BMJ Open

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

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2014-005575
Article Type:	Research
Date Submitted by the Author:	27-Apr-2014
Complete List of Authors:	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
Primary Subject Heading:	Respiratory medicine
Secondary Subject Heading:	Occupational and environmental medicine, Respiratory medicine
Keywords:	Emphysema < THORACIC MEDICINE, Chronic airways disease < THORACIC MEDICINE, Epidemiology < THORACIC MEDICINE

Enlarged Pulmonary Artery is Predicted by Vascular Injury Biomarkers and is Associated with an Abnormal FEV_1 in WTC Exposed Fire Fighters: A Case-Control Study

Edward J. Schenck, MD[1], Ghislaine C. Echevaria, MD[2,4], Francis G. Girvin,MD[7], Sophia Kwon, MPH[1], Ashley L. Comfort,BS[1], William N. Rom,MD[1,3], David J. Prezant, MD[4,6], Michael D. Weiden,MD[1,3,4], Anna Nolan,MD[1,3,4]

[1]Division of Pulmonary, Critical Care and Sleep, New York University, School of Medicine, New York, NY
[2]Department of Medicine, New York University, School of Medicine, New York, NY
[3]New York University, School of Medicine, Department of Environmental Medicine, Tuxedo Park, NY
[4]Bureau of Health Services and Office of Medical Affairs, Fire Department of New York, Brooklyn, NY
[5]División de Anestesiología, Escuela de Medicina, Pontificia Universidad Católica de Chile - Santiago, Chile
[6]Pulmonary Medicine Division, Department of Medicine, Montefiore Medical Center and Albert Einstein College of Medicine, Bronx, NY
[7]Department of Radiology, New York University Langone Medical Center, New York, NY

Corresponding Author:
Anna Nolan, MD
Assistant Professor of Medicine and Environmental Medicine
New York University School of Medicine
Division of Pulmonary, Critical Care and Sleep
462 1st Avenue
NY NY 10016
212-263-6479
anna.nolan@med.nyu.edu

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 FEV_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 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 FEV_1%predicted, entered FDNY-WTC Health Program within 200 days of 9/11/01 and had pre-9/11 FEV_1>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 FEV_1% 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 FEV_1% predicted of the cohort (N=100), an FEV_1 <77%. Controls (N=153) had an FEV_1 ≥77% predicted and were randomly selected from the study cohort after stratification based on body mass index (BMI) and FEV_1 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 FEV_1, 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 90th% 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 ≥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 ≥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 FEV_1.[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 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.[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 O_2.[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 90^{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.[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 (FEV_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

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 (FEV_1 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

1 Naveed B, Weiden MD, Kwon S, et al. Metabolic Syndrome Biomarkers Predict Lung Function Impairment: A Nested Case-Control Study. *Am J Respir Crit Care Med* 2012;**185**:392-9.

2 Nolan A, Naveed B, Comfort A, et al. Inflammatory Biomarkers Predict Airflow Obstruction After Exposure to World Trade Center Dust. *Chest* 2012;**142**:412-8.

3 Weiden MD, Naveed B, Kwon S, et al. Cardiovascular disease biomarkers predict susceptibility or resistance to lung injury in World Trade Center dust exposed firefighters. *Eur Respir J* 2013;**41**:1023-30.

4 Zureik M, Benetos A, Neukirch C, et al. Reduced pulmonary function is associated with central arterial stiffness in men. *Am J Respir Crit Care Med* 2001;**164**:2181-5.

5 Tockman MS, Pearson JD, Fleg JL, et al. Rapid decline in FEV1. A new risk factor for coronary heart disease mortality. *Am J Respir Crit Care Med* 1995;1**51**:390-8.

6 Mannino DM, Thorn D, Swensen A, et al. Prevalence and outcomes of diabetes, hypertension and cardiovascular disease in COPD. *Eur Respir J* 2008;**32**:962-9.

7 Rodriguez-Roisin R, Drakulovic M, Rodriguez DA, et al. Ventilation-perfusion imbalance and chronic obstructive pulmonary disease staging severity. *J Appl Physiol* 2009;**106**:1902-8.

8 Liebow AA. Pulmonary emphysema with special reference to vascular changes. *Am Rev Resp Dis* 1959;**80**:67-93.

9 Barbera JA, Riverola A, Roca J, et al. Pulmonary vascular abnormalities and ventilation-perfusion relationships in mild chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1994;**149**:423-9.

10 Caplan-Shaw CE, Yee H, Rogers L, et al. Lung pathologic findings in a local residential and working community exposed to World Trade Center dust, gas, and fumes. *J Occup Env Med* 2011;**53**:981-91.

11 King MS, Eisenberg R, Newman JH, et al. Constrictive bronchiolitis in soldiers returning from Iraq and Afghanistan. *N Engl J Med* 2011;**365**:222-30.

12 Wells JM, Washko GR, Han MK, et al. Pulmonary arterial enlargement and acute exacerbations of COPD. *N Engl J Med* 2012;**367**:913-21.

13 Weiden MD, Ferrier N, Nolan A, et al. Obstructive airways disease with air trapping among firefighters exposed to World Trade Center dust. *Chest* 2010;**137**:566-74.

14 Mahammedi A, Oshmyansky A, Hassoun PM, et al. Pulmonary artery measurements in pulmonary hypertension: the role of computed tomography. *J Thorac Imaging* 2013;**28**:96-103.

15 Fluss R, Faraggi D, Reiser B. Estimation of the Youden Index and its associated cutoff point. *Biom J* 2005;**47**:458-72.

16 Truong QA, Massaro JM, Rogers IS, et al. Reference values for normal pulmonary artery dimensions by noncontrast cardiac computed tomography: the Framingham Heart Study. *Circ Cardiovasc Imaging* 2012;**5**:147-54.

17 Devaraj A, Wells AU, Meister MG, et al. Detection of pulmonary hypertension with multidetector CT and echocardiography alone and in combination. *Radiology* 2010;**254**:609-16.

18 Mahammedi A, Oshmyansky A, Hassoun PM, et al. Pulmonary Artery Measurements in Pulmonary Hypertension: The Role of Computed Tomography. *J Thorac Imaging* 2013; **28**:96-103.

19 Nakanishi R, Rana JS, Shalev A, et al. Mortality Risk as a Function of the Ratio of Pulmonary Trunk to Ascending Aorta Diameter In Patients With Suspected Coronary Artery Disease. *Am J Cardiol* 2013; **111**:1259-63.

20 Gan WQ, Man SF, Senthilselvan A, et al. Association between chronic obstructive pulmonary disease and systemic inflammation: a systematic review and a meta-analysis. *Thorax* 2004;**59**:574-80.

21 Koutsokera A, Stolz D, Loukides S, et al. Systemic biomarkers in exacerbations of COPD: the evolving clinical challenge. *Chest* 2012;**141**:396-405.

22 Celli BR, Locantore N, Yates J, et al. Inflammatory biomarkers improve clinical prediction of mortality in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2012;**185**:1065-72.

23 Perros F, Hoogsteden HC, Coyle AJ, et al. Blockade of CCR4 in a humanized model of asthma reveals a critical role for DC-derived CCL17 and CCL22 in attracting Th2 cells and inducing airway inflammation. *Allergy* 2009;**64**:995-1002.

24 Gear AR, Suttitanamongkol S, Viisoreanu D, et al. Adenosine diphosphate strongly potentiates the ability of the chemokines MDC, TARC, and SDF-1 to stimulate platelet function. *Blood* 2001;**97**:937-45.

25 Golledge J, Clancy P, Moran C, et al. The novel association of the chemokine CCL22 with abdominal aortic aneurysm. *Am J Path* 2010;**176**:2098-106.

26 Roldan V, Marin F, Lip GY, et al. Soluble E-selectin in cardiovascular disease and its risk factors. A review of the literature. *Thromb Haemostasis* 2003;**90**:1007-20.

27 Hwang SJ, Ballantyne CM, Sharrett AR, et al. Circulating adhesion molecules VCAM-1, ICAM-1, and E-selectin in carotid atherosclerosis and incident coronary heart disease cases: the Atherosclerosis Risk In Communities (ARIC) study. *Circulation* 1997;**96**:4219-25.

28 Blann AD, Tse W, Maxwell SJ, et al. Increased levels of the soluble adhesion molecule E-selectin in essential hypertension. *J Hypertens* 1994;**12**:925-8.

29 Sisson TH, Simon RH. The plasminogen activation system in lung disease. *Curr Drug Targets* 2007;**8**:1016-29.

30 Flores J, Garcia-Avello A, Flores VM, et al. Tissue plasminogen activator plasma levels as a potential diagnostic aid in acute pulmonary embolism. *Arch Pathol Lab Med* 2003;**127**:310-5.

31 Gutte H, Mortensen J, Hag AM, et al. Limited value of novel pulmonary embolism biomarkers in patients with coronary atherosclerosis. *Clin Physiol Funct Imaging.* 2011;**31**:452-7.

32 Tashiro Y, Nishida C, Sato-Kusubata K, et al. Inhibition of PAI-1 induces neutrophil-driven neoangiogenesis and promotes tissue regeneration via production of angiocrine factors in mice. *Blood.* 2012;**119**:6382-93.

33 Kawut SM, Barr RG, Johnson WC, et al. Matrix metalloproteinase-9 and plasminogen activator inhibitor-1 are associated with right ventricular structure and function: the MESA-RV Study. *Biomarkers*. 2010;**15**:731-8.

34 Diebold I, Kraicun D, Bonello S, et al. The 'PAI-1 paradox' in vascular remodeling. *Thromb Haemostasis*. 2008;**100**:984-91.

Table 1: Demographics, CT and Pulmonary Function Test Data

Event	Measure	Cases	N	Controls	N	p
Pre 9/11	FVC, %	84 (80-94)	34	97 (89-111)	63	<0.001
	FEV_1, %	86 (82-95)	34	104 (93-114)	63	<0.001
	FEV_1/FVC	84 (79-87)	34	85 (82-89)	63	0.960
At 9/11	Present at Collapse*	12 (35)	34	18 (29)	63	0.494
	Arrived Later*	22 (65)	34	45 (71)	63	
	Years of Service	15 (7-20)	34	13 (6-18)	63	0.317
Pulm-Eval	9/11 to Pulm-Eval, Months	34 (25-52)	34	33 (24-57)	63	0.928
	FVC ,%	76 (72-86)	34	96 (90-104)	63	<0.001
	FEV_1, %	72 (64-74)	34	96 (90-102)	63	<0.001
	FEV_1/FVC	74 (65-78)	34	78 (75-82)	63	0.004
	BD Response	15 (6-25)	30	4 (1-9)	33	0.001
	TLC, %	86 (80-101)	28	105 (98-112)	32	<0.001
	FRC , %	84 (76-100)	28	102 (91-109)	32	0.002
	RV, %	130 (107-145)	28	129 (115-141)	32	0.859
	DL_{CO}, %	95 (85-106)	27	106 (99-113)	31	0.006
	BMI, kg/m^2	31 (29-34)	34	29 (27-31)	63	0.004
CT	PA†	29.22 (3.24)	34	28.19 (3.23)	63	0.138
	A†	32.09 (3.68)	34	31.95 (3.19)	63	0.850
	PA/A†	0.92 (0.11)	34	0.89 (0.10)	63	0.151
	BWT*	12 (36)	33	21(34)	61	0.851
	Air Trapping*	19 (58)	33	25 (41)	61	0.124
	Age	47 (41-51)	34	46 (42-51)	63	0.791
	Height, cm	182 (178-183)	34	178 (173-183)	63	0.041
	BSA, m^2	2.24 (2.17-2.43)	34	2.09 (2.01-2.28)	63	0.001

Abbreviations: Pulm-Eval Pulmonary Evaluation; BSA Body Surface Area; BD Bronchodilator; DL_{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

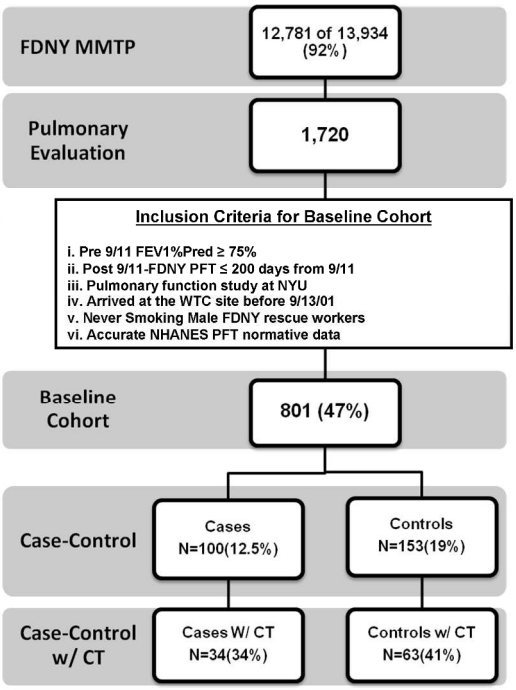
Analyte ng/mL	PA/A ≥0.92 N=37	PA/A <0.92 N=59	p
MDC	1.51 (1.21-2.01)	1.41 (0.99-1.77)	0.101
Adiponectin	12770 (9510-20941)	13218 (10037-18537)	0.789
sE-Selectin	49.7 (36.5-63.2)	42.8 (35.3-56.1)	0.153
tPAI-1	119.8 (84.5-152.4)	139.7 (119.5-173.3)	0.057
MMP-9	345.7 (265.8-465.3)	352.4 (267.9-517.9)	0.949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

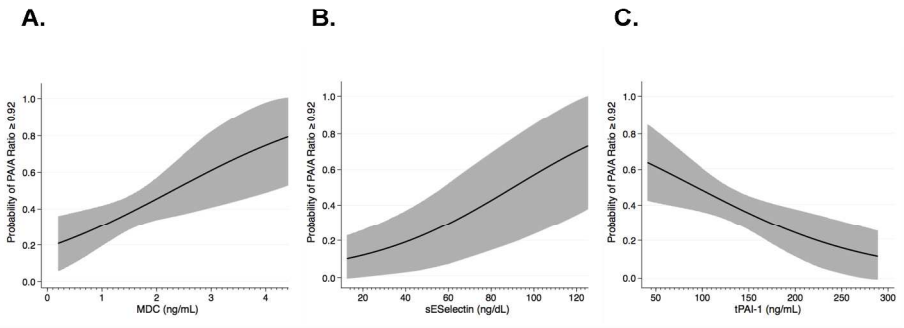
Abbreviations: MDC Macrophage Derived Chemokine; sE-Selectin Soluble Endothelial Selectin; tPAI Total Plasminogen Activator Inhibitor; MMP Matrix Metaloproteinase; MPO Myeloperoxidase; sICAM Soluble Intercellular Adhesion Molecule; sVCAM Soluble Vascular Cell Adhesion Molecule
Values expressed as median (IQR)
MDC Ratio ≥0.92N=38 Ratio <0.92 N=59

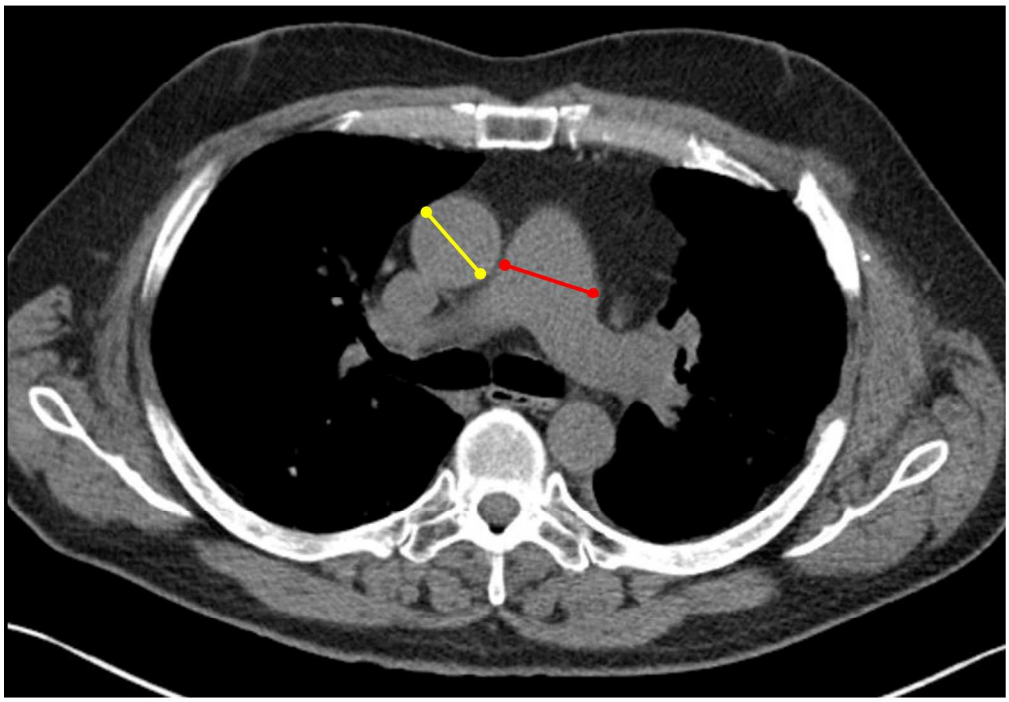
Table 3. Serum Biomarker Models Predicting PA/A ≥0.92

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

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)







Supplemental Table 1: CT Data Across 0.92

	PA/A		p
	≥0.92 N=38	<0.92 N=59	
PA mm*	30.2 (2.46)	27.5 (3.29)	<0.001
A mm*	30.3 (2.41)	33.1 (3.46)	<0.001
Ratio*	1 (0.06)	0.83 (0.07)	<0.001
BWT; N(%)	8 (21)	25 (45)	0.019
Air Trapping; N(%)	16 (42)	28(50)	0.452
Age CT Scan†	45 (40-50)	47 (42-51)	0.119
9/11 to CT Scan, Months†	52 (33-67)	58 (32-73)	0.286

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

BMJ Open

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

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2014-005575.R1
Article Type:	Research
Date Submitted by the Author:	10-Jul-2014
Complete List of Authors:	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
Primary Subject Heading:	Respiratory medicine
Secondary Subject Heading:	Occupational and environmental medicine, Respiratory medicine
Keywords:	Emphysema < THORACIC MEDICINE, Chronic airways disease < THORACIC MEDICINE, Epidemiology < THORACIC MEDICINE

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, MD[1], Ghislaine C. Echevaria, MD[2,5], Francis G. Girvin,MD[7], Sophia Kwon, DO, MPH[1], Ashley L. Comfort,BS[1], William N. Rom,MD[1,3], David J. Prezant, MD[4,6], Michael D. Weiden,MD[1,3,4], Anna Nolan,MD[1,3,4]

[1]Division of Pulmonary, Critical Care and Sleep, New York University, School of Medicine, New York, NY
[2]Department of Medicine, New York University, School of Medicine, New York, NY
[3]New York University, School of Medicine, Department of Environmental Medicine, Tuxedo Park, NY
[4]Bureau of Health Services and Office of Medical Affairs, Fire Department of New York, Brooklyn, NY
[5]División de Anestesiología, Escuela de Medicina, Pontificia Universidad Católica de Chile - Santiago, Chile
[6]Pulmonary Medicine Division, Department of Medicine, Montefiore Medical Center and Albert Einstein College of Medicine, Bronx, NY
[7]Department of Radiology, New York University Langone Medical Center, New York, NY

Corresponding Author:
Anna Nolan, MD, MS
Assistant Professor of Medicine and Environmental Medicine
New York University School of Medicine
Division of Pulmonary, Critical Care and Sleep
462 1st Avenue
NY NY 10016
212-263-6479
anna.nolan@med.nyu.edu

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 $FEV_1 \geq 75\%$, 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 $FEV_1 \leq 77\%$ 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).[1 2] 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.[1 2] It is characterized by primarily obstructive respiratory dysfunction with substantial and persistent losses in FEV_1% 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.[2-4]

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.[5-7]

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.[8-10] 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.[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.[13] The PA/A has been associated with a decreased FEV_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.[1] Symptomatic subjects referred for subspecialty pulmonary evaluation (SPE) between 10/1/2001 and 3/10/2008, underwent specialized pulmonary function testing as previously described.[1] 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≤77%). 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. [1]

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 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.[1] 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.[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).

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 -1) was used for predicting the development of WTC-LI.[15] 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_1 than controls at pre-9/11, and at SPE; Table 1. Cases also had a lower FEV_1/FVC ratio, TLC, FRC and DLCO at SPE. 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 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_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 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.[16] The mean PA/A in cases was 0.92, similar to the 90th% 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 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 ≥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 ≥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). 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.[10] 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.[13] 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]

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 O_2.[13] 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.[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.

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.[1] 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.[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] sE-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. 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_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

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

on behalf of all authors, a full copyright assignment to Thorax as set out in the copyright assignment at: http://group.bmj.com/products/journals/instructions-for-authors/licence-forms.

REFERENCES

1. Weiden MD, Ferrier N, Nolan A, et al. Obstructive Airways Disease with Air Trapping among Firefighters Exposed to World Trade Center Dust. *Chest* 2010;137(3):566-74.

2. Weiden MD, Naveed B, Kwon S, et al. Cardiovascular Biomarkers Predict Susceptibility to Lung Injury in World Trade Center Dust-Exposed Firefighters. *Eur Respir J* 2013;41(5):1023-30.

3. Naveed B, Weiden M, Kwon S, et al.Metabolic Syndrome Biomarkers Predict Lung Function Impairment: A Nested Case-Control Study. *American journal of respiratory & critical care medicine* 2012;185(4):392-99.

4. Nolan A, Naveed B, Comfort A, et al. Inflammatory Biomarkers Predict Airflow Obstruction after Exposure to World Trade Center Dust. *Chest* 2012:412-18.

5. Zureik M, Benetos A, Neukirch C, et al. Reduced Pulmonary Function Is Associated with Central Arterial Stiffness in Men. *Am J Respir Crit Care Med* 2001;164(12):2181-5.

6. Tockman MS, Pearson JD, Fleg JL, et al. Rapid Decline in Fev1. A New Risk Factor for Coronary Heart Disease Mortality. *Am J Respir Crit Care Med* 1995;151(2 Pt 1):390-8.

7. Mannino DM, Thorn D, Swensen A, et al. Prevalence and Outcomes of Diabetes, Hypertension and Cardiovascular Disease in Copd. *Eur Respir J* 2008;32(4):962-9.

8. Rodriguez-Roisin R, Drakulovic M, Rodriguez DA, et al. Ventilation-Perfusion Imbalance and Chronic Obstructive Pulmonary Disease Staging Severity. *J Appl Physiol* 2009;106(6):1902-8.

9. Liebow AA. Pulmonary Emphysema with Special Reference to Vascular Changes. *Am Rev Respir Dis* 1959;80(1, Part 2):67-93.

10. Barbera JA, Riverola A, Roca J, et al. Pulmonary Vascular Abnormalities and Ventilation-Perfusion Relationships in Mild Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med* 1994;149(2 Pt 1):423-9.

11. Caplan-Shaw CE, Yee H, Rogers L, et al. Lung Pathologic Findings in a Local Residential and Working Community Exposed to World Trade Center Dust, Gas, and Fumes. *J Occup Environ Med* 2011;53(9):981-91.

12. King MS, Eisenberg R, Newman JH, T et al.Constrictive Bronchiolitis in Soldiers Returning from Iraq and Afghanistan. *N Engl J Med* 2011;365(3):222-30.

13. Wells JM, Washko GR, Han MK, et al.Pulmonary Arterial Enlargement and Acute Exacerbations of Copd. *N Engl J Med* 2012;367(10):913-21.

14. Mahammedi A, Oshmyansky A, Hassoun PM, et al.Pulmonary Artery Measurements in Pulmonary Hypertension: The Role of Computed Tomography. *J Thorac Imaging* 2013;28(2):96-103.

15. Fluss R, Faraggi D, Reiser B. Estimation of the Youden Index and Its Associated Cutoff Point. *Biom J* 2005;47(4):458-72.

16. Truong QA, Massaro JM, Rogers IS, et al.Reference Values for Normal Pulmonary Artery Dimensions by Noncontrast Cardiac Computed Tomography: The Framingham Heart Study. *Circ Cardiovasc Imaging* 2012;5(1):147-54.

17. Devaraj A, Wells AU, Meister MG, et al.Detection of Pulmonary Hypertension with Multidetector Ct and Echocardiography Alone and in Combination. *Radiology* 2010;254(2):609-16.

18. Mahammedi A, Oshmyansky A, Hassoun PM, et al.Pulmonary Artery Measurements in Pulmonary Hypertension: The Role of Computed Tomography. *J Thorac Imaging* 2012.

19. Nakanishi R, Rana JS, Shalev A, et al. Mortality Risk as a Function of the Ratio of Pulmonary Trunk to Ascending Aorta Diameter in Patients with Suspected Coronary Artery Disease. *Am J Cardiol* 2013.

20. Gan WQ, Man SF, Senthilselvan A, et al. Association between Chronic Obstructive Pulmonary Disease and Systemic Inflammation: A Systematic Review and a Meta-Analysis. *Thorax* 2004;59(7):574-80.

21. Koutsokera A, Stolz D, Loukides S, et al. Systemic Biomarkers in Exacerbations of Copd: The Evolving Clinical Challenge. *Chest* 2012;141(2):396-405.

22. Celli BR, Locantore N, Yates J, et al. Inflammatory Biomarkers Improve Clinical Prediction of Mortality in Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med* 2012;185(10):1065-72.

23. Perros F, Hoogsteden HC, Coyle AJ, et al. Blockade of Ccr4 in a Humanized Model of Asthma Reveals a Critical Role for Dc-Derived Ccl17 and Ccl22 in Attracting Th2 Cells and Inducing Airway Inflammation. *Allergy* 2009;64(7):995-1002.

24. Gear AR, Suttitanamongkol S, Viisoreanu D, et al. Adenosine Diphosphate Strongly Potentiates the Ability of the Chemokines Mdc, Tarc, and Sdf-1 to Stimulate Platelet Function. *Blood* 2001;97(4):937-45.

25. Golledge J, Clancy P, Moran C, et al. The Novel Association of the Chemokine Ccl22 with Abdominal Aortic Aneurysm. *Am J Pathol* 2010;176(5):2098-106.

26. Roldan V, Marin F, Lip GY, et al. Soluble E-Selectin in Cardiovascular Disease and Its Risk Factors. A Review of the Literature. *Thromb Haemost* 2003;90(6):1007-20.

27. Hwang SJ, Ballantyne CM, Sharrett AR, et al. Circulating Adhesion Molecules Vcam-1, Icam-1, and E-Selectin in Carotid Atherosclerosis and Incident Coronary Heart Disease Cases: The Atherosclerosis Risk in Communities (Aric) Study. *Circulation* 1997;96(12):4219-25.

28. Blann AD, Tse W, Maxwell SJ, et al.ncreased Levels of the Soluble Adhesion Molecule E-Selectin in Essential Hypertension. *J Hypertens* 1994;12(8):925-8.

29. Sisson TH, Simon RH. The Plasminogen Activation System in Lung Disease. *Curr Drug Targets* 2007;8(9):1016-29.

30. Flores J, Garcia-Avello A, Flores VM, et al. Tissue Plasminogen Activator Plasma Levels as a Potential Diagnostic Aid in Acute Pulmonary Embolism. *Arch Pathol Lab Med* 2003;127(3):310-5.

31. Gutte H, Mortensen J, Hag AM, et al.Limited Value of Novel Pulmonary Embolism Biomarkers in Patients with Coronary Atherosclerosis. *Clin Physiol Funct Imaging* 2011;31(6):452-7.

32. Tashiro Y, Nishida C, Sato-Kusubata K, et al.Inhibition of Pai-1 Induces Neutrophil-Driven Neoangiogenesis and Promotes Tissue Regeneration Via Production of Angiocrine Factors in Mice. *Blood* 2012;119(26):6382-93.

33. Kawut SM, Barr RG, Johnson WC, et al. Matrix Metalloproteinase-9 and Plasminogen Activator Inhibitor-1 Are Associated with Right Ventricular Structure and Function: The Mesa-Rv Study. *Biomarkers* 2010;15(8):731-8.

34. Diebold I, Kraicun D, Bonello S, et al. The 'Pai-1 Paradox' in Vascular Remodeling. *Thromb Haemost* 2008;100(6):984-91.

Table 1: Demographics, CT and Pulmonary Function Test Data

Event	Measure	Cases	N	Controls	N	p
Pre 9/11	FVC, %	84 (80-94)	34	97 (89-111)	63	<0.001
	FEV_1, %	86 (82-95)	34	104 (93-114)	63	<0.001
	FEV_1/FVC	84 (79-87)	34	85 (82-89)	63	0.960
At 9/11	Present at Collapse*	12 (35)	34	18 (29)	63	0.494
	Arrived Later*	22 (65)	34	45 (71)	63	
	Years of Service	15 (7-20)	34	13 (6-18)	63	0.317
SPE	9/11 to SPE, Months	34 (25-52)	34	33 (24-57)	63	0.928
	FVC ,%	76 (72-86)	34	96 (90-104)	63	<0.001
	FEV_1, %	72 (64-74)	34	96 (90-102)	63	<0.001
	FEV_1/FVC	74 (65-78)	34	78 (75-82)	63	0.004
	BD Response	15 (6-25)	30	4 (1-9)	33	0.001
	TLC, %	86 (80-101)	28	105 (98-112)	32	<0.001
	FRC , %	84 (76-100)	28	102 (91-109)	32	0.002
	RV, %	130 (107-145)	28	129 (115-141)	32	0.859
	DL_{CO}, %	95 (85-106)	27	106 (99-113)	31	0.006
	BMI, kg/m^2	31 (29-34)	34	29 (27-31)	63	0.004
CT	PA†	29.22 (3.24)	34	28.19 (3.23)	63	0.138
	A†	32.09 (3.68)	34	31.95 (3.19)	63	0.850
	PA/A†	0.92 (0.11)	34	0.89 (0.10)	63	0.151
	PA/A≥0.92*	18(53)	34	20(32)	63	0.042
	BWT*	12 (36)	33	21(34)	61	0.851
	Air Trapping*	19 (58)	33	25 (41)	61	0.124
	Age	47 (41-51)	34	46 (42-51)	63	0.791
	Height, cm	182 (178-183)	34	178 (173-183)	63	0.041
	BSA, m^2	2.24 (2.17-2.43)	34	2.09 (2.01-2.28)	63	0.001

Abbreviations: SPE Pulmonary Evaluation; BSA: Body Surface Area; BD Bronchodilator; DL_{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

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

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 (FEV_1 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 PAA ≥0.92

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

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$_1$~~WTC-Lung Injury in ~~WTC~~ Exposed Fire Fighters:

A Case-Control Study

Edward J. Schenck, MD[1], Ghislaine C. Echevaria, MD[2,5~~4~~], Francis G. Girvin,MD[7], Sophia Kwon, DO, ~~-~~MPH[1], Ashley L. Comfort,BS[1], William N. Rom,MD[1,3], David J. Prezant, MD[4,6], Michael D. Weiden,MD[1,3,4], Anna Nolan,MD[1,3,4]

[1]Division of Pulmonary, Critical Care and Sleep, New York University, School of Medicine, New York, NY
[2]Department of Medicine, New York University, School of Medicine, New York, NY
[3]New York University, School of Medicine, Department of Environmental Medicine, Tuxedo Park, NY
[4]Bureau of Health Services and Office of Medical Affairs, Fire Department of New York, Brooklyn, NY
[5]División de Anestesiología, Escuela de Medicina, Pontificia Universidad Católica de Chile - Santiago, Chile
[6]Pulmonary Medicine Division, Department of Medicine, Montefiore Medical Center and Albert Einstein College of Medicine, Bronx, NY
[7]Department of Radiology, New York University Langone Medical Center, New York, NY

Corresponding Author:
Anna Nolan, MD, MS
Assistant Professor of Medicine and Environmental Medicine
New York University School of Medicine
Division of Pulmonary, Critical Care and Sleep
462 1st Avenue
NY NY 10016
212-263-6479
anna.nolan@med.nyu.edu

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: ~~2705~~2942

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

Formatted: Left

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)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 $FEV_1 \geq 75\%$, 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 $FEV_1 \leq 77\%$ 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).

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).

CONCLUSIONSConclusions: Elevated PA/A was associated with WTC-related decline in

Formatted: Font: Bold
Formatted: Font: Bold
Formatted: Subscript
Formatted: Font: Bold
Formatted: Font: Not Bold
Formatted: Font: Bold

~~FEV_1~~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 ~~obstructive lung dysfunction~~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.

~~**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.~~

Formatted: Bulleted + Level: 1 + Aligned at: 0.25" + Indent at: 0.5"

Formatted: Font: Not Bold

Formatted: Indent: Left: 0.25"

BACKGROUND

Development of ~~abnormal spirometry~~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).[1 2] 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~~{1{Weiden, 2013 #708}}~~.[1 2] It is characterized by primarily obstructive respiratory dysfunction with substantial and persistent losses in FEV_1% 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.[2-4]

~~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.[5-7]

Formatted: Line spacing: Double
Formatted: Font: 11 pt
Formatted: Font: 11 pt
Formatted: Font: 11 pt
Formatted: Font: 11 pt
Formatted: Font: 11 pt, Subscript
Formatted: Font: 11 pt
Formatted: Font: 11 pt
Field Code Changed
Formatted: Font: 11 pt

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$_1$~~WTC-LI.[8-10] 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.[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.[13] The PA/A has been associated with a decreased FEV$_1$ 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 ~~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.[1] Symptomatic subjects referred for subspecialty pulmonary evaluation (~~Pulm-Eval~~SPE) between 10/1/2001 and 3/10/2008, underwent specialized pulmonary function testing as previously described.[1] 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 FEV_1%predicted, entered FDNY-WTC Health Program within 200 days of 9/11/01 and had pre-9/11 FEV_1>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).

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≤77%). ~~Cases and controls were defined by their post-9/11 FEV_1% Predicted measured at Pulm-Eval using NHANES III criteria. Cases, those susceptible to WTC lung injury, were defined as being within~~

Formatted: Subscript

Formatted: Subscript

~~one SD of the lowest FEV_1% predicted of the cohort (N=100), an FEV_1 <77%.~~ 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. [1] ~~had an FEV_1 >77% predicted and were randomly selected from the study cohort 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.[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-Eval~~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. ~~97 individuals.~~ 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

Formatted: Subscript

(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.[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 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.[4]

Formatted: Font: 12 pt

Formatted: Font: 12 pt

Formatted: Font: 12 pt

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 ~~lung injury~~.[15] 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.~~the relationships between the CT derived measurements, abnormal FEV_1, 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-~~C~~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~~SPE, years of service, and age at 9/11. BMI, body surface area (BSA) and height of cases were higher than controls at ~~Pulm-Eval~~SPE.

Lung Function

Cases had a lower FEV_1 than controls at pre-9/11, and at ~~Pulm-Eval~~SPE; Table 1. Cases also had a lower FEV_1/FVC ratio, TLC, FRC and DLCO at ~~Pulm-Eval~~SPE. 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~~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_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~~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.[16] The mean PA/A in cases was 0.92, similar to the 90th% 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 WTC-LI ~~Abnormal FEV$_1$~~

Formatted: Not Superscript/ Subscript

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~~SPE and exposure, the odds of having a low FEV$_1$ at ~~Pulm-Eval~~SPE 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 ≥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).

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 ~~an abnormal FEV_1~~WTC-LI.[10] 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.[13] 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]

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 ~~predict~~ability to predict PA/A≥0.92~~ed 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.~~[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 O_2.[13] 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_2.[13] 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.[13]~~ 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.

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.[1] 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.

Formatted: Indent: First line: 0.5"

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] sE-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. 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_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

Figure Legends

Figure 1. Study Design. Subjects in the FDNY-WTC health program presented for pulmonary evaluation (~~Pulm Eval~~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 ~~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 (FEV_1 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

1. Weiden MD, Ferrier N, Nolan A, Rom WN, Comfort A, Gustave J, et al. Obstructive Airways Disease with Air Trapping among Firefighters Exposed to World Trade Center Dust. *Chest* 2010;137(3):566-74.

2. Weiden MD, Naveed B, Kwon S, Cho SJ, Comfort AL, Prezant DJ, et al. Cardiovascular Biomarkers Predict Susceptibility to Lung Injury in World Trade Center Dust-Exposed Firefighters. *Eur Respir J* 2013;41(5):1023-30.

3. Naveed B, Weiden M, Kwon S, Gracely E, Comfort A, Ferrier N, et al. Metabolic Syndrome Biomarkers Predict Lung Function Impairment: A Nested Case-Control Study. *American journal of respiratory & critical care medicine* 2012;185(4):392-99.

4. Nolan A, Naveed B, Comfort A, Ferrier N, Hall C, Kwon S, et al. Inflammatory Biomarkers Predict Airflow Obstruction after Exposure to World Trade Center Dust. *Chest* 2012:412-18.

5. Zureik M, Benetos A, Neukirch C, Courbon D, Bean K, Thomas F, et al. Reduced Pulmonary Function Is Associated with Central Arterial Stiffness in Men. *Am J Respir Crit Care Med* 2001;164(12):2181-5.

6. Tockman MS, Pearson JD, Fleg JL, Metter EJ, Kao SY, Rampal KG, et al. Rapid Decline in Fev1. A New Risk Factor for Coronary Heart Disease Mortality. *Am J Respir Crit Care Med* 1995;151(2 Pt 1):390-8.

7. Mannino DM, Thorn D, Swensen A, Holguin F. Prevalence and Outcomes of Diabetes, Hypertension and Cardiovascular Disease in Copd. *Eur Respir J* 2008;32(4):962-9.

8. Rodriguez-Roisin R, Drakulovic M, Rodriguez DA, Roca J, Barbera JA, Wagner PD. Ventilation-Perfusion Imbalance and Chronic Obstructive Pulmonary Disease Staging Severity. *J Appl Physiol* 2009;106(6):1902-8.

9. Liebow AA. Pulmonary Emphysema with Special Reference to Vascular Changes. *Am Rev Respir Dis* 1959;80(1, Part 2):67-93.

10. Barbera JA, Riverola A, Roca J, Ramirez J, Wagner PD, Ros D, et al. Pulmonary Vascular Abnormalities and Ventilation-Perfusion Relationships in Mild Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med* 1994;149(2 Pt 1):423-9.

11. Caplan-Shaw CE, Yee H, Rogers L, Abraham JL, Parsia SS, Naidich DP, et al. Lung Pathologic Findings in a Local Residential and Working Community Exposed to World Trade Center Dust, Gas, and Fumes. *J Occup Environ Med* 2011;53(9):981-91.

12. King MS, Eisenberg R, Newman JH, Tolle JJ, Harrell FE, Jr., Nian H, et al. Constrictive Bronchiolitis in Soldiers Returning from Iraq and Afghanistan. *N Engl J Med* 2011;365(3):222-30.

13. Wells JM, Washko GR, Han MK, Abbas N, Nath H, Mamary AJ, et al. Pulmonary Arterial Enlargement and Acute Exacerbations of Copd. *N Engl J Med* 2012;367(10):913-21.

14. Mahammedi A, Oshmyansky A, Hassoun PM, Thiemann DR, Siegelman SS. Pulmonary Artery Measurements in Pulmonary Hypertension: The Role of Computed Tomography. *J Thorac Imaging* 2013;28(2):96-103.

15. Fluss R, Faraggi D, Reiser B. Estimation of the Youden Index and Its Associated Cutoff Point. *Biom J* 2005;47(4):458-72.

16. Truong QA, Massaro JM, Rogers IS, Mahabadi AA, Kriegel MF, Fox CS, et al. Reference Values for Normal Pulmonary Artery Dimensions by Noncontrast Cardiac Computed Tomography: The Framingham Heart Study. *Circ Cardiovasc Imaging* 2012;5(1):147-54.

17. Devaraj A, Wells AU, Meister MG, Corte TJ, Wort SJ, Hansell DM. Detection of Pulmonary Hypertension with Multidetector Ct and Echocardiography Alone and in Combination. *Radiology* 2010;254(2):609-16.

18. Mahammedi A, Oshmyansky A, Hassoun PM, Thiemann DR, Siegelman SS. Pulmonary Artery Measurements in Pulmonary Hypertension: The Role of Computed Tomography. *J Thorac Imaging* 2012.

19. Nakanishi R, Rana JS, Shalev A, Gransar H, Hayes SW, Labounty TM, et al. Mortality Risk as a Function of the Ratio of Pulmonary Trunk to Ascending Aorta Diameter in Patients with Suspected Coronary Artery Disease. *Am J Cardiol* 2013.

20. Gan WQ, Man SF, Senthilselvan A, Sin DD. Association between Chronic Obstructive Pulmonary Disease and Systemic Inflammation: A Systematic Review and a Meta-Analysis. *Thorax* 2004;59(7):574-80.

21. Koutsokera A, Stolz D, Loukides S, Kostikas K. Systemic Biomarkers in Exacerbations of Copd: The Evolving Clinical Challenge. *Chest* 2012;141(2):396-405.

22. Celli BR, Locantore N, Yates J, Tal-Singer R, Miller BE, Bakke P, et al. Inflammatory Biomarkers Improve Clinical Prediction of Mortality in Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med* 2012;185(10):1065-72.

23. Perros F, Hoogsteden HC, Coyle AJ, Lambrecht BN, Hammad H. Blockade of Ccr4 in a Humanized Model of Asthma Reveals a Critical Role for Dc-Derived Ccl17 and Ccl22 in Attracting Th2 Cells and Inducing Airway Inflammation. *Allergy* 2009;64(7):995-1002.

24. Gear AR, Suttitanamongkol S, Viisoreanu D, Polanowska-Grabowska RK, Raha S, Camerini D. Adenosine Diphosphate Strongly Potentiates the Ability of the Chemokines Mdc, Tarc, and Sdf-1 to Stimulate Platelet Function. *Blood* 2001;97(4):937-45.

25. Golledge J, Clancy P, Moran C, Biros E, Rush C, Walker P, et al. The Novel Association of the Chemokine Ccl22 with Abdominal Aortic Aneurysm. *Am J Pathol* 2010;176(5):2098-106.

26. Roldan V, Marin F, Lip GY, Blann AD. Soluble E-Selectin in Cardiovascular Disease and Its Risk Factors. A Review of the Literature. *Thromb Haemost* 2003;90(6):1007-20.

27. Hwang SJ, Ballantyne CM, Sharrett AR, Smith LC, Davis CE, Gotto AM, Jr., et al. Circulating Adhesion Molecules Vcam-1, Icam-1, and E-Selectin in Carotid Atherosclerosis and Incident Coronary Heart Disease Cases: The Atherosclerosis Risk in Communities (Aric) Study. *Circulation* 1997;96(12):4219-25.

28. Blann AD, Tse W, Maxwell SJ, Waite MA. Increased Levels of the Soluble Adhesion Molecule E-Selectin in Essential Hypertension. *J Hypertens* 1994;12(8):925-8.

29. Sisson TH, Simon RH. The Plasminogen Activation System in Lung Disease. *Curr Drug Targets* 2007;8(9):1016-29.

30. Flores J, Garcia-Avello A, Flores VM, Navarro JL, Canseco F, Perez-Rodriguez E. Tissue Plasminogen Activator Plasma Levels as a Potential Diagnostic Aid in Acute Pulmonary Embolism. *Arch Pathol Lab Med* 2003;127(3):310-5.

31. Gutte H, Mortensen J, Hag AM, Jensen CV, Kristoffersen US, Brinth L, et al. Limited Value of Novel Pulmonary Embolism Biomarkers in Patients with Coronary Atherosclerosis. *Clin Physiol Funct Imaging* 2011;31(6):452-7.

32. Tashiro Y, Nishida C, Sato-Kusubata K, Ohki-Koizumi M, Ishihara M, Sato A, et al. Inhibition of Pai-1 Induces Neutrophil-Driven Neoangiogenesis and Promotes Tissue Regeneration Via Production of Angiocrine Factors in Mice. *Blood* 2012;119(26):6382-93.

33. Kawut SM, Barr RG, Johnson WC, Chahal H, Tandri H, Jain A, et al. Matrix Metalloproteinase-9 and Plasminogen Activator Inhibitor-1 Are Associated with Right Ventricular Structure and Function: The Mesa-Rv Study. *Biomarkers* 2010;15(8):731-8.

34. Diebold I, Kraicun D, Bonello S, Gorlach A. The 'Pai-1 Paradox' in Vascular Remodeling. *Thromb Haemost* 2008;100(6):984-91.

Table 1: Demographics, CT and Pulmonary Function Test Data

Event	Measure	Cases	N	Controls	N	p
Pre 9/11	FVC, %	84 (80-94)	34	97 (89-111)	63	<0.001
	FEV_1, %	86 (82-95)	34	104 (93-114)	63	<0.001
	FEV_1/FVC	84 (79-87)	34	85 (82-89)	63	0.960
At 9/11	Present at Collapse*	12 (35)	34	18 (29)	63	0.494
	Arrived Later*	22 (65)	34	45 (71)	63	
	Years of Service	15 (7-20)	34	13 (6-18)	63	0.317
~~Pulm-Eval~~SPE	9/11 to ~~Pulm-Eval~~SPE, Months	34 (25-52)	34	33 (24-57)	63	0.928
	FVC ,%	76 (72-86)	34	96 (90-104)	63	<0.001
	FEV_1, %	72 (64-74)	34	96 (90-102)	63	<0.001
	FEV_1/FVC	74 (65-78)	34	78 (75-82)	63	0.004
	BD Response	15 (6-25)	30	4 (1-9)	33	0.001
	TLC, %	86 (80-101)	28	105 (98-112)	32	<0.001
	FRC , %	84 (76-100)	28	102 (91-109)	32	0.002
	RV, %	130 (107-145)	28	129 (115-141)	32	0.859
	DL_{CO}, %	95 (85-106)	27	106 (99-113)	31	0.006
	BMI, kg/m^2	31 (29-34)	34	29 (27-31)	63	0.004
CT	PA†	29.22 (3.24)	34	28.19 (3.23)	63	0.138
	A†	32.09 (3.68)	34	31.95 (3.19)	63	0.850
	PA/A†	0.92 (0.11)	34	0.89 (0.10)	63	0.151
	PA/A≥0.92*	18(53)	34	20(32)	63	0.042
	BWT*	12 (36)	33	21(34)	61	0.851
	Air Trapping*	19 (58)	33	25 (41)	61	0.124
	Age	47 (41-51)	34	46 (42-51)	63	0.791
	Height, cm	182 (178-183)	34	178 (173-183)	63	0.041
	BSA, m^2	2.24 (2.17-2.43)	34	2.09 (2.01-2.28)	63	0.001

Abbreviations: ~~Pulm-Eval~~SPE Pulmonary Evaluation; BSA:~~-~~ Body Surface Area; BD Bronchodilator; DL_{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)

Formatted Table
Formatted: Centered
Formatted: Centered
Formatted: Centered
Formatted: Centered
Formatted: Centered
Formatted: Centered
Formatted: Centered
Formatted: Centered
Formatted: Centered
Formatted: Centered
Formatted: Centered
Formatted: Centered
Formatted: Centered
Formatted: Centered
Formatted: Centered
Formatted: Centered
Formatted: Font: 11 pt
Formatted: Font: 11 pt
Formatted: Centered
Formatted: Centered
Formatted: Centered
Formatted: Centered
Formatted: Centered
Formatted: Centered

Table 2: Biomarker PA/A relationship

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

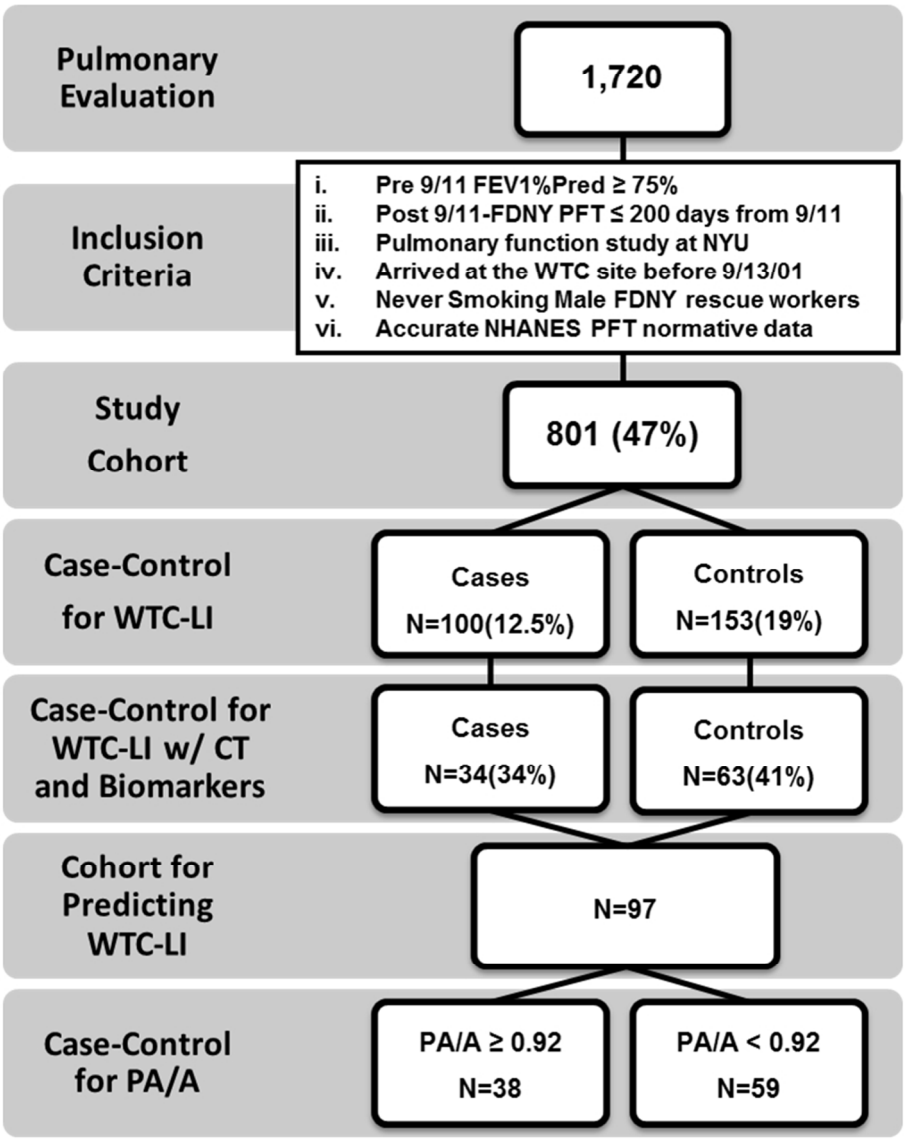
Abbreviations: MDC Macrophage Derived Chemokine; sE-Selectin Soluble Endothelial Selectin; tPAI Total Plasminogen Activator Inhibitor; MMP Matrix Metaloproteinase; MPO Myeloperoxidase; sICAM Soluble Intercellular Adhesion Molecule; sVCAM Soluble Vascular Cell Adhesion Molecule
Values expressed as median (IQR)
~~**MDC Ratio ≥0.92N=38 Ratio <0.92 N=59**~~

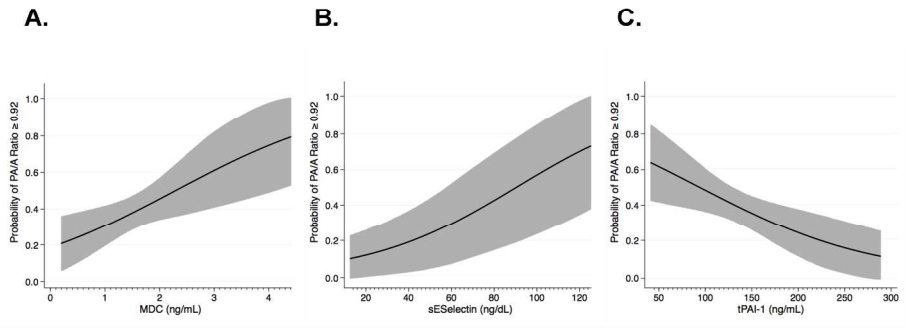
Table 3. Serum Biomarker Models Predicting PA/A ≥0.92

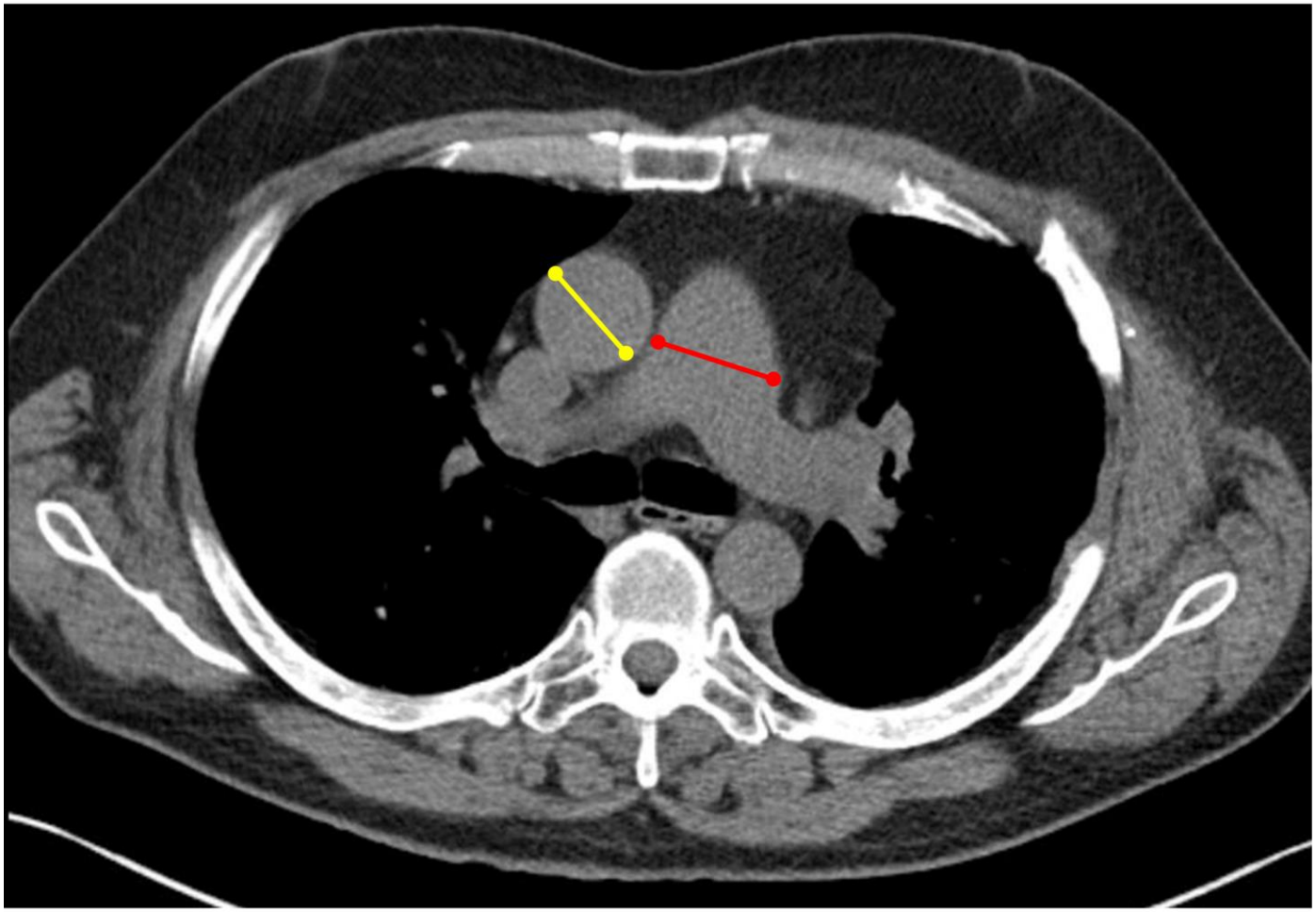
Model	Analytes	Crude		Adjusted*	
		OR(95%CI)	p	OR(95%CI)	p
Uni-Variable	MDC	1.70 (0.90-3.19)	0.100	1.78 (0.93-3.37)	0.080
	sE-Selectin	1.18 (0.97-1.44)	0.093	1.20 (0.98-1.47)	0.075
	tPAI-1	0.92 (0.84-1.01)	0.093	0.93 (0.85-1.03)	0.148
Multi-Variable	MDC	1.99 (1.02-3.88)	0.043	2.08 (1.05-4.11)	0.036
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

Formatted 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)


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

	PA/A		p
	≥0.92 N=38	<0.92 N=59	
PA mm*	30.2 (2.46)	27.5 (3.29)	<0.001
A mm*	30.3 (2.41)	33.1 (3.46)	<0.001
Ratio*	1 (0.06)	0.83 (0.07)	<0.001
BWT; N(%)	8 (21)	25 (45)	0.019
Air Trapping; N(%)	16 (42)	28(50)	0.452
Age CT Scan†	45 (40-50)	47 (42-51)	0.119
9/11 to CT Scan, Months†	52 (33-67)	58 (32-73)	0.286

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