guidelines as described. The first available post-9/11 CT was retrieved for the 34 cases and 63 controls. Contrast studies and CT scans obtained after 2009 were not included. Bronchial wall thickening (BWT) and air trapping (AT) were previously assessed.¹ Inspiratory images, collected with a BF40 algorithm and viewed on standard mediastinal windows were reviewed using iSite PACS.
The diameter of the main pulmonary artery (PA) at the level of its bifurcation and the diameter of the ascending aorta (A) in its maximum dimension were recorded using the same image. Supplementary Figure 1. The reader, trained by a board certified radiologist in this method, was blinded to case status.13 14

Serum Sampling and Analysis

Fasting blood was drawn at the first post-9/11 FDNY-WTC monitoring exam, processed and stored (Bio-Reference Laboratories, Inc. Elmwood Park, NJ) as previously described.

Serum was analyzed using a CVD-1(HCVD1-67AK) and a 39-Plex Human Pro-inflammatory Panel according to manufacturer’s instructions (Millipore, Billerica, MA) on a Luminex 200IS (Luminex Corporation, Austin, TX).

Serum was analyzed using a CVD-1(HCVD1-67AK) panel and a previous analyte macrophage derived chemokine (MDC) found associated with an abnormal FEV1 from a 39-Plex Human Pro-inflammatory Panel according to manufacturer’s instructions (Millipore, Billerica, MA) on a Luminex 200IS (Luminex Corporation, Austin, TX). Data analyzed with MasterPlex QT software (Version 1.2; MiraiBio, Inc.). Each batch of samples processed contained controls and cases in an approximate 12/7 ratio as previously described.4

Statistical Analysis

SPSS 20 (IBM, Armonk, NY) and STATA 12.1 (StataCorp LP, College Station, Texas) were used for database management and statistics. Demographics, CT data and analyte levels were compared by Student’s T-test, Mann-Whitney U and Chi-squared test where appropriate. The cutoff point of the PA/A ratio that maximized the value of the Youden index (Sensitivity +
Specificity R1) was used for predicting the development of WTC-ILI lung injury. Logistic regression was used to first see if the PA/A ratio was a marker of WTC-ILI. Then, a separate logistic regression was used to analyze if serum biomarkers could predict PA/A ratio. The relationships between the CT derived measurements, abnormal FEV1, and serum biomarkers.

Variables identified as potential confounding factors in previous studies and those with a P value <0.20 in the univariable analysis were included in the multivariable logistic regression model. A backward stepwise approach was used to determine the most parsimonious model for the serum biomarkers, with a pre-specified p-value <0.10. The Hosmer-Lemeshow goodness-of-fit test was used to assess calibration of the model. The model discrimination was evaluated through the receiver operating characteristic area under the curve (AUC). To test the robustness of the serum biomarker models, internal validation was performed using bootstrapping (10,000 bootstrap samples). Data are expressed as mean (standard deviation, SD), median (interquartile range, IQR) or Odds Ratio (95% confidence interval), unless otherwise stated. A two-sided p value less than 0.05 was considered significant.
RESULTS

Demographics of Case-Control Study

This case control study was drawn from a population of 801 never-smokers with normal pre-9/11 lung function. 253/801 individuals had serum available from their first post-9/11 monitoring exam. Non-contrast chest CT was available for review from 97 subjects (34/100 cases and 63/153 controls). The demographics of individuals with available serum and CT’s are summarized in Table 1. The control and case groups had similar WTC exposure; time from 9/11 to first post-9/11 monitoring exam, time from 9/11 to Pulm-EvalSPE, years of service, and age at 9/11. BMI, body surface area (BSA) and height of cases were higher than controls at Pulm-EvalSPE.

Lung Function

Cases had a lower FEV₁ than controls at pre-9/11, and at Pulm-EvalSPE; Table 1. Cases also had a lower FEV₁/FVC ratio, TLC, FRC and DLCO at Pulm-EvalSPE. Cases had an increased bronchodilator response. At pre-9/11 testing the FEV₁/FVC ratio was similar. FEV₁ in cases and controls declined from pre-9/11 to Pulm-EvalSPE (104% to 96% and 86% to 72 % respectively; p<0.001 all comparisons). FVC demonstrated a similar pattern. To confirm that the median FEV₁ difference represented individual changes we used patients as their own controls. The mean ratio of FEV₁ was 0.92 vs 0.77 in controls vs cases respectively between pre-9/11 and Pulm-EvalSPE testing, p<0.001, demonstrating a significantly greater loss of lung function in cases as compared to controls, even when analyzed individually.

CT Scan Measurements

PA and A measurements on available CT’s from cases and controls are outlined in Table 1. The PA diameter was modestly correlated with BSA and BMI (r = 0.219, 0.265 p = 0.031, 0.009
respectively), but did not vary with height or age. The A diameter demonstrated modest correlation with age and BMI ($r = 0.376, 0.284 \ p = <0.001, 0.005$ respectively). The measured PA/A declined with increased age ($r = -0.256, \ p = 0.011$) but was not significantly associated with height, BSA or BMI, which is similar to reported associations in a larger cohort study. The mean PA/A in cases was 0.92, similar to the 90th% upper limit of normal in the Framingham Heart Study. Cases had similar proportions of AT and BWT to controls.

**PA/A as a biomarker of WTC-LI Abnormal FEV**

After calculating the Youden index, a PA/A value of 0.92 was selected as the cutoff for predicting the development of WTC lung injury in a logistic regression analysis. After adjusting for age at CT, pre-9/11 FEV1, BMI at Pulm-EvalSPE and exposure, the odds of having a low FEV1 at Pulm-EvalSPE in patients with a value of PA/A ≥0.92 was 4.02 (95% CI: 1.21-13.41, $p = 0.023$) times larger than the odds of those patients with a value <0.92 (AUC = 0.854 (95% CI: 0.773-0.934), Hosmer-Lemeshow’s $p=0.55$).

**Vascular Disease Biomarkers and PA/A Association**

CT measurements across the PA/A of 0.92 are displayed in Supplemental Table 1. There were 38 individuals with a ratio ≥0.92 and 59 with a ratio <0.92. There was a higher proportion of measured BWT in the low ratio group. There was a similar amount of AT in both groups. Age and time since 9/11 were similar in both groups. Height, age, BSA, BMI and exposure intensity were similar across the PA/A ratio of 0.92 (data not shown).

Levels of analytes across the ratio of 0.92 are displayed in Table 2. The results of multivariable logistic analysis using backward stepwise approach are shown in Table 3. For each 1 ng/ml increased of serum MDC, the odds of have a subsequent PA/A ratio ≥0.92 increased by 2.08 fold (95%CI: 1.05, 4.11). For each 10ng/ml increase in soluble endothelial selectin (sE-selectin)
and total plasminogen activator inhibitor 1 (tPAI-1), the odds of having a PA/A ratio \( \geq 0.92 \) increased by 33\% (95\%CI: 6\%, 68\%) and decreased by 12\% (95\%CI: 2\%, 21\%) respectively.

The probability of having PA/A ratio \( \geq 0.92 \) was determined for each of our three analytes when holding all other variables in the model constant, Figure 2. The probability increased from 0.21 to 0.79 as the concentration of MDC increased from 0.20 ng/ml to 4.41 ng/ml. (Figure 2A) The probability of an increased PA/A increased from 0.14 to 0.75 as the concentration of sE-selectin increased from 12.7 ng/ml to 125.4 ng/ml (Figure 2B). Increase in the concentration of tPAI-1 from 41.4 ng/ml to 288.9 ng/ml decreased the probability of having an increased PA/A ratio from 0.63 to 0.11 (Figure 2C).

When using biomarkers to predict WTC-LI, MDC remains significant with an OR of 2.1, data not shown. However, sE-selectin and TPAI-1 were not significant predictors of WTC-LI.
DISCUSSION

We have identified biomarkers of cardiovascular risk, inflammation and metabolic syndrome expressed within 6 months of 9/11/2001 predict the development of WTC-LI. Vascular changes occur early in COPD, prior to development of an abnormal FEV$_1$. In a recently published analysis of the Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE)/COPDGene cohorts, in patients with moderate to severe disease, a PA/A $>1$ was significantly associated with COPD exacerbations. A unique advantage to observing the PA/A ratio by non-contrast CT is that it is a non-invasive mechanism that provides insight into the pulmonary and systemic circulation.

In this nested case control study of our well-phenotyped WTC exposed FDNY cohort, we found that an elevated PA/A $\geq 0.92$ was associated with an observed development of WTC-LI, with odds of 4.02. Early levels of MDC, sE-selectin and tPAI-1 showed predictability to predict PA/A $\geq 0.92$ and an abnormal PA/A ratio measured years later. This elevated PA/A ratio was also associated with a contemporaneously observed decline in FEV$_1$. Combining these three biomarkers together in a multivariable model adjusted for confounders strengthened the former results. A unique advantage to observing the PA/A ratio by non-contrast CT is that it is a non-invasive mechanism that provides insight into the pulmonary and systemic circulation.

Recently, CT measurements of the PA/A have been associated with outcomes other than pulmonary hypertension. The ECLIPSE/COPDGene cohort examined patients with advanced disease, many requiring supplemental O$_2$. Although our cohort’s mean PA and A were similar to those measured in ECLIPSE/COPDGene, 81% of the cohort did not meet GOLD COPD criteria. Therefore, it was expected that the PA/A would be less than previously reported ratios of 1. The ECLIPSE/COPDGene cohort examined patients with advanced disease, many
requiring supplemental O\textsubscript{2}. Our cohort’s mean PA and A were similar to those measured in ECLIPSE/COPDGene. Since our population had less advanced COPD, mostly GOLD 0, it was expected that the PA/A would be less abnormal. When comparing our cohort to the Framingham Heart Study, our case mean PA/A and PA values are similar to their 90\textsuperscript{th} % upper limit of normal. Although we found a similar inverse relationship with PA/A and age, there were only weak associations with height and BSA. In a very recent study, an abnormal PA/A has been associated with increased mortality in patients with coronary artery disease. Our study would indicate that abnormalities in the PA/A may represent a marker of early vascular injury in particulate matter related lung disease and is in line with these recent publications.

Bronchial wall thickening was also found to be more prevalent in those with PA/A>0.92. Our previous work has linked this indicator of proximal airway inflammation and/or remodeling, with WTC-LI. Our new finding may show that bronchial wall thickening is related to early vascular changes. However, it is unclear what the temporal relationship between bronchial wall thickening and the vascular changes is.

Our group and others have linked biomarkers of systemic inflammation and vascular disease with COPD. The proposed pathobiologic mechanism of this relationship is related to inflammatory loss of lung parenchyma and vascular beds. COPD is systemic process affecting not only the airways but also affecting lung parenchyma, vascular structures and other organ systems such as skeletal muscle and adipose tissue. Non-invasive biomarkers guiding management and prognostication in COPD are needed.

We chose to study a limited number of biologically plausible vasoactive biomarkers, to examine the link between their expression in a serum soon after massive particulate matter exposure and PA/A. MDC, also known as CCL22, is an inflammatory mediator that has been linked to obstructive lung dysfunction by our lab and others. It is important in platelet activation and has
been associated with systemic vascular phenomenon.\textsuperscript{24-25} sE-selectin is a member of the selectin family of carbohydrate binding lectins, and is specifically produced by activated endothelial cells.\textsuperscript{26} It is one of the main endothelial neutrophil adhesion molecules and has been linked to poor CVD outcomes. Additionally, well-known metabolic risk factors for CVD are associated with increase in the soluble form sE-selectin.\textsuperscript{27-28} TPAI-1, the main inhibitor of plasminogen activator, has been associated with lung diseases,\textsuperscript{29} atherosclerosis, thrombosis, and vascular remodeling.\textsuperscript{30-32} It has been analyzed in pulmonary hypertension and right heart failure, although whether it is inhibitory or stimulatory in these disease states has yet to be elucidated and is under investigation.\textsuperscript{33-34}

In our population, we found that elevated levels of MDC, sE-selectin were significantly associated with an increased ratio PA/A ratio. TPAI-1 was inversely related to this ratio. These associations correlate with previous reports on cardiovascular disease. This association is novel because of it is the first time these biomarkers have been associated with a non-invasive marker of vascular injury in lung disease. However, in using the biomarkers to predict WTC-LI, only MDC remained a predictive biomarker. This may indicate that sE-Selectin and TPA-I may be involved with the vascular remodeling after exposure, but may not be directly related to the lung damage seen in those with WTC-LI. The population size may also have limited the ability to find significant differences in the two groups.

There are several limitations to this study. Understandably, this cohort of FDNY firefighters did not have pre-exposure serum banked for future analyses. We did achieve the next best option by obtaining serum samples within a few months post-exposure. There were no unexposed or asymptomatic exposed controls in our study. Replication of these findings in other populations with and without particulate matter exposure will be important. We were only able to obtain CT images on 97 subjects with available serum. We do not have longitudinal follow-up data (FEV\textsubscript{1}, CTs, serum biomarkers) for additional correlation. Finally, we have no data on the prevalence of
important co-morbidities such as sleep apnea, left heart failure and thromboembolic disease in this population. Thus, the finding linking an elevated PA/A to a decreased FEV$_1$ may be influenced by the presence of these unaccounted for confounders.

In this nested case-cohort study, we were able to identify cardiovascular related serum biomarkers drawn within six months of 9/11/01 that predicted an abnormal PA/A ratio. The observation that biomarkers predict changes in PA/A and that PA/A was a non-invasive marker lung injury (FEV$_1$ loss) in this post-exposure population, provides a novel association to well characterized processes in vascular biology and inflammation secondary to particulate matter exposure. Importantly, these biomarkers were expressed during the early stages of WTC lung injury and reflect potential processes leading to disease susceptibility. This insight on protein expression and its relationship to FEV$_1$ loss and vascular injury may guide future mechanistic and therapeutic studies in the field.
Acknowledgments

The authors would like to thank the firefighters and rescue workers for their participation in this study and for their selfless contributions.

Competing interests: The authors report no financial or competing interests.

Funding Sources: This work was supported by NIH-NHLBI K23HL084191(AN), NIAID K24A1080298(MDW), NIH-R01HL057879 (MDW), and NIOSH (U10-OH008243, U10-OH008242) and UL1RR029893 (DJP). This work was also partially funded by the NYU-HHC CTSI UL1TR000038 from the National Center for Advancing Translational Sciences of the National Institutes of Health. The funding agencies did not participate in the study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

Contributorship: AN, MDW, EJS, DJP participated in study conception and design; AN, EJS were the primary investigators; EJS, AN, SK, AC were responsible for data collection; AN, EJS, FGG, SK were responsible for data validation; AN, EJS, SK participated in data analysis; AN, GCE, EJS undertook the statistical analysis. All authors participated in data interpretation, writing and revision of the report and approval of the final version.

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Figure Legends

**Figure 1. Study Design.** Subjects in the FDNY-WTC health program presented for pulmonary evaluation (*Pulm-EvalSPE*). Baseline Cohort met the listed inclusion criteria. Cases (N=34) and Controls (N=63) had CT and biomarkers available.

**Figure 2. Probability Plots.** Probability of having PA/A ratio≥0.92 developing WTC lung injury over the range of MDC (A), sE-Selectin (B) and tPAI-1 (C) are represented when adjusting for the covariates of exposure, and age. Plots express probability isopleths for the development of WTC lung injury (FEV₁ loss) with all other covariates held constant.

**Supplemental Figure 1. PA/A Measurement.** Inspiratory series were evaluated at the level of the main pulmonary artery bifurcation. The main pulmonary artery trunk was measured perpendicular to its axial longitudinal axis (red) and the ascending thoracic aorta was measured in its widest dimension (yellow).
REFERENCES


Table 1: Demographics, CT and Pulmonary Function Test Data

<table>
<thead>
<tr>
<th>Event</th>
<th>Measure</th>
<th>Cases</th>
<th>N</th>
<th>Controls</th>
<th>N</th>
<th>p</th>
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<tbody>
<tr>
<td>Pre 9/11</td>
<td>FVC, %</td>
<td>84 (80-94)</td>
<td>34</td>
<td>97 (89-111)</td>
<td>63</td>
<td>&lt;0.001</td>
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<td></td>
<td>FEV\textsubscript{1}, %</td>
<td>86 (82-95)</td>
<td>34</td>
<td>104 (93-114)</td>
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<td>&lt;0.001</td>
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<td></td>
<td>FEV\textsubscript{1}/FVC</td>
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<td>85 (82-89)</td>
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<td>At 9/11</td>
<td>Present at Collapse*</td>
<td>12 (35)</td>
<td>34</td>
<td>18 (29)</td>
<td>63</td>
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<tr>
<td></td>
<td>Arrived Later*</td>
<td>22 (65)</td>
<td>34</td>
<td>45 (71)</td>
<td>63</td>
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<tr>
<td></td>
<td>Years of Service</td>
<td>15 (7-20)</td>
<td>34</td>
<td>13 (6-18)</td>
<td>63</td>
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<td>9/11 to Pulm-EvalSPE, Months</td>
<td>34 (25-52)</td>
<td>34</td>
<td>33 (24-57)</td>
<td>63</td>
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<td></td>
<td>FVC, %</td>
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<td>96 (90-104)</td>
<td>63</td>
<td>&lt;0.001</td>
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<tr>
<td></td>
<td>FEV\textsubscript{1}, %</td>
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<td>96 (90-102)</td>
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<td></td>
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<td>Pulm-EvalSPE</td>
<td>BD Response</td>
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<td>4 (1-9)</td>
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<td>TLC, %</td>
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<td>105 (98-112)</td>
<td>32</td>
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<td></td>
<td>FRC, %</td>
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<td>102 (91-109)</td>
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<td>BMI, kg/m\textsuperscript{2}</td>
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<td>29 (27-31)</td>
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<td>PA\textsuperscript{1}</td>
<td>29.22 (3.24)</td>
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<td>28.19 (3.23)</td>
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<td>0.89 (0.10)</td>
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<td>Air Trapping*</td>
<td>19 (58)</td>
<td>33</td>
<td>25 (41)</td>
<td>61</td>
<td>0.124</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>47 (41-51)</td>
<td>34</td>
<td>46 (42-51)</td>
<td>63</td>
<td>0.791</td>
</tr>
<tr>
<td></td>
<td>Height, cm</td>
<td>182 (178-183)</td>
<td>34</td>
<td>178 (173-183)</td>
<td>63</td>
<td>0.041</td>
</tr>
<tr>
<td></td>
<td>BSA, m\textsuperscript{2}</td>
<td>2.24 (2.17-2.43)</td>
<td>34</td>
<td>2.09 (2.01-2.28)</td>
<td>63</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Abbreviations: Pulm-EvalSPE Pulmonary Evaluation; BSA: Body Surface Area; BD Bronchodilator; DL\textsubscript{CO}: Diffusion Capacity of the Lung for Carbon Monoxide. PA = Pulmonary Artery, A = aorta, BWT = Bronchial Wall Thickening

Expressed as Median (IQR) except *Expressed as N (%), †Expressed as Mean (SD)
Table 2: Biomarker PA/A relationship

<table>
<thead>
<tr>
<th>Analyte</th>
<th>PA/A</th>
<th></th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≥0.92</td>
<td>&lt;0.92</td>
<td></td>
</tr>
<tr>
<td>MDC</td>
<td>1.51 (1.21-2.01)</td>
<td>1.41 (0.99-1.77)</td>
<td>0.101</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>12770 (9510-20941)</td>
<td>13218 (10037-18537)</td>
<td>0.789</td>
</tr>
<tr>
<td>sE-Selectin</td>
<td>49.7 (36.5-63.2)</td>
<td>42.8 (35.3-56.1)</td>
<td>0.153</td>
</tr>
<tr>
<td>tPAI-1</td>
<td>119.8 (84.5-152.4)</td>
<td>139.7 (119.5-173.3)</td>
<td>0.057</td>
</tr>
<tr>
<td>MMP-9</td>
<td>345.7 (265.8-465.3)</td>
<td>352.4 (267.9-517.9)</td>
<td>0.949</td>
</tr>
<tr>
<td>MPO</td>
<td>141.0 (104.3-226.1)</td>
<td>135.3 (95.1-289.0)</td>
<td>0.865</td>
</tr>
<tr>
<td>sICAM-1</td>
<td>165.2 (130.4-197.4)</td>
<td>157.8 (136.1-201.8)</td>
<td>0.952</td>
</tr>
<tr>
<td>sVCAM-1</td>
<td>1355 (1119-1679)</td>
<td>1335 (1108-1602)</td>
<td>0.617</td>
</tr>
</tbody>
</table>

Abbreviations: MDC Macrophage Derived Chemokine; sE-Selectin Soluble Endothelial Selectin; tPAI Total Plasminogen Activator Inhibitor; MMP Matrix Metalloproteinase; MPO Myeloperoxidase; sICAM Soluble Intercellular Adhesion Molecule; sVCAM Soluble Vascular Cell Adhesion Molecule

Values expressed as median (IQR)

MDC Ratio ≥0.92 N=37 Ratio <0.92 N=59
Table 3. Serum Biomarker Models Predicting PA/A ≥0.92

<table>
<thead>
<tr>
<th>Model</th>
<th>Analytes</th>
<th>Crude OR(95%CI)</th>
<th>p</th>
<th>Adjusted* OR(95%CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uni-Variable</td>
<td>MDC</td>
<td>1.70 (0.90-3.19)</td>
<td>0.100</td>
<td>1.78 (0.93-3.37)</td>
<td>0.080</td>
</tr>
<tr>
<td></td>
<td>sE-Selectin</td>
<td>1.18 (0.97-1.44)</td>
<td>0.093</td>
<td>1.20 (0.98-1.47)</td>
<td>0.075</td>
</tr>
<tr>
<td></td>
<td>tPAI-1</td>
<td>0.92 (0.84-1.01)</td>
<td>0.093</td>
<td>0.93 (0.85-1.03)</td>
<td>0.148</td>
</tr>
<tr>
<td>Multi-Variable</td>
<td>MDC</td>
<td>1.99 (1.02-3.88)</td>
<td>0.043</td>
<td>2.08 (1.05-4.11)</td>
<td>0.036</td>
</tr>
<tr>
<td></td>
<td>sE-Selectin</td>
<td>1.32 (1.05-1.65)</td>
<td>0.018</td>
<td>1.33 (1.06-1.68)</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>tPAI-1</td>
<td>0.87 (0.78-0.97)</td>
<td>0.013</td>
<td>0.88 (0.79-0.98)</td>
<td>0.024</td>
</tr>
</tbody>
</table>

Abbreviations: MDC Macrophage Derived Chemokine; sE-Selectin Soluable Endothelial Selectin; tPAI Total Plasminogen Activator Inhibitor

*Adjusted for age at CT and Exposure Group
Per 1 ng/ml MDC, Per 10 ng/ml sE-Selectin, tPAI-1

$X^2 (5) = 15.69, p = 0.008$. Hosmer and Lemeshow’s goodness-of-fit test $P = 0.25$.
Area Under ROC curve=0.728 (0.623-0.834)
Pulmonary Evaluation

1,720

Inclusion Criteria

i. Pre 9/11 FEV1%Pred ≥ 75%
ii. Post 9/11-FDNY PFT ≤ 200 days from 9/11
iii. Pulmonary function study at NYU
iv. Arrived at the WTC site before 9/13/01
v. Never Smoking Male FDNY rescue workers
vi. Accurate NHANES PFT normative data

Study Cohort

801 (47%)

Case-Control for WTC-LI

Cases
N=100 (12.5%)

Controls
N=153 (19%)

Case-Control for WTC-LI w/ CT and Biomarkers

Cases
N=34 (34%)

Controls
N=63 (41%)

Cohort for Predicting WTC-LI

N=97

Case-Control for PA/A

PA/A ≥ 0.92
N=38

PA/A < 0.92
N=59
### Supplemental Table 1: CT Data Across 0.92

<table>
<thead>
<tr>
<th></th>
<th>PA/A</th>
<th></th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≥0.92</td>
<td>&lt;0.92</td>
<td></td>
</tr>
<tr>
<td>N=38</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA mm*</td>
<td>30.2 (2.46)</td>
<td>27.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(3.29)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A mm*</td>
<td>30.3 (2.41)</td>
<td>33.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(3.46)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ratio*</td>
<td>1 (0.06)</td>
<td>0.83</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(0.07)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BWT; N(%)</td>
<td>8 (21)</td>
<td>25 (45)</td>
<td>0.019</td>
</tr>
<tr>
<td>Air Trapping; N(%)</td>
<td>16 (42)</td>
<td>28 (50)</td>
<td>0.452</td>
</tr>
<tr>
<td>Age CT Scan†</td>
<td>45 (40-50)</td>
<td>47 (42-51)</td>
<td>0.119</td>
</tr>
<tr>
<td>9/11 to CT Scan, Months†</td>
<td>52 (33-67)</td>
<td>58 (32-73)</td>
<td>0.286</td>
</tr>
</tbody>
</table>

**Abbreviations:** PA = Pulmonary Artery, A = aorta, BWT = Bronchial Wall Thickening, Expressed as Mean (SD) †Expressed as Median (IQR) Air Trapping/Bronchial Wall Thickening PA/A <0.92 N=56, PA/A ≥0.92 N=38