ASSESSING AMBIENT FINE PARTICULATE MATTER EXPOSURE AND ASSOCIATIONS WITH CORONARY ARTERY CALCIFICATION

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Abstract

Long-term exposure ambient particulate matter ($PM_{2.5}$) may be associated with atherosclerosis, increasing the risk of adverse cardiovascular outcomes. Few studies have previously examined this relationship, with most research focusing on two different cohorts: the Multi-Ethnic Study and Atherosclerosis (MESA) and the Heinz-Nixdorf Recall Study (HNRS). While several methods are used to assess the risk of cardiovascular outcomes, this study focuses on coronary artery calcification (CAC) as an indicator of atherosclerosis and, therefore, poor cardiovascular health. This study aimed to find associations between ambient $PM_{2.5}$ concentrations, inflammatory and cardiovascular-specific biomarkers, and CAC. Utilizing data from the Heart Strategies Concentrating on Risk Evaluation (Heart SCORE) cohort in Allegheny County, PA, CAC scores were collected from 724 participants in cohort from 2003-2008 (aged 45-75 years). A general inflammatory marker, Interleukin-6, the cardiovascular-specific augmentation index normalized to 75 beats per minute (AI75), and the Framingham Index (FRHI) were also collected. $PM_{2.5}$ exposure concentrations were determined via active sampling and Land Use Regression (LUR). Each participant's exposure was designated as the $PM_{2.5}$ concentration from the prior year within 300 meters of their address.

All examinations considered potential confounding from age, sex, and race. Statistically significant ($p < 0.05$) associations were found for $PM_{2.5}$ and IL-6 (0.092), as well as between AI75 and CAC (-0.009). Pairwise correlations between $PM_{2.5}$ and IL-6 (0.05) as well AI75 and

FRHI (0.16) were also significant. Comparison of the 90th and 10th percentiles of $PM_{2.5}$ exposure showed a 74.55 HU difference in Agatston score for individuals with presence of CAC. No significant association was found between these exposure percentiles and the whether an individual developed CAC.

To date, this is one of the few studies to examine $PM_{2.5}$ exposure associations to atherosclerotic risk using CAC as opposed to CIMT outside of MESA and HNRS. While the analysis found suggestive evidence of a direct link between CAC and ambient $PM_{2.5}$, the results were not statistically significant. Pairwise correlations between components of the hypothesized pathway were statistically significant, albeit weak. Understanding the association between $PM_{2.5}$ and CAC can impact primary and secondary public health prevention efforts for cardiovascular disease.

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I. Introduction

Air quality has been a major area of concern in environmental health since well before the introduction of the Air Pollution Control Act in 1955 [1]. Subsequent legislation, and creation of the Clean Air Act of 1970, created the standards by which regions seek attainment on several different measures of air quality. Amendments to the Clean Air Act in 1977 and 1990 provided additional research support to assess the public health effects of various stationary and mobile pollution sources [1]. More recently, concern about the effects of ambient air pollution on health endpoints such as atherosclerosis, asthma, chronic obstructive pulmonary disease (COPD), mortality, stroke, myocardial infarction (MI), and diabetes have grown, as evidenced by diverse research within this arena in the past 10 years [2,3].

One emerging area of study pertains to the cardiovascular effects of ambient air pollution. In a 2010 published statement, the American Heart Association linked poor air quality to increased cardiovascular morbidity and mortality rates when compared to areas of higher air quality [2]. A key index of air quality is particulate matter (PM), which is one of the six criteria pollutants regulated by the US Environmental Protection Agency (EPA) National Ambient Air Quality Standards [1]. This paper focuses on the cardiovascular effects of particulate matter that is less than 2.5 microns in diameter, also known as fine particulate matter or $PM_{2.5}$. Effects of high $PM_{2.5}$ exposure include an increased risk of mortality in people with heart or lung disease, an increased incidence of asthma, decreased lung function, and a wide-range of respiratory effects [4]. Various cardiovascular effects due to $PM_{2.5}$ exposure include heart rate variability, stroke, ischemia, and myocardial infarctions (MI) [5]. As such, cardiovascular morbidity and mortality rates have been shown to be elevated with acute increases in ambient $PM_{2.5}$ [6]. Many previous studies have focused on short-term effects of ambient air pollution; however, long-term

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exposure to ambient fine particulate matter may also lead to increased risk of the adverse health effects [6-10, 13].

Chronic exposures to ambient air pollution may be associated with atherosclerosis, or the thickening of artery walls due to high accumulation of white blood cells, and this health effect is also indicative of high risk for acute events such as stroke or MI [12]. Atherosclerosis is considered one of the prominent cardiovascular pathologies and can lead to permanent structural and functional changes of the arteries [5]. While an individual's degree of atherosclerosis increases over time, other factors can influence the rate of progression [5, 7-9, 11-13]. Potential environmental contributors of atherosclerosis have been examined in various cohort studies, including the Multi-Ethnic Study of Atherosclerosis (MESA) and the Heinz Nixdorf Recall Study (HNRS) [7-9, 11-13]. Animal studies have also provided ample evidence into the toxicological effects and biological plausibility of increasing $PM_{2.5}$ exposure on atherosclerosis progression [5, 13]. Investigations into the long-term effects of ambient $PM_{2.5}$ exposure on atherosclerosis are needed to fully understand the risks associated with specific air quality levels. According to Kunzli *et al*. (2011), preventing the development of atherosclerosis holds larger public health significance than focusing on incidences that trigger acute events such as stroke or MI as primary prevention efforts have an arguably larger impact than secondary [5].

The pathogenesis of atherosclerosis starts at birth and progresses over an individual's lifetime, presumably with additional factors influencing the rate of development. Additionally, the degree of atherosclerosis in any individual would reflect a combination of all factors influencing an increase in progression. These factors would include genetic predisposition, internal (i.e. Body Mass Index), and external influences (i.e. environmental exposures) [5, 10-12,

14]. While several noninvasive, subclinical measures have been established to measure the degree of atherosclerosis, reliability issues hinder their employment.

Kunzli *et al*. aimed to aggregate current findings and outlined five key criteria for surrogate, subclinical markers for atherosclerosis (2011). First, the marker should be continuous, indicating a consistent trend with progression of the disease. Repeated measures of the subclinical marker would then indicate progression of atherosclerosis. Second, the desired marker should be indicative of a long-term load, not just recent exposures. By this measure, markers geared solely towards short-term exposures would not adequately capture the chronic nature of the exposure. Third, the marker must have high specificity to the cardiovascular system, not simply indicative of general inflammation. Fourth, an ideal measure should have a causal relationship with the progression of atherosclerosis. Fifth and finally, the marker should be affordable, noninvasive, accurate, and reliable [5]. Biomarkers fitting all criteria have been utilized in previous clinical assessment studies and primarily focus on two main indicators of atherosclerosis [7-9, 11-13].

Carotid intima-media thickness (CIMT), broadly, is the measurement of the tunica intima and tunica media within the carotid artery [5]. In theory, these inner layers of the carotid artery would increase in thickness with the progression of atherosclerosis. In keeping with the guidelines established by Kunzli *et al*. (2011), the CIMT marker is non-invasive and measured via a real-time B-mode ultrasound. CIMT also examines a specific anatomical region across all measurements and can be used as a marker for long-term exposure, as increased thickness is not very sensitive to acute changes that may occur in day-to-day variation in ambient air pollution concentrations. A drawback to the CIMT marker is the possibility of variability in measurement

interpretation and quantification. At later stages of atherosclerosis, plaque may cause clinicians to overestimate and skew interpretation of CIMT. Therefore, detailed descriptions of individual measurements must be considered when comparing across multiple studies [5]. With clear explanations of CIMT measurement techniques and standardization of protocols, the negative aspects of this measure are nullified and, therefore, can be relied upon for subclinical analysis of atherosclerosis.

The second main marker utilized as an indicator of atherosclerosis is the amount of coronary artery calcification (CAC), measured via the quantity of calcified plaque found in the coronary artery. The most common quantification of this measure is the Agatston score, measured in Hounsfield Units, and calculated by electron-beam multidetector computed tomography [5, 15]. Use of CAC to predict atherosclerosis is not as common as utilization of CIMT, but multiple studies have concluded that CAC is a better predictor of cardiovascular events compared to CIMT [5, 15]. Although CAC may be more reliable, this marker is largely undetectable in individuals under the age of 50 or is very low [5]. For cohorts with a larger proportion of young participants under age 50, relying solely on this marker may result in many data values of zero, or a positive skew. Use of CAC as an indicator of atherosclerosis does fit the other four Kunzli criteria for effective atherosclerosis markers, however.

This paper aims to examine the relationship between outdoor $PM_{2.5}$ exposure and subclinical measures of atherosclerosis. A comprehensive review of literature regarding this link will be conducted, focusing on studies examining associations between CAC measures with ambient $PM_{2.5}$ concentrations. Following the review, a study of $PM_{2.5}$ exposures relating to CAC measures within the Heart Strategies Concentrating on Risk Evaluation (Heart SCORE) cohort

will be conducted. Findings from the Heart SCORE cohort will subsequently be compared to existing conclusions from previous studies.

II: Literature Review

The literature review regarding the link between ambient air pollution and atherosclerosis included all articles from a query on PubMed (National Center for Biotechnology Information, operated by the National Library of Medicine) and PittCat+ (The University of Pittsburgh University Library system). Searches were not limited by date and included articles published through January 2015. Searches were conducted using the following terms: "CAC", "coronary artery calcification", "coronary calcification", "coronary calcium", and "augmentation index" in all combinations with "particulate matter", "fine particulates", or "PM$_{2.5}$."

The literature search returned five original research articles pertaining to CAC and PM$_{2.5}$ within the context of cohort studies on human populations. Animal studies focusing on the pathogenicity of coronary calcification were excluded. Of the five relevant studies, population data came from two cohorts: the Multi-Ethnic Study of Atherosclerosis (MESA) and the Heinz Nixdorf Recall Study (HNRS). Both studies have been approved by appropriate institutional ethics boards prior to investigation, and participants gave informed consent [13, 16]. Although all five articles included measurements of coronary calcification, only two studies, one from each cohort, focused on CAC as an endpoint. Additionally, a review article summarizing several measures of atherosclerosis in relation to air pollution was written by Kunzli *et al.* in 2011, but this article did not go into detail about any associations between subclinical measures and PM$_{2.5}$. These following sections will review each cohort and the relevant studies separately, and then compare methodologies and results.

A. The Multi-Ethnic Study of Atherosclerosis (MESA)

MESA is a longitudinal cohort of men and women from 45-84 years of age with initial recruitment from 2000-2002 [7, 11]. Participants were initially free of cardiovascular disease at the beginning of the study and have been followed over time with current assessment as recent as 2014. Individuals were recruited from six centers across the United States: Baltimore, Maryland (via Johns Hopkins University); Chicago, Illinois (via Northwestern University); Forsyth County, North Carolina (via Wake Forest University); Los Angeles, California (via University of California-Los Angeles); New York, New York (via Columbia University); and St. Paul Minnesota (via the University of Minnesota) [16]. At baseline, 6,814 individuals were included, providing residential addresses for exposure assessment and screening for CAC measurements [11]. After exclusions, the number of participants in each study center was statistically similar to the others. The cohort is 47.4% male and 52.6% female, and the racial distribution of the final study size (n=5,172) is: 30% Black, 6.9% Chinese, 20.3% Hispanic, and 42.8% White [11]. While some studies of this cohort considered age as a continuous measure, others utilized 10-year groups (e.g. 44-55 years old) [7-9, 11, 13]. Additionally, some researchers chose to focus on certain timeframes to study the cohort, as the original baseline data was collected from 2000-2002 [16]. The MESA cohort also collects data on smoking status, cholesterol, medication use, and hypertension, all of which may influence results [9].

1. MESA Methods and Study Design

Three of the four studies examined the relationship of CAC with $PM_{2.5}$ within the MESA cohort. Diez Roux *et al*. (2007) investigated 20-year exposure windows of particulate matter (PM_{10} and $PM_{2.5}$) related to multiple measures of subclinical atherosclerosis, including CAC. This study examined the full effect size of the MESA cohort (n=5,172) and included exposure

estimates based on residential addresses. The $PM_{2.5}$ concentrations used for exposure assignment were accessed from the US EPA's Aerometric Information Retrieval Service database. The researchers used spatio-temporal modeling to predict residential exposures for all participants based off of monthly $PM_{2.5}$ averages from the database. Participants residing beyond 10km from an air monitor were excluded from analysis, and 20-year exposure estimates were compared with ambient $PM_{2.5}$ concentrations from 2001 (the mid-point of the CAC scoring). The 2001 exposure estimates were then used to investigate possible associations with the CAC values collected during the baseline examination. The 20-year exposure estimates were not used. CAC values were derived from the MESA testing centers, collected using the same methods, and quantified using the Agatston score [11].

The next study to utilize the MESA cohort is a prospective study conducted by Kaufman *et al*. published in 2012. This study examined the progression of subclinical atherosclerosis and exposure to ambient $PM_{2.5}$, with the goal of establishing a relationship between ambient PM and development of cardiovascular disease. This study utilized the same baseline cohort data as Diez Roux *et al*. 2011, including the CAC levels initially collected from baseline [7, 11]. The Kaufman study, however, examined different exposure windows using follow-up examinations of the original baseline participants. In addition to the baseline MESA participants, researchers also recruited from the MESA Family study, and additional subjects were enrolled for the direct purpose of the Kaufman study. In total, the investigation included 6,226 participants from across the six MESA study centers – while also adding two more study areas in California and one additional in New York State. Follow-up CAC examinations of baseline MESA participants will be conducted for 10 years after the initial testing. All other recruits (MESA Family and new recruits) were examined from 2005-2007 [7]. $PM_{2.5}$ data used for exposure assignment will be

collected via several methods, mainly relying on outdoor monitoring from integrated samples, deterministic models, and geospatial data [17]. Personal exposures for each participant were then determined by weighting the external air monitoring measurements with estimated contributions from indoor residential pollutant monitoring and reported time-location information [7].

Sun *et al.* (2013) conducted a cross-sectional study investigating exposures of long-term $PM_{2.5}$ (and components) to CAC and CIMT, again in the MESA cohort. Identical to the Diez Roux study, researchers used baseline examination data from the original recruitment of MESA participants from 2000-2002. Residential addresses were manually cleaned and geocoded after participant consent (n= 6,256). Initial exposure assessments were performed in a similar manner to the Kaufman study, using air monitoring data from the MESA Air study fixed air monitors [9]. The Sun group, however, estimated residential exposure using three different methods: 1) annual average concentration of two-week $PM_{2.5}$ measurements at the monitor nearest to each address, 2) inverse distance weighting of all annual average $PM_{2.5}$ monitor concentrations in each area, and 3) city-wide averaging of $PM_{2.5}$ concentrations based on all monitors within the specified area [9]. Additionally, the researchers considered the proximity to roadways for monitors due to the potential influence from mobile sources. To compensate for this potential confounder, monitors within 100 meters of a major road or 50 meters of a secondary road were assigned the average value from the area's MESA Air roadside monitor [9].

Kim *et al.* in 2014 used a cross-sectional design similar to the Diez Roux and Sun studies [8, 9, 11]. Baseline data from the original MESA participant examination were used for clinical outcome measures. Residential addresses were geocoded, and the resulting sample size was 5,488 after the exclusion of participants residing more than 10km away from a sampling monitor. The spatio-temporal model of $PM_{2.5}$ utilized two-week averages from data sampled outdoors

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MESA Air. The resulting exposure estimates consisted of a spatially-varying long-term mean, with a single temporal tendency. The researchers also developed a national spatial model via kriging from all monitors across the study domain. Additional details of the procedure are described in other papers [8].

All but one of the studies utilized a cross-sectional study design. Only the Kaufman study examines the related exposure and outcome measure in a longitudinal fashion, though this claim is limited by studies found as of February 2015. Of the studies using data from the MESA cohort, each used different methods to determine exposure estimates. Initially, Diez Roux used data from the US EPA's Aerometric Information Retrieval Service database and utilized spatio-temporal modeling to predict residential exposures. Exposure assessments were then improved by Kaufman *et al*. (2012) by not only relying on outdoor monitoring via integrated samples, but also incorporating deterministic models, personal exposures for each participant, contributions from indoor pollutant monitoring, and reported time-location information [7]. Sun *et al*. (2013) utilized the same outdoor sampling monitors as Kaufman (MESA Air), but examined exposure using three different methods: 1) annual average $PM_{2.5}$ from the nearest monitor, 2) inverse distance weighting, and 3) city-wide averaging [9]. This study also introduced traffic factors such as density as a possible influence on the outcome measure. The final, related MESA study by Kim *et al*. (2014) used the same MESA Air monitors as the previous studies, but excluded participants living more than 10km away from the nearest monitor as pollutant concentration estimates are not as accurate at these distances. Spatio-temporal modeling via universal kriging was then conducted to create exposure estimates for the included participants [8].

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2. MESA Statistical Analysis and Results

Each of the four studies comparing CAC and PM$_{2.5}$ exposure considered unique methods to determine possible associations. Diez Roux *et al*. identified potential confounders as age, sex, race/ethnicity, and associated cardiovascular risk factors (e.g. hypertension, cholesterol levels, and smoking status) [11]. To investigate the relationship between the exposure to PM$_{2.5}$ and presence of CAC, investigators used binomial regression after adjustment for the covariates. Linear regression was used to compare participants with non-zero CAC scores, which were log-transformed to normalize the distribution. The investigators also compared the 10th percentile of exposure to the 90th percentile to determine the odds ratio of the difference.

Diez Roux *et al*. found that 50% of their study population tested positive for detectable CAC, with the mean non-zero Agatston score of 90.3 units [Interquartile Range (IQR) = 285.64 units]. Of the MESA cohort, 43% remained at the same address for the entirety of the 20-year exposure window, and 69% remained within 10km of an outdoor monitor for the study period. Imputed particulate matter results from the predictive model showed a 0.93 correlation with observed concentrations. Mean 20-year exposures for each participant were used in the analysis and also were correlated to the 2001 estimates (0.64, Pearson). From this correlation, researchers compared the 2001 estimates to the baseline CAC measures and compared the 10th decile to the 90th decile of exposure and comparted via relative difference ratios. Diez Roux *et al*. found that PM$_{2.5}$ exposures were weakly associated with the presence of CAC but were not significant, with a ratio of 1.00-1.06 depending on exclusions. The relative difference ratio was also negatively associated with CAC for individuals with any calcification, but these results were not significant as the confidence intervals included the null value [11].

Kaufman *et al.* 2012 also considered the effect modifiers of age, race/ethnicity, gender, obesity, diabetes, hypertension, and medication status. Results of this analysis were not available as of February 2015. Analysis was conducted separately in a cross-sectional function, as well as a longitudinal examination. CAC measurements were compared to the individually-determined exposures for each participant. While the Agatston score was utilized for the cross-sectional analysis, the longitudinal study required a different quantification of coronary artery calcification to show progression of the condition. Investigators noted the same 50% prevalence of CAC in the baseline MESA participants, and hypothesized a rate change of +0.074 Agatston units per year [7]. Though this study is prospective, the fundamental methodology for exposure assessment and outcome estimation should be considered for this review.

The next study utilizing the MESA cohort was a cross-sectional design by Sun *et al.* who used linear regression, controlling for several different variables, to estimate associations between subclinical measures and $PM_{2.5}$ exposures. Several models controlling for separate confounding factors were considered by this study, however Model 1, controlling for age, sex, and race/ethnicity is the most comparable to other studies. This study also considered Agatston scores greater than zero (49% of cohort), and overall presence of CAC independently. Based on the "near roadway" variable described previously, 28.4% of the PM2.5 concentrations used for exposure assignment to cohort participants were influenced by traffic-related factors. The other methods of exposure assessment included utilizing the value of the nearest monitor, inverse distance weighting, and applying the daily, city-wide $PM_{2.5}$ average for a year-long exposure window. While the study also examined some components of $PM_{2.5}$, this paper will solely focus on the Sun *et al.*'s results regarding the influence on CAC by total $PM_{2.5}$, which ranged from 10.3-16.2 $\mu g/m^3$. The relative risk for presence of CAC by each of the three previously

mentioned exposure estimation methods (Section II, Part A: Study Design, Cohort Selection and Exposure Estimation) were not significant in any of the models, with ratios ranging from 0.99 to 1.02. The investigators also log-transformed positive CAC scores to test for associations with $PM_{2.5}$, but did not find that analysis to be statistically significant [9].

Kim *et al.* utilized linear regression to examine possible relevant associations of $PM_{2.5}$ concentrations and log-transformed CAC measures, similar to previous studies. The researchers also conducted a relative risk assessment for presence of CAC within their cohort. As in the Kaufman study, four separate models were considered for regression analysis, which were identical to those in the Kaufman *et al.* study (2012). The mean non-zero Agatston score was 281.7 (SD = 519.4) in the 48.9% of included participants with measurable CAC. Associations were reported for an increase of one interquartile range in each exposure model. The log(CAC) associations ranged from 0.956-0.983 for the spatiotemporal model and 0.894-1.020 for the national model, with all confidence intervals spanning the null value. Similarly, relative risk for presence of CAC ranged 0.997-1.028 for the spatiotemporal model and 1.021-1.106 for the national model, again with confidence intervals containing the null value. Based on these results, there is very little evidence for an association between CAC and $PM_{2.5}$ [8].

B. The Heinz-Nixdorf Recall Study (HNRS)

The HNRS is a prospective cohort study that includes 4,814 individuals from three major cities in Germany: Essen, Mulheim, and Bochum. These cities are located in the highly industrialized and highly populated Ruhr valley. This specific region covers approximately $600km^2$ ($\sim232mi^2$) and has a population 1.2 million [13]. Baseline testing for the participants was from 2000-2003. As in the MESA study, data were also collected on factors that may affect progression of atherosclerosis, including smoking status, cholesterol, disease status, and

hypertension [12, 13]. Interestingly, the HNRS did not collect data on race/ethnicity of the participants.

1. HNRS Methods and Study Design

The only relevant study to utilize the Heinz Nixdorf Recall Study was Hoffmann *et al*. (2007), which assessed long-term ambient fine particulate matter exposure and distance to roadway with CAC. The sample size for this study was 4,494 after preliminary exclusions for address geolocation. Similar to the MESA cohort, the CAC scores were quantified using the Agatston score and were conducted at baseline recruitment. Measured concentrations of $PM_{2.5}$ were collected daily in 2002, and the average concentration for that year was used for exposure estimates as it was the midpoint year of the study recruitment (2002). The European Air Pollution Dispersion Model (EURAD) was used to estimate daily values from official emission inventories, meteorological information, and regional topographic data. The yearly $PM_{2.5}$ concentrations were visualized on 5km grids for the region to illustrate spatial variability. The estimated values showed a 0.86-0.88 correlation with actual collected $PM_{2.5}$ concentrations from sampled sites [13]. The residential addresses were geo-located and overlaid with the grid estimates, resulting in the ambient $PM_{2.5}$ concentrations used for exposure assessment. Distance to roadway was also used as an exposure contributor of $PM_{2.5}$ and categorized with respect to major roads [13]. Bauer *et al*. (2010) was able to improve the specificity of the estimates by reducing the grid size from 5 to 1km^2, but did not specifically examine CAC as an endpoint [12].

2. HNRS Statistical Analysis and Results

Hoffman *et al*. adjusted for age and sex as potential confounders, but did not include race / ethnicity, as the MESA study did. The reason for this omission is possibly due to a homogeneous racial makeup of the cohort. Similarly to previous studies, multiple models were

derived for the purpose of linear regression to determine associations on those individuals with a natural logarithm CAC score greater than one. $PM_{2.5}$ measurements, originally continuous, were then classified into quartiles for interval analysis. Associations were also examined between CAC and each classification of road distance: 0-50, 51-100, 101-200, and 200+ meters from residential address. Many models were used to separately control for confounding factors. Age and sex considered as potential confounders and were accounted for in Model 3. The researchers concluded that individuals living less than 50m to a major road have higher CAC scores, as well as increased risk of adverse cardiovascular effects [13]. Interestingly, the researchers did find that living within 50m of major road is associated with a 10.2% increase in CAC. However, Hoffman *et al*. found that $PM_{2.5}$ exposure and distance to a major road were not correlated. Throughout the five different models examined, each controlling for potential confounders, percent increases in CAC with reduced distance to road were 7-10.1%. Odds ratios for scores above the age- and sex-specific 75th percentile were elevated but not statistically significant, as they included the null effect. The findings from this study suggest an association between $PM_{2.5}$ exposure and CAC, but researchers claim the potential for exposure misclassification as simple distance measurements did not account for influences from multiple roads [13].

C. Key Points

Table 1 provides a summary of comparable points from each of the previous studies. Each study did find a weak positive correlation between ambient $PM_{2.5}$ and CAC; all confidence intervals from each model in each study contained the null value, however, making the results not statistically significant [8, 9, 11, 13]. Diez-Roux, Sun, and Kim all tested the relative risk of detectable CAC in participants of the top tier of $PM_{2.5}$ exposure compared to those in the lowest tier, each with slightly positive, but not statistically significant results [8, 9, 11]. These separate

cohorts examined populations in different parts of the world, but baseline data and subsequent outcome assessment were performed at the same time as each study was cross-sectional. Each group collected possible confounders in addition to the indices of interest. The age of participants also was consistent across both cohorts, as was the methodology of collecting CAC measurements. This methodology used the same scanner in the entire HNRS and three (New York, Chicago, and Los Angeles) of the MESA cohorts (GE Imatron C-150, San Francisco, California) [9, 12, 13]. Though the equipment may not have been the same, the methodologies were consistent and considering the total CAC score is reported as opposed to the Agatston score, the differences are considered to be negligible.

The results from all five studies vary but share some similarities which can be compared. The intra-cohort MESA studies drew from the same baseline CAC Agatston scores and method of collection, but differed in their separate exposure assessment methods. Fortunately, Hoffmann *et al*. utilized the same equipment and scoring method as the initial MESA baseline testing, though the exposure analysis was different. All research groups examined the association of log-transformed non-zero Agatston scores with continuous $PM_{2.5}$ exposure estimates. Hoffmann *et al*. found a significant correlation between distance to roadway and CAC, but the findings did not correlate significantly with ambient $PM_{2.5}$ exposure. The results are comparable, even though the method of assessing exposure for each of the participants varied, along with the study domain between the two cohorts. While not definitive individually, collective insights from the studies' approaches and results can be applied to future research examining the association between $PM_{2.5}$ and CAC.

Table 1. Summary of Articles Examining Ambient PM2.5 Exposure and CAC

Author, year, cohort	Sample Size	Exposure Assessment	Examination Method	Associations	Comments
Diez-Roux *et al.* **2007,** MESA	5,172	20-year average of residential $PM_{2.5}$ from USEPA Aerometric Information Retrieval System; spatio-temporal modeling with monthly averages; corresponding to midpoint year of CAC collection (2001)	Differences between 90th and 10th percentile of exposure; RR	RR: 1.00-1.06; Not statistically significant	No air sampling for exposure assessment; limited by sensitivity of pollutant estimation
Kaufman *et al.*, **2012,** MESA	6,226	10-year prospective study; outdoor monitoring, integrated sampling, deterministic models, geospatial data, indoor monitoring	Linear regression of exposure with CAC progression in scores >0	NA	Longitudinal study with unpublished results as of February 2015
Sun *et al.*, **2013,** MESA	6,256	Exposure estimates during collection; used 1-year, 2-week, and city-wide averaging	Linear regression with nonzero CAC; RR within IQR	Linear regression and IQR RR: 0.99-1.02, not statistically significant	Did not exclude participants living far away from monitors
Kim *et al.*, **2014,** MESA	5,488	Exposure during baseline testing; compared 2-week city-wide averages to a national exposure model	RR assessed between IQRs of each exposure model	RR: 0.997-1.03 for the 2 week average; 1.02-1.1 for national average; neither statistically significant	Did not directly monitor the regions for exposure estimates and used data from MESA but did exclude individuals 10km away from site due to inaccurate exposure assessment
Hoffmann *et al.*, **2007,** HNRS	4,494	Mean daily values from EURAD for the midpoint year (2002); also considered proximity to roadway	Linear regression; CAC prevalence with proximity to roadway	No linear associations; 10.2% increase in CAC for those within 50m of a road	Did not examine race as a confounder. Positive association between distance to road and CAC, but not $PM_{2.5}$ to road.

Considering the strong associations between ambient air pollution and adverse cardiovascular health outcomes, and the established subclinical marker of CAC for atherosclerosis, relatively few studies have investigated the direct link between exposure to ambient fine particulate matter and calcification [5]. The previous studies in this area have utilized data from two cohorts, each with unique exposure assessment methods. The large majority of research has focused on less reliable subclinical markers as they show a stronger association with increases in ambient $PM_{2.5}$ concentration [5, 7-9, 11-13].

III: Testing Associations between $PM_{2.5}$ and CAC within the Heart SCORE Cohort

A. Introduction

Ambient air pollution has been associated with adverse cardiovascular health effects, and elevated concentrations of $PM_{2.5}$ have been linked with increases in mortality and morbidity rates [2-6]. Previous studies have examined the association between short-term exposures and acute health effects such as stroke and MI, but very few have examined the effects of long-term $PM_{2.5}$ exposures [5, 8, 9, 11, 13]. Outcomes associated with long-term $PM_{2.5}$ exposure can be assessed via subclinical measures and do not necessarily produce the hard endpoints associated with acute events. Previous studies have examined the CIMT as a subclinical measure of atherosclerosis, a major factor in cardiovascular disease [5, 6-9, 11-13]. Though this measurement was found to positively correlate with ambient $PM_{2.5}$, other more deterministic subclinical measures are rarely examined. CAC is considered to be a better subclinical measure of atherosclerosis than CIMT, though very few studies have examined the relationship between $PM_{2.5}$ and CAC [5].

Of the few studies investigating the association between $PM_{2.5}$ and CAC, three utilized the American MESA cohort and the other utilized data from the German HNRS [5, 7-9, 11, 13].

These cross-sectional studies examined different exposure windows and utilized varying methods for determining residential exposure for study participants. All studies found a weak, positive association between $PM_{2.5}$ and CAC, though none of the results were significant [8, 9, 11, 13]. This study hypothesizes that exposure to higher ambient $PM_{2.5}$ concentrations will result in increased inflammation, as well as a higher CAC score within the Heart Strategies Concentrating on Risk Evaluation (Heart SCORE) cohort. A pathway will be investigated for associations directly between $PM_{2.5}$ and CAC, as well as associations between $PM_{2.5}$, various biomarkers, and CAC.

B. Methods

The Heart SCORE cohort, originally 2,000 individuals located in southwest Pennsylvania, was recruited from 2003-2006. Demographic data and inflammatory marker Interlukin-6 (IL-6) were collected at the first clinical visit. Participants also provided their residential address at this date. Several exclusions, made based off geocoded address and availability of biomarkers are described in 4. Biological Markers2. Geolocating Addresses. CAC examination was conducted from 2003-2008 using electron beam tomography, similar to the MESA and HNRS cohorts [18, 19]. This group has previously been classified as having less than ideal cardiovascular health classified by smoking status, body mass index (BMI), physical activity, diet, cholesterol, blood pressure, and glucose level. The Heart SCORE cohort is considered high risk group for adverse cardiovascular health events [20].

1. Exposure Study Domain

The study domain was limited to Allegheny County, Pennsylvania. A spatially saturated air monitoring campaign was conducted at the University of Pittsburgh, across an established domain capturing major industrial and traffic sources surrounding the Pittsburgh metropolitan

area [21, 22]. Researchers collected weeklong integrated samples of $PM_{2.5}$ throughout the domain during summer 2012 and winter 2013. This domain captured 209 of the 1175 km^2 within Allegheny County (20.5%). Land use regression (LUR) was used, which is based on the principle that concentrations of pollutants at any location are dependent on the environmental characteristics of the surrounding area, and is often used to predict air pollution exposures of research study participants. The final model estimates $PM_{2.5}$ concentrations based on weekly sampling sessions within the previously defined domain that were used in building a merged annual LUR model, and then extrapolated to derive an exposure surface for Allegheny County. The impact of sources outside of Allegheny County could cause inaccurate exposure estimates, since we did not sample across other surrounding counties. Therefore, study domain was not extrapolated outside of Allegheny County as predicted concentrations farther away from the actual sampling domain may not be accurate.

2. Geolocating Addresses

Participant addresses were first standardized for consistency and then further cleaned using the ZP4 address identifier (Semaphore Corporation, 2014). Of the original 2,000 addresses, 31 were post office (P.O.) addresses and excluded. Of the remaining geo-codable addresses, 98% (n = 1,930) were accurately matched with an address locator developed from TeleAtlas in GIS ArcInfo (Version 10.1, ESRI, Redlands, CA). To determine exposure estimates (described in 3. Population Exposure Estimates), a 300m buffer was constructed around each geolocated address point, which considers the average $PM_{2.5}$ concentration within a 300m radius of a participant's address. Participants residences with buffer regions contained completely within the boundary of Allegheny County were included in our study population. After all exclusions, the total sample size represents 88.2% of the original cohort (n = 1,766) (Figure 1).
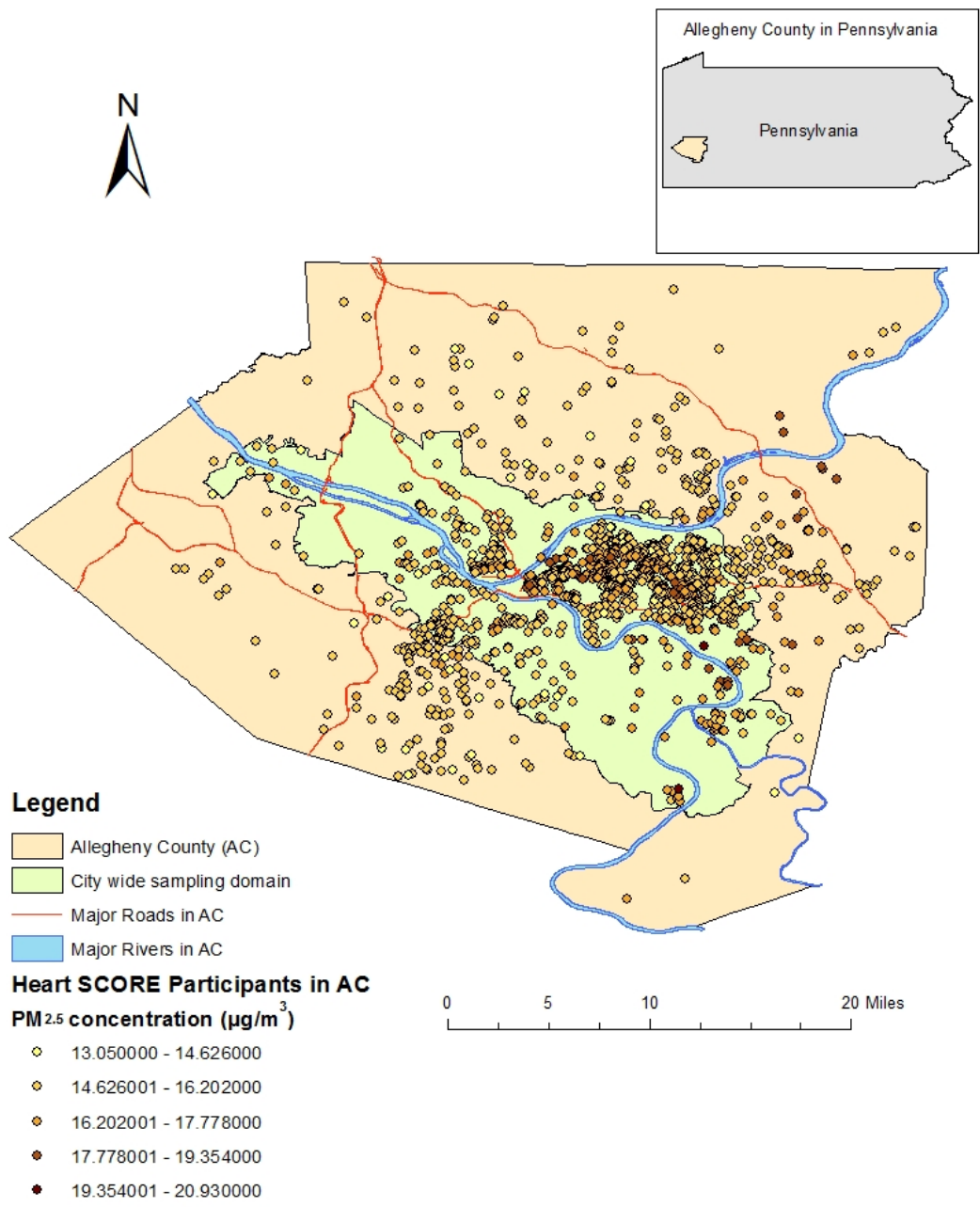
Figure 1. PM$_{2.5}$ Concentrations for 1-year Exposure Window

3. Population Exposure Estimates

The LUR predictive model provides pollutant concentration estimates on a $100m^2$ grid of Allegheny County. A centroid placed in the center of each grid block represents the average predicted concentration of $PM_{2.5}$ for that $100m^2$ location. For each participant's residential address, the mean predicted $PM_{2.5}$ concentration from all centroids contained within the 300m buffer previously described represents the estimated $PM_{2.5}$ exposure for that individual.

The concentrations estimates from this prediction model is only relative to when the air monitoring was conducted, however. To temporally adjust for the exposure window relevant to when the cohort was tested, a monitoring station in Lawrenceville was considered. Particulate matter concentrations have been collected consistently at this permanent EPA monitoring station since 2001, which is maintained and operated by the Allegheny County Health Department. Use of this monitor was for temporal variability across years not sampled by Tunno *et al.* as the site was contained within the original sampling domain and is considered representative of the area. Using monthly average $PM_{2.5}$ concentrations for the Lawrenceville site, exposure windows of 1-year and 2-years prior to when each participant was examined were considered. These exposure windows were chosen as $PM_{2.5}$ concentrations. Average $PM_{2.5}$ concentrations from these exposure windows are relative to when each biological markers were collected. As the concentrations from the exposure windows are only relative to the Lawrenceville site, a ratio is needed to adjust for spatial differences across the domain. Concentration estimates for each participant's address were divided by the median concentration of $PM_{2.5}$ across the entire domain. The subsequent spatial ratio represents the concentration of $PM_{2.5}$ within a 300m buffer of a person's residence to the median concentration of $PM_{2.5}$ of the entire domain. The spatial ratios derived from LUR model remain the same across the investigated time period. The spatial

ratio was then multiplied by the average concentration for each participant's exposure window calculated from the Lawrenceville site. The resulting concentration represents an individual's estimated $PM_{2.5}$ exposure 1- or 2-years prior to when each biological marker was collected.

4. Biological Markers

The marker interleukin-6 (IL-6) was measured at baseline examination and is indicative of general inflammation. Though IL-6 is not specifically indicative of cardiovascular inflammation, it was examined as a first step in the attempt to associate $PM_{2.5}$ to CAC by way of increasing atherosclerotic risk. As a biological marker specific to cardiovascular health, the augmentation index normalized to 75 beats per minute (AI75) was collected at either baseline or a follow-up date. Based on these and other measurements, a Framingham reactive hyperemia index (FRHI) was calculated. Both of these indices are indicative of atherosclerotic risk as well as presence of CAC [23]. Of the original 1,766 participants included after geolocating exclusions, 1,658 (93.9%) were tested for IL-6, 41% (724) were tested for CAC, 72.9% (1,288) were measured for AI75, and 71.6% (1,264) were scored on FRHI. Of those individuals tested for the presence of CAC, 552 of 724 (76%) had a total Agatston score greater than zero. Of these participants initially included in the study, 411 have all three cardiovascular measures in common. The final sample size of 381 includes participants with all IL-6, AI75, FRHI, and CAC markers. Only non-zero CAC scores were included in the final 381-person sample size. Demographic information for the cohort before and after exclusions for location and biomarker availability is described in Table 2.

Table 2: Demographic differences between Heart SCORE cohorts before and after exclusions

	Original Heart SCORE Cohort	Heart SCORE Cohort after exclusions
Age*		
Mean	59.2	61.4
SD	7.6	7.4
Range	45-75	45-74
Race (n, %)		
Black	854 (42.7%)	166 (43.6%)
White	1,095 (54.8%)	198 (52.0%)
Other	51 (2.5%)	17 (4.4%)
Sex (n, %)*		
Male	693 (34.7%)	237 (62.2%)
Female	1,307 (65.3%)	144 (37.8%)
Statistically different between total and sample groups		
Total	2,000	381

For analysis, the results of the final group of 381 participants will be compared to specific larger groups of all individuals who had that measure. Figure 2 shows a flow chart of exclusions. While a correlation and association via linear regression will be examined for participants in the final sample size, these results will be compared to the specific larger groups of all participants who share that measure.
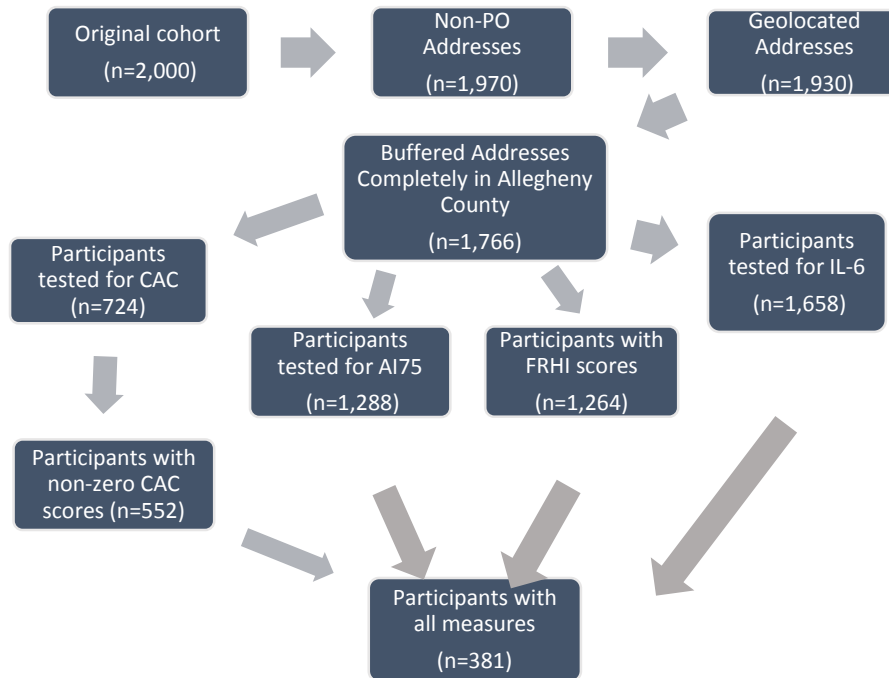
Figure 2. Flowchart of exclusions

5. Statistical Analysis

The mean concentration from monthly averages for PM$_{2.5}$ concentration for each participant's residential location were examined within either a 1- or 2-year exposure window of each biomarker's collection date. The baseline date used for each participant corresponded with the date of examination for AI75, FRHI, or CAC, which were all collected at different times. The initial exposure window is relative to each participant's baseline examination date for which IL-6 and demographic data (age, sex, and race) were collected. The second exposure window is relative to exposures for CAC-related measures. The third and final exposure window applies to AI75 and FRHI scores. Linear regression will be used to test for associations between all measures Variables will be treated as continuous, with non-zero CAC scores logarithmically transformed. Pairwise correlation will also be conducted for participants with common measures.

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All analyses with exposure as a variable will use concentrations relative to when that measure was collected. Additionally, non-zero CAC scores in the 90th percentile of exposure will be compared via a Risk Ratio to those in the 10th percentile. For comparative purposes, all analysis will be performed on all individuals with the common measures, as well as those in the smallest sample size who have all measures in common.

C. Results

Initial correlations between one-year and two-year exposure estimates were highly positively correlated (r = 0.92). Based on this high correlation, one-year exposure windows were considered for the remaining analyses. Exposure estimates for the previous year were made with respect to collection of all measurements (Table 3). IL-6 levels ranged from 0.04-12 pg/ml (mean = 2.2; SD = 1.7). Additional information on markers is shown in Table 4. Linear regression between IL-6 and $PM_{2.5}$ showed positive association (beta values) of 0.095 (CI: 0.004-0.186) in crude models and 0.092 (CI: 0.001-0.183) while controlling for age, sex, and race (p<0.05). IL-6 and $PM_{2.5}$ also showed a 0.05 correlation (p < 0.05). Scatter plots of tested associations are show in Figure 3.

Table 3. Associated PM2.5 exposure with collected biomarkers

	Biomarker					
	IL-6		CAC		AI75 and FRHI	
	All measures	Final Sample Size	All measures	Final Sample Size	All measures	Final Sample Size
Participants with measure	1,766	381	552	381	1,288	381
Associated PM₂.₅ exposure (µg/m³)						
Mean	15.73	15.79	15.73	15.74	15.78	15.81
SD	0.91	0.94	0.88	0.91	0.88	0.88
Range	13.05 – 20.93	13.42 – 20.93	13.65 – 21.27	13.65 – 21.27	14.07 – 21.01	14.07 – 21.01
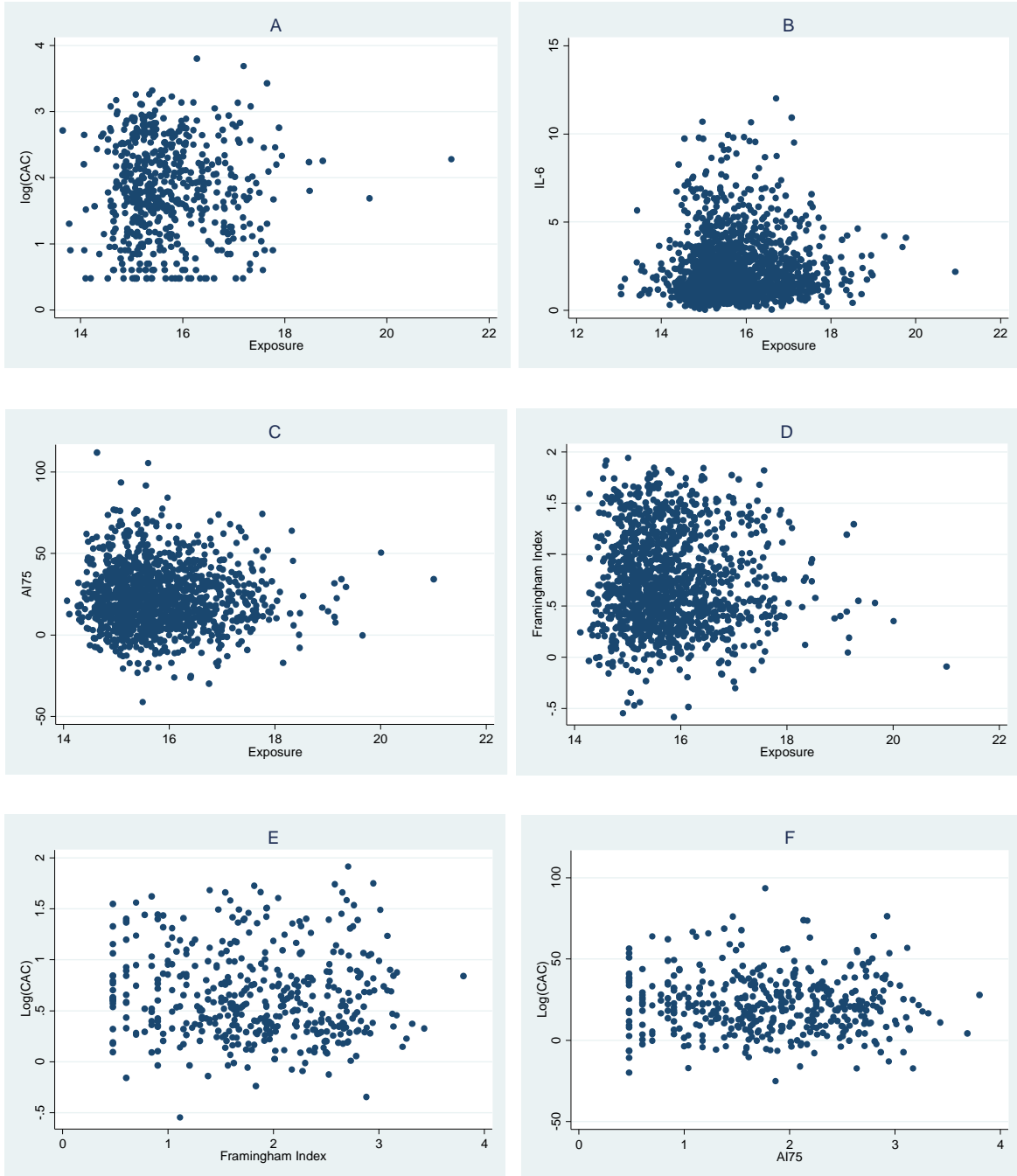
Figure 3: Scatter plots of tested associations

A) $PM_{2.5}$ ($\mu g/m^3$) exposure and log(CAC), B) $PM_{2.5}$ ($\mu g/m^3$) and IL-6 (pg/ml), C) $PM_{2.5}$ ($\mu g/m^3$) and AI75, D) $PM_{2.5}$ ($\mu g/m^3$) and Framingham index, E) Framingham index and log(CAC), and F) AI75 and log(CAC).

Table 4. Biomarker data for all tested participants and those with all markers

	Biomarker (All measures / final sample size)			
	IL-6 (pg/ml) (n = 1,658 / 381)	CAC (total) (n = 552 / 381)	AI75 (n = 1,288 / 381)	FRHI (n = 1,264 / 381)
Mean	2.20 / 2.09	222.1 / 187.8	22.82 / 21.3	0.73 / 0.66
SD	1.7 / 1.5	458.8 / 269.8	18.6 / 17.1	0.46 / 0.43
Range	0.04 – 12 / 0.04 – 9.36	3 – 6332 / 3 – 1379	-41.2 – 111.9 / -25.1 – 76.3	-0.58 – 1.94 / -0.54 – 1.91

Nonzero CAC scores were log-transformed and analyzed via linear regression, controlling for age, sex, and race. This analysis showed a positive 0.016 association (beta value) equating to a 1.04 increase in CAC score for each increase of 1 µg/m^3 of PM$_{2.5}$. This measures had a 95% confidence interval of -0.05-0.08, but was statistically not significant from the *p*-value ($p = 0.61$) and the confidence interval containing the null effect (Table 5 and

Table 6: Associations via linear regression and pairwise correlation individuals with all measures in common (n=381).).The effects of age, sex, and race, on each correlation and association were different and unique. All three variables appeared to have an effect on correlations with log(CAC) (p < 0.05) except for IL-6. Correlations with AI75 were influenced by all three variables when comparing the largest possible pairs, but race was not statistically significant in the final sample size. A positive correlation between AI75 and FRHI was found in both the larger comparison and smallest sample group to be statistically significant.

Nonzero CAC scores were measured in 552 of the 724 participants. To determine relative prevalence of coronary artery calcification, exposure ranges for the 90th and the 10th percentile of those tested for CAC were calculated (Table 7). Of those within these percentiles, a positive relative risk ratio was calculated (1.04), though is not statistically significant (Table 8).

Table 5. Associations via linear regression and pairwise correlation for all individuals with each measure

	Biomarker Statistically significant measures are in **bold**			
	IL-6 (n=1,766)	Log(CAC) (n=552)	AI75 (n=1,288)	FRHI (n=1,264)
Association with PM$_{2.5}$ concentration via linear regression				
Beta	**0.092**	0.016	-0.37	-0.01
p-value	**0.04**	0.6	0.5	0.41
r^2	0.006	0.19	0.14	0.05
CI	**0.001-0.183**	-0.05-0.08	-1.45 – 0.7	-0.04 – 0.016
Correlations				
PM$_{2.5}$ (*p*-value)	**0.05 (0.04)**	0.008 (0.85)[1,2,3]	-0.03 (0.34)	-0.04 (0.2)[2,3]
IL-6 (*p*-value)				
Log(CAC) (*p*-value)	0.007 (0.87)			
AI75 (*p*-value)	-0.04 (0.19)[1,2,3]	-0.03 (0.53)[1,2,3]		
FRHI (*p*-value)	-0.03 (0.2)	-0.06 (0.2)[1,2,3]	**0.08 (0.006)[1,2,3]**	

Table 6: Associations via linear regression and pairwise correlation individuals with all measures in common (n=381)

	Biomarker Statistically significant measures are in **bold**			
	IL-6	Log(CAC)	AI75	FRHI
Association with PM$_{2.5}$ concentration via linear regression				
Beta	0.12	0.01[1,2]	-0.5[1,2,3]	-0.014[2,3]
p-value	0.14	0.83	0.59	0.55
r^2	0.012	0.19	0.14	0.03
CI	-0.04 – 0.28	-0.06 – 0.08	-2.32 – 1.33	-0.06 – 0.03
Correlations				
PM$_{2.5}$ (*p*-value)	0.008 (0.11)	-0.013 (0.8)[1,2,3]	-0.04 (0.4)	-0.04 (0.4)
IL-6 (*p*-value)				
Log(CAC) (*p*-value)	-0.03 (0.66)			
AI75 (*p*-value)	-0.08 (0.1)[1,2]	-0.001 (0.98)[1,2]		
FRHI (*p*-value)	0.01 (0.89)	-0.04 (0.4)[1,2,3]	**0.16 (0.001)[1,2]**	

[1]Age statistically significant

[2]Sex statistically significant

[3]Race statistically significant

Table 7. Relative difference in average CAC of individuals in the 90th and 10th exposure percentile

	# Individuals	Average CAC
90% exposure (>16.96)	73	226.82
10% exposure (<14.82)	70	152.27

Table 8. Prevalence of CAC in individuals in the 90th and 10th exposure percentiles

	CAC present	No CAC	Totals
90% exposure (>16.96)	54	19	73
10% exposure (<14.82)	50	20	70
Totals	104	39	143

Relative risk	1.04
95 % CI	0.847 to 1.266
Significance (95%)	P = 0.733

Measurements of AI75 were collected from 72.9% (n = 1,288) of the geolocated participants. Exposure estimates were calculated for the previous year, with respect to when the AI75 measurement was calculated and a single outlier was removed using the following equation: μ +/- $3*\sigma^2$, where μ = mean of the population and σ^2 = standard deviation. This equation was used to identify outliers from the other markers, though none were found. Linear regression between this exposure window and AI75 measures were not statistically significant in either a crude model, or one controlling for age, sex, and race.

Finally, FRHI scores were calculated on 71.6% (n = 1,264) of the participants not previously excluded. No index measures were deemed to be outliers, as determined by the previous equation. Associations via betas from linear regression, both crude and controlling for potential confounders, was not statistically significant ($p = 0.59$ and $p = 0.76$), with both confidence intervals also including the null value. While sex and race were significant modifiers ($p = 0.01$ and $p = 0.02$), age was not a statistically significant modifier in the model ($p = 0.14$).

Considering the 90th and 10th percentiles for $PM_{2.5}$ exposure, differences in AI75 and FRHI are summarized in Table 9. No statistically significant difference was found between the groups, however.

Table 9. Relative differences between the 90th and 10th percentiles of exposure for average AI75 and average FRHI

	# Individuals	Average AI75	Average FRHI
90% exposure (>16.96)	131	22.73	0.71
10% exposure (<14.84)	125	22.98	0.73

AI75 and FRHI are markers of cardiovascular health, but they may be a better indicator of atherosclerosis than the general inflammatory marker IL-6. Linear regression was also performed on AI75 values and logarithmically-transformed, non-zero CAC measures, while controlling for age, sex, and race. All groups of values had been "trimmed" for outliers and only individuals with both measures were included (n = 381). An association via linear regression of -0.009 was found between AI75 and log-transformed, non-zero CAC score (CI: -0.018 - -0.001), and was statistically significant ($p = 0.03$). Potentially confounding variables of sex and age had a significant effect ($p < 0.05$), but not race. Results for linear regression were similar when correlating FRHI and CAC scores. An association of -0.102 (CI: -0.248 – 0.043) was calculated but not statistically significant ($p = 0.17$). As with the other regression model, FRHI had significant confounding from age and sex, but not race ($p = 0.18$).

D. Discussion

The initial aim of this study was to examine a possible association between $PM_{2.5}$ and the subclinical outcome, CAC. Though CIMT has been associated with elevated ambient $PM_{2.5}$ concentrations [5], CAC is proven to be a better marker of cardiovascular disease [5, 8, 9, 11, 13]. Additionally, indicators of cardiovascular disease (AI75 and FRHI) were also examined. In

theory, higher concentrations of ambient $PM_{2.5}$ exposure would be indicative of a higher chance

of CAC. The examined pathways in this study are illustrated in Figure 4. All examined pathways

were examined as crude models and controlled for the potential confounders of age, sex, and

race.
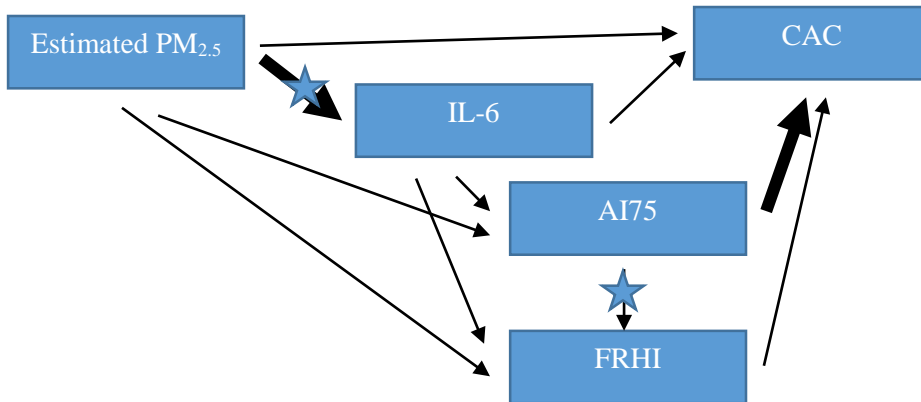
Figure 4. Possible associations between estimated $PM_{2.5}$ exposure and various clinical measures.

Heavily weighted arrows indicate a statistically significant association from linear regression ($p<0.05$). Arrows with stars indicate a statistically significant pairwise correlation.

While an association was found between $PM_{2.5}$ and the general inflammatory marker IL-

6, no statistically significant associations with $PM_{2.5}$ was found for either of the cardiovascular

clinical measures. Previous studies, including the MESA cohort [24], have reported positive

correlations between general inflammation and ambient $PM_{2.5}$ concentrations. As AI75

decreases, the log(CAC) scores increase, similar to that reported by Avalos *et al.* (2007) [25].

Attempts to directly associate ambient $PM_{2.5}$ exposure and nonzero CAC scores proved to not be

statistically significant. Potential confounders of age and sex were significant in all linear

regression models and are consistent with the controlling models from previous studies [8, 9, 11,

13]. Race as a confounding variable did not appear statistically significant in either the

exposure→IL-6 model, or the AI75→log(CAC) linear regression. This finding is different from the previous studies, where race was a significant confounder [8, 9, 11, 13].

The second method of analysis examined the 10th vs. the 90th percentiles of $PM_{2.5}$ exposure within the cohort. This method was used by Diez-Roux *et al.* and Sun *et al.* to compare presence of CAC in the highest and lowest 10% exposed participants in the study [9, 11]. The risk ratio was found to be 1.035, but was not statistically significant. This finding is consistent with the 1.02 ratio found by Diez-Roux while controlling for the same confounding factors [11]. Sun *et al.* utilized a different method of analysis, determining percentage increases of CAC with an increase in the interquartile range (IQR). Their tests found a negative percentage change in CAC with increasing $PM_{2.5}$ exposure (-0.85 - -1.52%, depending on exposure estimation method) [9]. A similar analysis within this current study resulted in a -17.16% change in IQR. The sample size used for this statistic is smaller than the MESA cohort utilized by Sun *et al.*, (about 1/6th the number of participants) which may be the reason for the large difference. When comparing the 10th and 90th percentiles of exposure, analysis showed a 48.96% increase between the exposure ranges. Hoffmann *et al.* performed a similar analysis, but used adjusted odds ratios for comparing their top quartile of exposure to the standard age and gender specific CAC score. Their findings also showed a slightly positive (1.22), but not statistically significant relationship between the higher $PM_{2.5}$ exposures and increased CAC [13].

The results of the analysis were consistent with some of the previous studies examining ambient $PM_{2.5}$ concentrations with CAC [8, 9, 11, 13]. While our study utilized a different cohort and unique exposure data, the association between the estimated concentration and outcome was not statistically significant when analyzed via linear regression. The model used in this analysis controlled for the same potential confounders of age, sex, and race. One possible explanation for

the results is the relatively smaller sample size as the MESA and HNRS cohorts were much larger. Examination of the 10th and 90th percentile of exposure for relative difference in CAC showed similar results to previous studies using the same comparison method [9, 11].

Hoffmann *et al.* were able to find a statistically significant odds ratio (1.45) when comparing the CAC values of those living within 100 meters of a road to the 7th percentile of age- and sex- specific cardiovascular measures [13]. According to the US EPA – 45 million Americans live less than 100 meters from a highway [25]. The Heart SCORE cohort is primarily in an urban environment so this variable may have some effect although previous studies have shown traffic to not be the main source of $PM_{2.5}$ in this region [21]. Examination into the effect of proximity to roadway may elucidate other associations with CAC, but were not examined in this study.

While race was controlled for as a potential confounder in all regression models, it was not statistically significant in either of the main linear regressions. Within the MESA study, race was determined to be a major factor in determining CAC values, which did not seem to be consistent with data from the Heart SCORE cohort [27]. The proportion of races was different between the cohorts and a larger sample size or more equal racial ratios might show statistical significance for race within the Heart SCORE cohort.

1. Study Strengths and Weaknesses

One of the main strengths of this study is the use of a different cohort than the MESA or the HNRS. The Heart SCORE cohort represents a racially diverse population living in a complex terrain with current and historic industrial activity [19, 21, 22]. This study is similar to the HNRS in that the domain encompasses a historically industrial area [13, 21]. The clinical measures were consistent with other studies, making the outcome variables comparable [8, 9, 11, 13, 19]. The

exposure assessment used techniques similar to the MESA cohort estimations, utilizing $PM_{2.5}$ concentrations from monitors and LUR models to create a layer of predicted pollutant concentration across the entire study domain [21, 22]. The one-year exposure window was also used, similar to previous studies [13]. This study also relied on a government monitoring site to temporally adjust for relative exposure windows. Another strength of this study is the similar results found to previous studies with larger sample sizes. Analysis methods consistent with the literature were used to directly compare relative differences, percentage changes, relative risk ratios, and correlations.

One of the drawbacks to the study is the comparatively small sample size used after exclusions for geolocation and collection of subclinical measures. Also, the population in the Heart SCORE cohort after exclusions was only 34.5% male, which may have skewed some of the correlations. The smallest sample size, which included participants will all markers, had this ratio reversed, with 62.7% male. Women have a lower prevalence of detectable CAC at every comparable age for blacks and whites. Additionally, in individuals with measurable CAC, Agatston scores were much higher in men than in women [27]. This study employed exposure estimation which may be more accurate that previous studies, but the high proportion of female participants may have influenced the weak, not statistically significant association between $PM_{2.5}$ and CAC in the larger comparisons. Participants in the study may have been misclassified with respect due to exposure if they failed to report a change of address at some point during the study as the address with respect to each exposure window was used for pollutant estimates.

Furthermore, this study is cross-sectional, not reflecting changes in CAC measure with the corresponding changes in $PM_{2.5}$ over an extended time period. The initial onset and progression of atherosclerosis may occur over a longer time period than examined in this study.

Only two years prior to the data collection were able to be examined, not necessarily reflective of an individual's lifetime exposure. The study domain of Allegheny County has an industrious past, with high concentrations of $PM_{2.5}$ prior to the examined exposure window. A limitation to this study is the lack of reliable exposure data from this time period.

E. Conclusion

To date, this study is one of the few to examine associations between ambient $PM_{2.5}$ and CAC as opposed to CIMT. A hypothesized pathway was investigated with statistically significant associations between pollutant concentration and a general inflammatory marker, as well as a cardiovascular indicator and CAC. The association found, however, was very low. Pairwise correlations were statistically significant between estimated $PM_{2.5}$ concentrations and IL-6. The cardiovascular indicators were weakly correlated with each other and a slight association via linear regression was found between AI75 and CAC.

The analysis did find suggestive evidence of a link between CAC and ambient $PM_{2.5}$, but the results were statistically not significant. Future research into this association will be significant to primary and secondary prevention efforts for cardiovascular disease. Considering intrapersonal influences and more overt external factors may elucidate potential confounders to strengthen the association between $PM_{2.5}$ and CAC (i.e. direct monitoring, residential air sampling, and family history). If a full pathway between primary exposure, inflammatory marker, cardiovascular indicator, and clinical outcome can be established, environmental public health efforts can then be targeted to specific areas in an effort to reduce the prevalence or severity of disease.

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