HHS Public Access

Author manuscript

Environ Res. Author manuscript; available in PMC 2017 May 19.

Published in final edited form as:

Environ Res. 2016 October ; 150: 398–402. doi:10.1016/j.envres.2016.06.025.

Indoor particulate matter exposure is associated with increased black carbon content in airway macrophages of former smokers with COPD

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Abstract

Introduction—Exposure to fine particulate matter ($PM_{2.5}$) is associated with worse morbidity in individuals with COPD. Inhaled PM is phagocytosed by airway macrophages (AM), and black carbon measured in AM may serve as a biomarker of air pollution exposure. As there is little data on how indoor PM exposure may influence AM black carbon content in those with respiratory disease, we investigated the association of indoor $PM_{2.5}$ concentration to AM black carbon content in adults with COPD.

Methods—Former smokers (> 10 pack-years smoking history, quit date > 1 year prior to enrollment) older than 40 years of age with moderate-severe COPD were eligible. Indoor air $PM_{2.5}$ concentrations were measured over 5–7 days at baseline, 3 month, and 6 month intervals. Sputum induction was performed during clinic visits concordant with home monitoring. A total of 50 macrophages per sputum specimen were photographed and quantified using appropriate software by trained staff blinded to PM concentrations. Longitudinal analyses using generalized estimating equations were used to assess the relationship between indoor PM exposure and AM black carbon content.

Results—Participants (n = 20) were older (mean (SD) age 67 (4) years), predominantly Caucasian (85%) and male (70%), with an average smoking history of 52 pack-years and mean (SD) quit date of 13 (9) years prior to enrollment. The majority of daily time was reported to be spent indoors (> 23 h). Mean indoor $PM_{2.5}$ concentration was 12.8 (13.5) $\mu g/m^3$. The mean area of black carbon quantified in airway macrophages was 1.2 (0.7) μm^2. In multivariate cross-sectional and longitudinal analyses, each 10 $\mu g/m^3$ increase in indoor $PM_{2.5}$ was significantly associated with a 0.26 μm^2 and 0.19 μm^2 increase in airway macrophage black carbon total area, respectively ($p < 0.05$).

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Conclusion—Higher indoor $PM_{2.5}$ concentration is associated with an increase in black carbon content of AM in individuals with COPD. These data support the potential for AM black carbon content to be a useful non-invasive biomarker of exposure to indoor PM.

Keywords

Black carbon; Particulate matter; Air pollution; Biomarker; COPD

1. Introduction

COPD, the third leading cause of death worldwide (Chronic respiratory diseases, 2015), is characterized by a progressive decline in lung function. In developed countries, the most strongly supported cause of COPD is cigarette smoking; however, there is growing evidence that environmental exposures other than tobacco smoke, for example, ambient air pollution, are linked to cardiopulmonary morbidity and mortality (Atkinson et al., 2014; Dominici et al., 2006). These studies have demonstrated the significant role of outdoor environmental exposures in worse health outcomes for COPD patients, but less is known about the health effects of airborne exposures present indoors, where Americans, especially those with respiratory illness, spend the majority of their time (Klepeis et al., 2001).

We have previously shown that in-home fine particulate matter (particles less than 2.5 μm diameter, or $PM_{2.5}$) exposure is associated with respiratory symptoms in former smokers with COPD (Hansel et al., 2013). Inhaled fine particulate matter has the ability to travel through the nasopharyngeal passage, avoid upper airway mucociliary trapping, and reach the lower airways where airway macrophages (AM) present along the luminal surface then phagocytize these particles and retain them in the cytoplasmic material. The carbonaceous core that commonly comprises $PM_{2.5}$ can be visualized in the AM using light microscopy (Bai et al., 2015). As a result, black carbon content within AM could serve as a marker for airborne pollutant exposure. To this purpose, cytopathologic techniques that examine macrophages collected from induced sputum and use their known phagocytic activity within the airway have the ability to assess an individual's inhaled dose of particles (Nwokoro et al., 2012; Brugha et al., 2014). As little is known about the ability of AM carbon content to reflect indoor PM exposure, we used these methods to investigate the association of indoor particulate matter with individual airway black carbon content among a cohort of former smokers with COPD.

2. Methods

2.1. Participant recruitment

Recruitment of participants has been previously described (Hansel et al., 2013). Briefly, a total of 84 former smokers with COPD from the Baltimore area were enrolled after meeting the following inclusion criteria: 1) age ≥ 40 years, 2) post bronchodilator $FEV_1 \leq 80\%$ predicted, 3) $FEV_1/FVC < 70\%$, and 4) > 10 pack years smoking, with quit date of > 1 year prior to enrollment and exhaled carbon monoxide level ≤ 6 ppm. Of the 84 subjects in the cohort, we randomly selected a subset of 20 subjects representing a distribution of high and low exposures of indoor PM (10 subjects were randomly selected from subjects with homes

above the median PM and 10 subjects randomly selected from those below the median), who were able to provide induced sputum samples and had adequate (> 50) macrophages identifiable in baseline sputum cytospins for further airway macrophage black carbon analysis. Participants provided written informed consent and the Johns Hopkins Medical Institutional Review Board approved the protocol.

2.2. Air quality assessment

One-week air sampling was performed concordant with the study clinic visit in the participant's main living area, identified as the room where the participant reported spending the most time outside of the bedroom. Indoor air sampling for $PM_{2.5}$ was conducted as previously described (Hansel et al., 2013). The limit of detection (LOD) for $PM_{2.5}$ was 0.64 $\mu g/m^3$.

Outdoor $PM_{2.5}$ concentrations corresponding to the indoor sampling period were obtained from the EPA's Aerometric Information Retrieval Service (now referred to as the Air Quality System database).

2.3. Clinical evaluation and macrophage carbon assessment

Clinic visits occurred between days 4–7 of the one-week airmonitoring period. Health information was collected through validated questionnaires. Spirometry, before and after albuterol administration, was performed according to American Thoracic Society (ATS) criteria (Miller et al., 2005). Induced sputum samples were used to obtain airway macrophages for assessment. Sputum induction was performed with the Aerosol Universal III nebulizer (Flaem, Brescia, Italy) using an established protocol. The sputum processing protocol included plug selection of airway cells, sample solubilization and treatment with Sputolysin (Calbiochem, EMD, USA), cytocentrifugation (Thermo Fisher Scientific, Waltham, MA) onto glass microscope slides and staining with Diff-Quick (Dade Behring, Deerfield, IL). Light microscopy was used to visualize the slides and trained staff performed differential cell counts to assess percent neutrophils, eosinophils, lymphocytes, macrophages and squamous epithelial cells.

Digital color images of macrophages were taken using the DinoEye Eyepiece microscope camera (Dino-Lite, Torrance, CA) at 100× magnification with oil immersion. The Dino-Lite software was used to capture the images and calibrate the known distance in the microscope field image using a measurement micrometer. A total of 50 randomly selected macrophages with an intact cell wall per participant were analyzed for black carbon content. Initial image processing was done using Dino-Lite and Microsoft Paint (Microsoft, Redmond, WA) to isolate the macrophage from other cells or debris and remove the nucleus, and ImageJ software (National Institute of Health, Bethesda, MD) was used to quantify the total area of black carbon (μm^2) in each macrophage. Trained staff that was blinded to indoor PM concentrations processed each image and used the threshold command to obtain the area of black carbon best fit for each macrophage.

2.4. Analysis

Baseline descriptive statistics were analyzed using Spearman correlations, χ^2 tests, and t-tests, as appropriate. At baseline, linear regression models were created to assess the effect of indoor and outdoor $PM_{2.5}$ on AM black carbon content. To investigate the longitudinal relationship between indoor and outdoor $PM_{2.5}$ and AM black carbon, generalized estimating equations (GEE) were used to account for the potential correlation of repeated measures over time (Diggle et al., 2002). A covariate analysis was conducted to assess potential confounding variables, including age, sex, race, education, BMI, time since smoking cessation, pack-years, and season of sampling. Covariates of pack-years of smoking and BMI were associated in either cross-sectional or longitudinal analyses with airway black carbon content in bivariate analyses with a $p < 0.10$ and were subsequently included in multivariate models. Indoor and outdoor $PM_{2.5}$ models were run independently and simultaneously to determine the independent effects of indoor $PM_{2.5}$. Analyses were performed with StataSE statistical software, version 12.0 (Stata Corp, College Station, TX). Statistical significance was defined as $p < 0.05$.

3. Results

This subset of participants from our larger published cohort (n=20) were predominately male (70%), had a mean (± SD) age of 66.6 (3.9) years of age and mean (± SD) pack-years of smoking of 52.4 (23.9). There were no significant differences between individuals included in this analysis and the main study cohort (data not shown). Mean cell percentages in sputum at baseline are shown in Table 1 and the overall mean (± SD) area of airway macrophage black carbon was 1.2 (0.7) μm^2. An example of images of airway macrophages exhibiting high and low airway black carbon loading is shown (Fig. 1). There was no correlation between sputum cell counts and AM black carbon content (data not shown). Additionally, time since smoking cessation was not significantly associated with AM black carbon content (data not shown).

Participants reported spending approximately 23 out of 24 daily hours indoors, with an overall average of less than an hour per day spent outside. Almost half (45%) of the cohort did not go outdoors at all during the monitoring period. The mean (± SD) baseline indoor $PM_{2.5}$ concentration from the main living area was 12.8 (13.5) $\mu g/m^3$. A correlation between indoor $PM_{2.5}$ and airway macrophage black carbon at baseline was statistically significant (rho=0.60, p=0.005). A plot of indoor PM and airway macrophage black carbon area shows increased AM black carbon content with higher indoor $PM_{2.5}$ concentrations (Fig. 2).

In the baseline bivariate analysis, each 10 $\mu g/m^3$ increase in indoor $PM_{2.5}$ was significantly associated with a 0.29 μm^2 increase in airway macrophage black carbon total area (p=0.01) (Table 2). In multivariate analysis, each 10 $\mu g/m^3$ increase in indoor $PM_{2.5}$ was associated with a 0.26 μm^2 increase in airway macrophage black carbon total area (p=0.02) (Table 3). The adjusted R-squared value for the bivariate and multivariate analysis was 0.31 and 0.36 respectively. These values suggest that a large amount of variability is explained through the regression models, and that indoor PM explains 31% of the variability in airway black carbon content, while the covariates explain an additional 5% of variability.

Of the original 20 participants selected for the baseline analysis, 11 and 8 participants were available and able to produce sputum at follow-up 3- and 6-month visits, respectively. The mean (± SD) indoor $PM_{2.5}$ concentrations at 3 months and 6 months were 10.9 (9.3) $\mu g/m^3$ and 13.0 (12.0) $\mu g/m^3$, respectively. The between home standard deviation of PM concentrations was 10.5 $\mu g/m^3$ and the with-in home standard deviation was 5.33 $\mu g/m^3$. In addition, the between and with-in person standard deviations of airway BC content was 0.59 μm^2 and 0.37 μm^2, respectively. In bivariate longitudinal models, each 10 $\mu g/m^3$ increase in indoor $PM_{2.5}$ was significantly associated with a 0.22 μm^2 increase in airway macrophage black carbon total area ($p = 0.01$) (Table 2). In multivariate longitudinal analysis, each 10 $\mu g/m^3$ increase in indoor $PM_{2.5}$ was significantly associated with a 0.19 μm^2 increase in airway macrophage black carbon total area ($p=0.01$) (Table 2).

The mean outdoor $PM_{2.5}$ concentration over the sampling period was 10.4 $\mu g/m^3$; outdoor $PM_{2.5}$ was not significantly associated with AM black carbon area in cross-sectional or longitudinal analyses (Tables 2 and 3). Furthermore, adjustment for outdoor $PM_{2.5}$ concentrations in multivariate indoor $PM_{2.5}$ models did not alter the outcomes (Table 3). Lastly, in both cross-sectional and longitudinal bivariate and multivariate analyses, air nicotine concentrations were not significantly associated with AM black carbon content (data not shown).

4. Discussion

Among former smokers with COPD who spend the predominant amount of their time indoors, we found that indoor $PM_{2.5}$ concentrations are associated with airway macrophage black carbon content both at a single time point and over repeated measures over a 6-month period. These results suggest the potential for airway macrophage black carbon area to serve as a biomarker of indoor particulate matter exposure. To our knowledge, this is the only report that investigates this potential application for patients with COPD. Given that we have previously published evidence of adverse health effects of indoor $PM_{2.5}$ in COPD patients (Hansel et al., 2013), it is not only essential to identify useful indicators of pollutant exposures, but also specifically establish ones that are relevant to exposures within the indoor environment where people with COPD spend a significant portion of time.

Our work builds upon prior epidemiologic studies that have demonstrated the potential for airway macrophage black carbon content to reflect the degree of outdoor air pollution exposure. In a London study of 28 healthy participants, higher airway macrophage black carbon areas were found in cyclists with higher environmental black carbon exposure compared to non-cyclists (Nwokoro et al., 2012). In addition, outdoor PM_{10} (PM with aerodynamic diameter ≤ 10 μm) concentrations have been shown to be associated with airway black carbon content in a cohort of healthy children (Miyata and van Eeden, 2011). However, whether indoor air pollution–which can originate in either outdoor or indoor environments and have multiple concurrent sources–contributes similarly to airway black carbon content in adults is unknown. Sources of airborne carbonaceous particles within the home have been linked to combustion events such as cooking and candle burning (LaRosa et al., 2002). Our results suggest that in a cohort of former smokers with COPD who spend over 95% of their time indoors, indoor PM concentrations are highly correlated with areas of

black carbon within airway macrophages, even in a setting of relatively low pollutant burden (12.8 μg/m^3). Of note, as previously published in this cohort (Hansel et al., 2013), levels of PM within the home did not significantly correlate with outdoor PM levels among participants who lived within a 3-mile radius of an ambient pollution monitoring station. In addition, there was no statistically significant relationship between indoor and outdoor PM concentrations in this subset of 20 participants, thereby strengthening the idea that airway macrophage black carbon content may be a useful biomarker of an individual's indoor exposure to harmful particulate matter in a cohort that is predominantly indoor-dwelling.

There is limited evidence to support the ability of airway macrophage black carbon area to predict pollutant exposure in populations specifically with respiratory disease. Brugha et al. reported airway macrophage black carbon content in a cohort of 36 children with moderate-to-severe asthma and found that black carbon content was 51% lower compared to healthy children and children with mild asthma (Brugha et al., 2014). Interestingly, associations between PM concentrations and black carbon content were not seen among asthmatic children in contrast to healthy children, possibly explained by an impaired airway phagocytosis feature of severe asthma. Similarly, Kulkarni et al. found that the carbon content of AM in children with asthma was lower than in healthy children (median 0.00 μm^2 vs. 0.41 μm^2) (Kulkarni et al., 2006), and healthy adults (0.71 μm^2) (Kulkarni et al., 2005). Such findings may describe relationships that, due to differing biologic mechanisms, may not be generalizable to all forms of obstructive lung disease. Indeed, our results support that airway macrophage black carbon in induced sputum may be a useful biomarker of PM exposure among those with fixed airways obstruction. Further studies are needed to elucidate whether phagocytic function, as well as other structural, physiologic, or mechanistic features of COPD play a role in airway black carbon deposition and/or retention in patients with COPD.

Our study homes had relatively low overall pollutant burden, therefore whether the relationship between indoor PM concentrations and AM BC will be similar in homes with higher overall pollution exposure remains unknown. Moreover, the ability of AM black carbon content to indicate the timing of pollutant exposure is also not yet well established. Other authors who have investigated whether carbon loading is a reflection of acute vs. chronic exposures have found positive associations between ambient 6-month pollution exposure and macrophage carbonaceous particles (Miyata and van Eeden, 2011) but not for exposures occurring over 24 h periods (Nwokoro et al., 2012; Kulkarni et al., 2006). Our study suggests that airway macrophage black carbon content varies over time (i.e, within 3 month intervals) and exposures captured during a 1-week monitoring period, reflective of the recent past, are linked to airway BC content. Thus, the longitudinal design of our study is therefore a strength that allows us to link particulate matter exposure over such a 6-month period with detected AM black carbon, and further supports the promise of airway black carbon measurements as an informative marker of recent exposures in the indoor environment.

Alveolar macrophages are essential for the clearance of foreign debris including PM and provide a first line of immune defense for the respiratory system. However, increased BC content derived from indoor PM exposure may result in dysfunctional macrophages.

Transition metals derived from PM exposure have been shown to generate reactive oxygen species that subsequently activate the innate immune response, however in the process also can induce TLR4-dependent tolerance in macrophages, inhibiting their ability to recognize and clear bacteria (Miyata and van Eeden, 2011). Such mechanisms of macrophage dysfunction may be particularly relevant to COPD, where bacterial exacerbations contribute to disease morbidity. Further studies are needed to explore mechanisms by which engulfed inhaled particles including BC may influence the function of these critical cell types in individuals with COPD.

There are a few limitations to consider with our study. The size of our subset (n=20) is relatively small to achieve strength in our models and restricts the ability to explore a wide variety of correlations. Larger studies in COPD patients are warranted to further characterize pollutant exposure in relation to AM black carbon content. Additional limitations are common to others who have employed such techniques to measure individual pollutant exposure and include, for example, the following: first, the implications of sampling macrophages from induced sputum instead of from bronchoalveolar lavage, which may ignore other fractions of black carbon resident in the lower airway and therefore limit the ability to estimate total absolute exposure to PM; second, unclear effects of sputum processing on intracellular carbon content, though in this study, consistent sputum processing techniques were utilized for all sputum samples and are similar to methods used in other investigations of AM black carbon content (Bai et al., 2015); and third, technical aspects of 2-D microscopy which may under- or over-estimate the amount of carbon particles contained in a darkly-pigmented area. However, with regards to technical matters, our results support consistent relationships between increasing household PM exposure and measured black carbon content, suggesting that common technical challenges of this assay did not play a significant role in our study. Of note, macrophages are not the only cells known to be phagocytic, and whether black carbon content in other phagocytic cells also reflects PM exposure is unknown. Lastly, due to limited sample size, we did not specifically evaluate the health implications of AM black carbon loading in our cohort. Prior studies have related the area of carbonaceous particles to health outcomes such as lung function, however results have been inconsistent (Bai et al., 2015; Kulkarni et al., 2006), and to our knowledge, not explored among those with COPD. Ongoing work to explore the health significance of sputum black carbon content in patients with COPD aims to fill these gaps.

5. Conclusion

Our findings suggest that in our cohort of former smokers with COPD, higher indoor PM exposure is associated with increased black carbon content in airway macrophages, supporting the potential for these measurements to represent a biomarker of exposure to indoor particulate matter. Future studies are needed to further explore the full health implications of airway carbon loading within the context of indoor exposures faced by patients with chronic respiratory disease.

Acknowledgments

Funding sources: This work was supported by Grants including NIH R01ES023500, R21ES015781, R01ES022607, P50MD010431, P50ES018176.

Participants provided written informed consent and the Johns Hopkins Medical Institutional Review Board approved the protocol.

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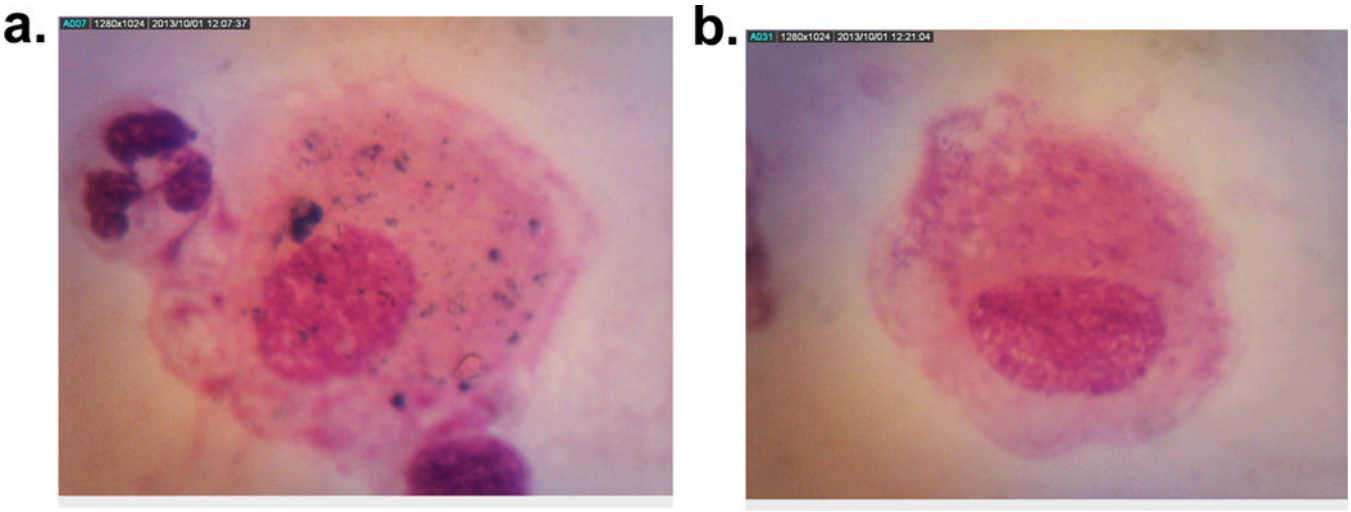

Fig. 1.

Airway Macrophage Black Carbon Content. Digital color images of airway macrophages (AM) in induced sputum. Magnification is at 2000×. Image (a) is an example of an AM where black carbon content is visible intracellularly. Image (b) has no black carbon content within the AM.

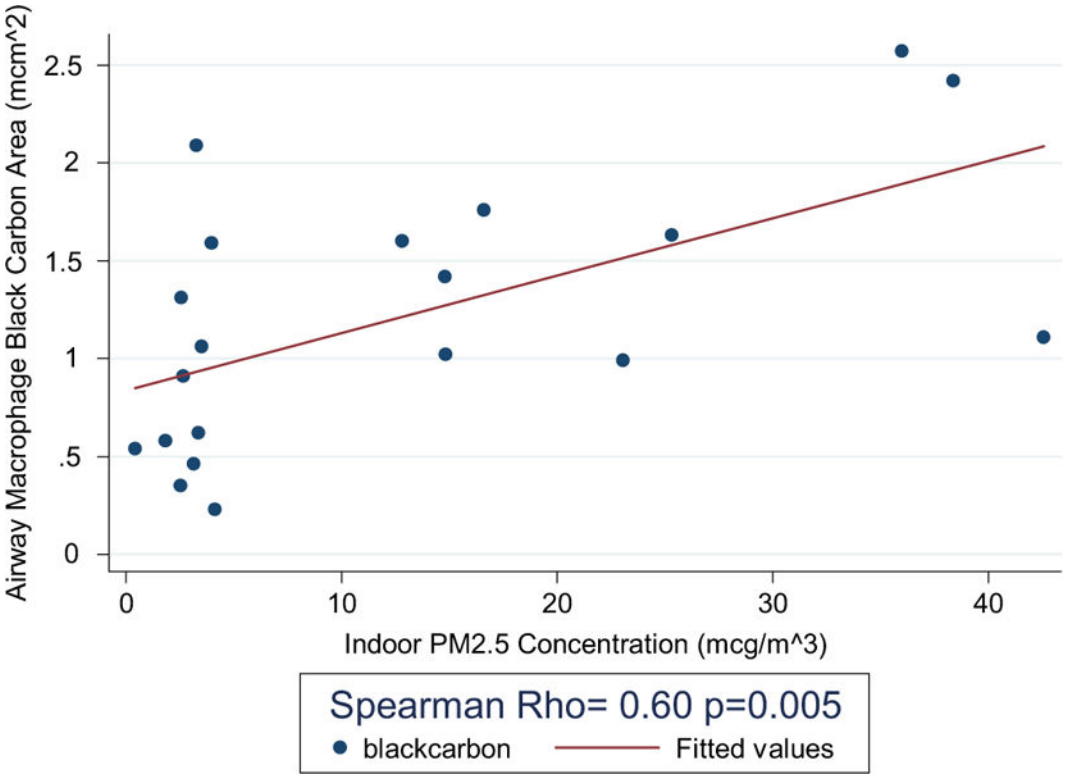

Fig. 2.

Correlation of $PM_{2.5}$ Concentrations and Airway Black Carbon Area at baseline visit. Higher concentration of indoor airborne particulate matter is significantly correlated with the area of black carbon visualized within airway macrophages (rho = 0.60, p = 0.005).

Table 1

Participant characteristics.

Participant characteristics	**n=20**
Age, mean years (SD)	66.6 (3.9)
Gender, n (%) male	14 (70)
Race, n (%)	
Caucasian	17 (85)
Black/African American	3(15)
Education, n (%)	
< High School	4 (20)
High School	7 (35)
Some College	8 (40)
Graduate Degree	1 (5)
Smoking History, mean (SD)	
Pack Years	52.4 (23.9)
Years smoked	36.0 (9.6)
Last Cigarette (years since)	12.4 (9.0)
Baseline Lung Function, mean (SD)	
Pre-BD FEV_1, L	1.51 (0.79)
Pre-BD FEV_1, % predicted	51.6 (19.6)
Pre-BD FEV_1/FVC	0.53 (0.12)
Post-BD FEV_1, L	1.61 (0.82)
Post-BD FEV_1, % predicted	54.8 (20.1)
Post-BD FEV_1/FVC	0.54 (0.11)
Sputum Inflammatory Cells, mean (SD)	1.5 (1.1)
Total cell count, $\times 10^5$ cells/mL	55.8 (24.3)
Neutrophils, %	38.4 (19.9)
Macrophages, %	3.5 (7.0)
Eosinophils, %	2.8 (6.8)
Lymphocytes, %	
Fine Particulate Matter ($PM_{2.5}$), $\mu g/m^3$ mean (SD)	12.8 (13.5)
Airway Black Carbon, μm^2 mean (SD)	1.2 (0.7)

Table 2

Bivariate analysis: alveolar macrophage black carbon content.

	β	p	95% CI
Cross-Sectional Analysis			
Indoor $PM_{2.5}$	**0.29**	**0.01**	(0.09, 0.49)
Outdoor $PM_{2.5}$	0.24	0.65	(−0.85,1.33)
Longitudinal Analysis			
Indoor $PM_{2.5}$	**0.22**	**0.01**	**(0.04, 0.40)**
Outdoor $PM_{2.5}$	0.11	0.74	(−0.52, 0.74)

Table 3

Multivariate analysis: alveolar macrophage black carbon content per 10 mcg/m3 increase in $PM_{2.5}$.

	Single pollutant adjustment			Models adjusted for both indoor and outdoor PM		
	β	**p**	**95% CI**	**β**	**p**	**95% CI**
Cross-Sectional Analysis						
Indoor $PM_{2.5}$	**0.26**	**0.02**	**(0.05, 0.46)**	**0.21**	**0.01**	**(0.05, 0.38)**
Outdoor $PM_{2.5}$	0.07	0.89	(−0.99, 1.13)	−0.33	0.32	(−1.0, 0.33)
Longitudinal Analysis						
Indoor $PM_{2.5}$	**0.19**	**0.01**	**(0.05, 0.34)**	**0.21**	**0.01**	**(0.06, 0.36)**
Outdoor $PM_{2.5}$	0.0004	1.0	(−0.59, 0.59)	−0.32	0.30	(−0.91, 0.27)

Environ Res. Author manuscript; available in PMC 2017 May 19.