PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (**http://bmjopen.bmj.com/site/about/resources/checklist.pdf**) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

This paper was submitted to the OEM but declined for publication following peer review. The authors addressed the reviewers' comments and submitted the revised paper to BMJ Open where it was re-reviewed and accepted.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Enlarged Pulmonary Artery is Predicted by Vascular Injury Biomarkers and is Associated with WTC-Lung Injury in Exposed Fire Fighters: A Case-Control Study
AUTHORS	Schenck, Edward; Echevaria, Ghislaine; Girvin, Francis; Kwon, Sophia; Comfort, Ashley; Rom, William; Prezant, David; Weiden, Michael; Nolan, Anna

VERSION 1 - REVIEW

REVIEWER	Wells, Michael
REVIEW RETURNED	21-Mar-2014

| GENERAL COMMENTS | The manuscript by Dr. Schenck, et al. utilizes a nested case-control model to analyze biomarkers and novel CT imaging techniques to identify subjects at risk for lung injury related to exposure to World Trade Center (WTC) particulate matter. The study has two central hypotheses: 1) In WTC exposed firefighters, CT measurements (specifically the PA/A ratio) will help identify individuals at highest risk of developing lung function decline; and 2) Biomarkers of vascular disease will correlate with development of PA enlargement on CT. This is a novel study concept and the authors are experts in the post-WTC field and are well suited to answer these questions.

Major critiques:
The major endpoint for hypothesis 1 of the present study is identification of patients with WTC-LI. However, the current manuscript is hampered by the unclear study design and ill-defined endpoints. The authors use "decline in FEV1" as the definition of WTC-LI (the reviewer assumes it to be a FEV1 %predicted of <77% per the methods). This should be clarified and further references to "decline in FEV1," "low FEV1," and "abnormal FEV1" should be avoided.
To this reviewer, it seems more logical to investigate the change in FEV1 from pre-911 to post-911 pulmonary evaluation (deltaFEV1) as a means to analyze "decline in FEV1." Alternative analyses relating PA/A values (as a continuous variable) to deltaFEV1 could be used to characterize the relationship between the magnitude of PA enlargement and "FEV1 decline."
The authors found no direct differences between the PA/A ratio in WTC-LI cases and controls. This may be a reflection of the relatively small sample size. They did, however, identify a PA/A threshold (0.92) that had the highest accuracy for identifying WTC-LI in the |

entire population. The prevalence of a PA/A ratio >0.92 for cases and controls is not reported and should be included in Table 1.

The second hypothesis is addressed through exploratory analyses of predefined systemic biomarkers and the PA/A ratio. As above, the authors separated the groups based on the presence of a PA/A ratio >0.92. There was a trend towards significance of lower tPAI-1 in the PA/A >0.92 group. The authors then used logistic regression analyses to find associations between three biomarkers of interest (tPAI-1, sE-selectin, and MDC) and the presence of a PA/A >0.92. The authors do not evaluate the relationships between biomarkers and PA/A ratio within case/control groups or relate these findings to development of WTC-LI. This would be of great interest to this reviewer.

Minor critiques:
- The abstract highlights the FEV1 below the lower limit of normal (LLN). This is not mentioned in the manuscript and should be changed to reflect the definition of WTC-LI used in the text.
- In the introduction (page 4), the sentence "Additionally, an elevated PA/A ratio has been associated with past and future exacerbations in patients with severe COPD." Should be changed to "moderate-to-severe COPD"
- The "Study Design" section in the methods should is unclear. The sentence beginning "The baseline cohort N=801…" is should be revised for clarity.
- Although the authors mention that all subjects enrolled were never-smokers, other comorbidities are not specifically addressed. Can the authors report on the prevalence of COPD, asthma, other pre-existing lung disease, pulmonary hypertension, and cardiovascular disease in this population?
- In the "Serum Sampling and Analysis" section, it is unclear to the reviewer what "MDC found associated with an abnormal FEV1 from a 39-Plex Human Pro-inflammatory Panel…" means. This should be clarified.
- The Figure Legend for Figure 2 seems incorrect as written. Should "Probability of developing WTC lung injury…" be "a PA/A ratio >0.92"?
- The finding of lower bronchial wall thickening in subjects with a PA/A >0.92 is interesting. How do the authors explain this finding?

VERSION 1 – AUTHOR RESPONSE

Reviewer 1

1. ***The major endpoint for hypothesis 1 of the present study is identification of patients with WTC-LI. However, the current manuscript is hampered by the unclear study design and ill-defined endpoints. The authors use "decline in FEV1" as the definition of WTC-LI (the reviewer assumes it to be a FEV1 %predicted of <77% per the methods). This should be clarified and further references to "decline in FEV1," "low FEV1," and "abnormal FEV1" should be avoided.***
The following has been clarified and amended as requested:

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

All mention of "decline in FEV1," "low FEV1," and "abnormal FEV1" have been removed from the abstract and body of the paper.

In the <u>background</u>, we discuss the definition of WTC-LI. The sentence reads:

"Our group has previously defined WTC-LI as the chronic inflammatory lung dysfunction experienced by a subcohort of firefighters with intense exposure to WTC dust. It is characterized by primarily obstructive respiratory dysfunction with substantial and persistent losses in FEV1% predicted to ≤77% in the subsequent 6.5 years post-exposure. In addition, systemic biomarkers of inflammation, metabolic derangement and cardiovascular disease predict WTC-LI."

In the <u>methods</u> section, we have removed the phrase "those susceptible to WTC-LI." We have also included the subcohort case numbers to be clear about how cases were defined by FEV1% predicted at the time of SPE. The sentence reads:

FEV1% predicted at SPE was used as a measurable, phenotypic marker of WTC-LI. Cases represented those who had continued lung dysfunction that we termed WTC-LI, whereas controls represented those who did not have WTC-LI. Cases (N=100) were defined as being within one SD of the lowest FEV1% predicted of the cohort at SPE (FEV1≤77%). Controls (N=153) were those who had FEV1>77% and were randomly selected after stratification based on body mass index (BMI) and FEV1 as previously defined.

In **Figure 1** and in the **methods**, we have elaborated the study design to include the derivation of the population of those with PA/A ratio ≥ and < 0.92. After cases and controls of WTC-LI were determined, we pooled the available population and re-stratified them by PA/A ratio. We have included this part in the methods and the sentence reads as follows: The population was then re-stratified by PA/A ratio using a cutoff of 0.92 for analysis of biomarkers predictive of PA/A ratio.

2. *To this reviewer, it seems more logical to investigate the change in FEV1 from pre-911 to post-911 pulmonary evaluation (deltaFEV1) as a means to analyze "decline in FEV1." Alternative analyses relating PA/A values (as a continuous variable) to deltaFEV1 could be used to characterize the relationship between the magnitude of PA enlargement and "FEV1 decline."*
   We were interested in correlating biomarkers to clinically relevant symptoms and manifestation of disease. Therefore, we chose to use World Trade Center Lung Injury as a dichotomous outcome and use logistic regression. We similarly chose the dichotomous cutoff values of PA/A ratio to define clinically abnormal vs normal PA/A ratios. The logistic model was adjusted for covariates that were used as continuous variables in order to preserve power (less degrees of freedom).

3. *The prevalence of a PA/A ratio >0.92 for cases and controls is not reported and should be included in Table 1.*

This information has now been included in the CT subsection in Table 1, represented as N(%).

4. ***The second hypothesis is addressed through exploratory analyses of predefined systemic biomarkers and the PA/A ratio. As above, the authors separated the groups based on the presence of a PA/A ratio >0.92. There was a trend towards significance of lower tPAI-1 in the PA/A >0.92 group. The authors then used logistic regression analyses to find associations between three biomarkers of interest (tPAI-1, sE-selectin, and MDC) and the presence of a PA/A >0.92. The authors do not evaluate the relationships between biomarkers and PA/A ratio within case/control groups or relate these findings to development of WTC-LI. This would be of great interest to this reviewer.***
We have included PA/A >0.92 as a demographic line in Table 1. There is statistical difference between cases and controls in their PA/A being above 0.92 (p=0.042).

When using biomarkers to predict WTC-LI, MDC remains significant with an OR of 2.1. This may indicate that se-Selectin and TPAI 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.

We have now included this explanation in the:

Results: 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: However, in using the biomarkers to predict WTC-LI, only MDC remained a predictive biomarker.This may indicate that se-Selectin and TPAI 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.

5. ***The abstract highlights the FEV1 below the lower limit of normal (LLN). This is not mentioned in the manuscript and should be changed to reflect the definition of WTC-LI used in the text.***
This was an error in the abstract and has now been fixed. All mention of the LLN has been changed to < or > 77% to reflect the true definition of the case / control population.

6. ***In the introduction (page 4), the sentence "Additionally, an elevated PA/A ratio has been associated with past and future exacerbations in patients with severe COPD." Should be changed to "moderate-to-severe COPD"***
This has now been corrected per reviewer's instructions. The sentence reads:

Additionally, an elevated PA/A has been associated with past and future exacerbations in patients with moderate-to-severe COPD.

7. ***The "Study Design" section in the methods is unclear. The sentence beginning "The baseline cohort N=801…" is should be revised for clarity.***
The sentence now reads: "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."

Furthermore we have clarified the case and control definition

"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."

8. ***Although the authors mention that all subjects enrolled were never-smokers, other comorbidities are not specifically addressed. Can the authors report on the prevalence of COPD, asthma, other pre-existing lung disease, pulmonary hypertension, and cardiovascular disease in this population?***
There is no evidence of pulmonary / cardiovascular disease based on screening measures with PFTs, EKGs, and measures of exercise capacity in this population prior to 9/11. We clarify this in the <u>study design</u> paragraph.

We now include the following: 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.

9. ***In the "Serum Sampling and Analysis" section, it is unclear to the reviewer what "MDC found associated with an abnormal FEV1 from a 39-Plex Human Pro-inflammatory Panel…" means. This should be clarified.***

The sentence now reads:

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).

10. ***The Figure Legend for Figure 2 seems incorrect as written. Should "Probability of developing WTC lung injury…" be "a PA/A ratio >0.92"?***
The sentence in the figure legend 2 reads: 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.

11. **The finding of lower bronchial wall thickening in subjects with a PA/A>0.92 is interesting. How do the authors explain this finding?**

GOLD criteria definition of our population now reflects the most recent guidelines. We then changed the sentence to read as the following:

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.

Therefore, when the Youden index was utilized to improve sensitivity in a population with less advanced COPD, the ratio was expected to drop to 0.92.

The discussion now reads: 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.2 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.

VERSION 2 – REVIEW

REVIEWER	Chung, In-sung School of Medicine, Keimyung university South Korea
REVIEW RETURNED	10-Jun-2014

| GENERAL COMMENTS | Major compulsory revisions:

1) It is not clear who are cases or controls. The authors defined cases and controls in page 5 by post-9/11 FEV1%, but cases or controls in final analysis or table 3 were not same them in page 5, Table 1. Also the terms such as case or controls are not clear in all of parts of article.

2) Was post-9/11 FEV1% predicted value used for gold-standard to decide the cutoff point of the PA/A ratio? Or the difference between pre and at Pulm-Eval individually was used? Was gold standard available for prediction of WTC lung injury?

3) How cases were diagnosed COPD? The authors described " Since our population had less advanced COPD, mostly GOLD 0…" in page 11.

4) How were job history and attribution by job characters managed at post-9/11 in study design? Means of years of service at FDNY in cases and controls in Table 1 were 15 and 13 years. Fire fighters are daily exposed to hazard materials which affect to lung function, so job history is |

	important factor for analysis about lung injury.

VERSION 2 – AUTHOR RESPONSE

Major compulsory revisions:

1. It is not clear who are cases or controls. The authors defined cases and controls in page 5 by post-9/11 FEV1%, but cases or controls in final analysis or table 3 were not same them in page 5, Table 1. Also the terms such as case or controls are not clear in all of parts of article.
We have addressed this in the abstract and background to be more lucid about the cases and controls. We have modified Figure 1 to show that there are cases of WTC-LI and controls. The cohort was then pooled and restratified by PA/A ratio to determine biomarkers that predict PA/A ratio abnormality.

The methods now reads: The population was then re-stratified by PA/A ratio using a cutoff of 0.92 for analysis of biomarkers predictive of PA/A.

2. Was post-9/11 FEV1% predicted value used for gold-standard to decide the cutoff point of the PA/A ratio? Or the difference between pre and at Pulm-Eval individually was used? Was gold standard available for prediction of WTC lung injury?
Cutoff for PA/A ratio was based on logistic regression to improve the specificity and sensitivity of the predictive model (Youden Index). Case definition was as stated in the methods section, and was determined by only FEV1% at SPE. WTC LI has further been clarified as being defined by a cutoff of FEV1≤77% as in previously published papers. We have clarified the case definition throughout the paper.

In the methods: FEV1% predicted at SPE was used as a measurable, phenotypic marker of WTC-LI. Cases represented those who had continued lung dysfunction that we termed WTC-LI, whereas controls represented those who did not have WTC-LI. Cases (N=100) were defined as being within one SD of the lowest FEV1% predicted of the cohort at SPE (FEV1≤77%). Controls (N=153) were those who had FEV1>77% and were randomly selected after stratification based on body mass index (BMI) and FEV1 as previously defined.

3. How cases were diagnosed COPD? The authors described " Since our population had less advanced COPD, mostly GOLD 0…" in page 11.
We recalculated GOLD criteria on the cohort to reflect the most recent recommendations.

GOLD 1, N=6 GOLD 2, N=11 GOLD 3, N=0 GOLD 4, N=1

We have now included the following sentence in the discussion:

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.

4. How were job history and attribution by job characters managed at post-9/11 in study design?
Means of years of service at FDNY in cases and controls in Table 1 were 15 and 13 years. Fire fighters are daily exposed to hazard materials which affect to lung function, so job history is important factor for analysis about lung injury.

We agree that job history is an important factor for analysis. However, there was no significant statistical difference between cases and controls in years of service. P value was 0.317 and was not a likely confounder in this population.

Further, the population had annual testing prior to 9/11 to ensure that there were no pre-existing lung disease based on PFTs and/or cardiovascular disease based on EKG. We have included this sentence in the Study Design and the sentence reads:

Annual physicals occurred prior to 9/11/2001, and active-duty firefighters had normal lung function testing, EKG assessment and measures of exercise capacity.