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Association of pulmonary black carbon accumulation with cardiac fibrosis in residents of Sao Paulo, Brazil

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ABSTRACT

Evidence suggests that myocardial interstitial fibrosis, resulting from cardiac remodeling, may possibly be influenced by mechanisms activated through the inhalation of airborne pollutants. However, limited studies have explored the relationship between lifetime exposure to carbon-based particles and cardiac fibrosis, specially using post-mortem samples. This study examined whether long-term exposure to air pollution (estimated by black carbon accumulated in the lungs) is associated with myocardial fibrosis in urban dwellers of megacity of Sao Paulo. Data collection included epidemiological and autopsy-based approaches. Information was obtained by interviewing the next of kin and through the pathologist's report. The individual index of exposure to carbon-based particles, which we designed as the fraction of black carbon (FBC), was estimated through quantification of particles on the macroscopic lung surface. Myocardium samples were collected for histopathological analysis to evaluate the fraction of cardiac fibrosis. The association between cardiac fibrosis and FBC, age, sex, smoking status and hypertension was assessed by means of multiple linear regression models. Our study demonstrated that the association of FBC with cardiac fibrosis is influenced by smoking status and hypertension. Among hypertensive individuals, the cardiac fibrosis fraction tended to increase with the increase of the FBC in both groups of smokers and non-smokers. In non-hypertensive individuals, the association between cardiac fibrosis fraction and FBC was observed primarily in smokers. Long-term exposure to tobacco smoke and environmental particles may contribute to the cardiac remodeling response in individuals with pre-existing hypertension. This highlights the importance of considering hypertension as an additional risk factor for the health effects of air pollution on the cardiovascular system. Moreover, the study endorses the role of autopsy to investigate the effects of urban environment and personal habits in determining human disease.

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1. Introduction

Cardiovascular diseases (CVD) are the leading cause of deaths worldwide. According to WHO reports, about four of five CVD deaths are due to ischemic events and three quarters occur in low- and middle-income countries (World Health Organization, 2021). A common outcome for nearly all forms of CVD is pathological cardiac remodeling with myocardial interstitial fibrosis.

Myocardial fibrosis is characterized by excessive production and deposition of collagen and other extracellular matrix proteins, which increase cardiac stiffness (Cokkinos and Pantos, 2011; Travers et al., 2016; González et al., 2018). Acute injuries such as myocardial infarction and chronic conditions as hypertensive heart disease have potential implications for the development of cardiac fibrosis and the risk of developing heart failure (Berk et al., 2007; Van den Borne et al., 2010).

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Although diverse risk factors may contribute to underlying pathogenesis and progression of CVD as well as their consequences in structural disarrangement, evidence is accumulating to support that exposure to environmental pollutants and tobacco smoke are potentially associated with this context. It has been largely known that individuals living in large urban areas inhale air particles with carbonaceous components, then resulting in deposition of dark pigments in the lung, described as black carbon (BC) or anthracosis. Previous data have confirmed that the longer residence time in areas with high levels of ambient particles results in large quantities of lung retention of ultrafine particle aggregates and carbonaceous particles in smokers and non-smokers (Brauer et al., 2001; Takano et al., 2019). Therefore, the macroscopic analysis of the lung surface particles may represent an interesting tool for measuring long-term exposure to ambient particles by individuals residing in large urban centers.

In view of the above mentioned, this study takes a step forward in investigating the association of pulmonary retained particles with cardiac fibrosis in individuals that lived in Sao Paulo, the biggest city of South America. Distinct from the majority of human studies correlating the exposure to environmental particles with cardiovascular outcomes, we have combined epidemiological and autopsy approaches to determine more precise assessment of individual responses to inhaled particles and the establishment of causal relationships.

2. Methods

2.1. Study design

The present study is part of the project entitled 'The Use of Modern Autopsy Techniques to Investigate Human Diseases (MODAU)'. Therefore, the study explores aspects of life in megacities on the pathogenesis of human diseases, integrating epidemiological and autopsy-based approaches. A flow diagram summarizing the steps of our study is shown in Fig. 1. The protocols were approved by the Research Ethics Committee of the University of Sao Paulo (numbers 537.195 and 2.955.304) and comply with the Federal requirements for research involving human subjects.

2.2. Setting and sampling strategy

The study was conducted at the Death Verification Service of Sao Paulo (SVOC), which is the largest autopsy center in Brazil, performing approximately 15,000 autopsies per year. The procedures are conducted in four daily shifts (9 a.m. to noon, 3–6 p.m., 9 p.m. to midnight, 3–6 a.m.), operating 24 h per day, 365 days per year. However, the collection of samples for research purposes occurred at a frequency of 2–3 days per week over the course of the 22-month collection period, spanning from March 2017 to December 2018. In addition, it was necessary to provide a humane and compassionate environment to conduct the interviews with the next of kin (NOK), in a scenario framed by pain and mourning. The complexity of the professional working in case selection necessarily limits large-scale sampling on a given day. Although an average of 40 individuals undergo autopsy each day, the fulfillment of the requirements could be achieved for 3 to 4 cases a day. Therefore, we had to employ the convenience sampling method (Golzar et al., 2022), which is a non-probabilistic sampling method.

2.3. Participants and data collection

Before the autopsy procedure, consent for providing information and collecting samples was obtained from the NOK of all the deceased subjects included in the study. A trained interviewer applied a questionnaire to the NOK prior to autopsy to collect reliable and complete information about the deceased, including residential address, socioeconomic index (GeoSES), occupation, daily commuting in the most recent occupation, time of residence in Sao Paulo, smoking status, and chronic comorbidities. The hypertension information was sourced from the family member, while additional cardiovascular diseases are documented in the pathologist's report. The GeoSES values range from −1 to 1, and high values indicate better socioeconomic conditions (Barrozo et al., 2020). Current smokers and former smokers were grouped in the '*yes*' category of tobacco, while non-smokers were grouped into the '*no*' category of

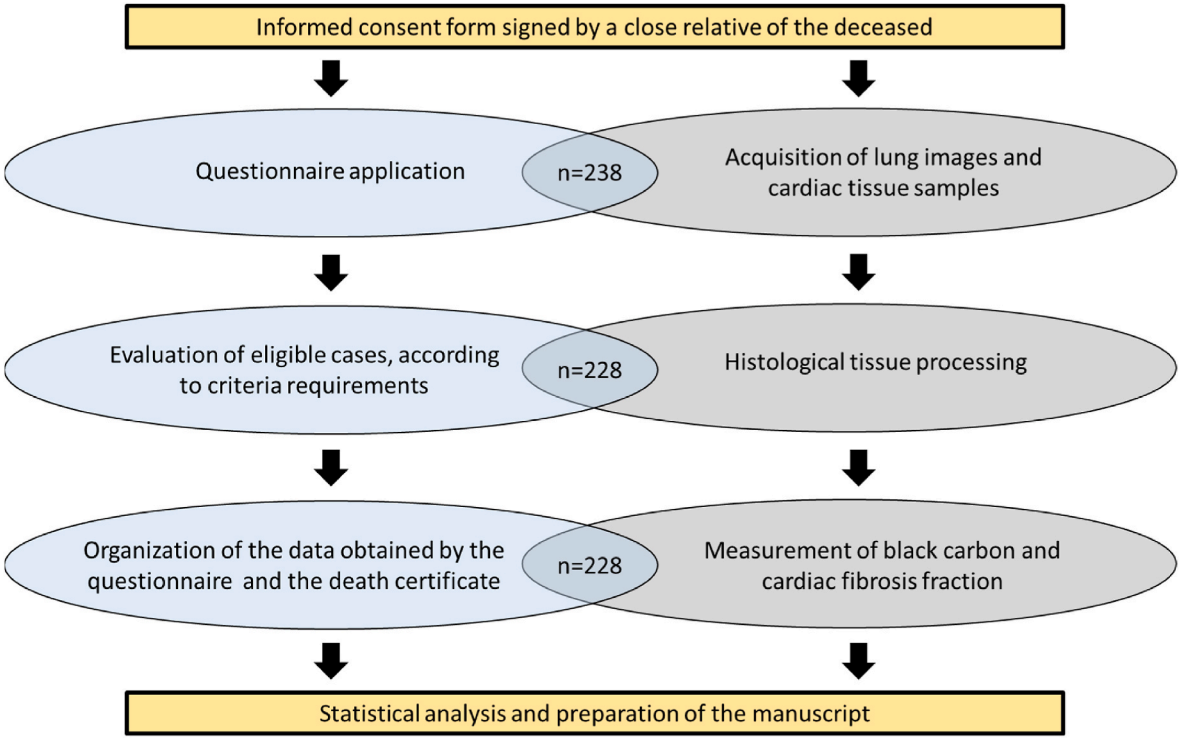

Fig. 1. Flow diagram with the steps of the study.

this variable. The occurrence of environmental tobacco smoking in the residence was also recorded. From the time of residence in Sao Paulo, it was possible to compute the proportion of lifespan living in Sao Paulo defined as the time of residence in Sao Paulo in years divided by age. The daily commuting time was evaluated by asking '*How long did he/she take to go from home to work?'*. When the deceased remained exposed to traffic during all his/her work shift, we adjusted his/her exposure by adding 8 h to his(her) daily commuting time. The cause of death and additional autopsy information were obtained from the SVOC pathologist's report. To be eligible, subjects should be 18 years or older, have died of natural causes, and have a NOK who can provide reliable information during the interview. The exclusion criteria include: refusal of consent by relatives, significant incomplete information provided during the interview and the presence of gross alterations in the lungs suggestive of pneumonia, severe chronic obstructive pulmonary disease (COPD or emphysema), pulmonary granulomatous or neoplastic diseases (primary or metastatic lung cancer). Additionally, cases with severe acute hemorrhagic edema were excluded, as this condition interferes with the color of the visceral pleura. Bodies for which the time elapsed between death and autopsy procedure exceeded 24 h were also excluded.

2.4. Measurement of black carbon deposition

The pigments observed on the lung surface served as valuable markers for particles that had been inhaled and retained in the individual's lungs throughout their lifetime. In this context, we have previously demonstrated a new strategy to quantify the accumulated lifetime exposure to urban air pollution and/or cigarette smoke by measuring carbon-based particle deposition (black carbon or anthracosis) on the pleural surface (Takano et al., 2019). The corresponding approach was used in the present study. Briefly, lungs were removed during the autopsy and the pleural surface was cleaned to place a Petri dish on the anterior surface of the upper and lower lobes of both lungs to flatten the observation area. Pictures of the pleura surface were taken with a high-resolution camera (Canon Power Shot SX400 IS). The ImageJ software was used to generate the point test system on the images of the four lobes. The fraction of black carbon (FBC) of each lobe was estimated by the following: number of black patches/(number of black patches + number of clean pleura). In sequence, the values were used to calculate the mean of the FBC of the whole lung. It is important to note that BC is often used as a proxy to estimate the complex mixture of urban ambient particles.

2.5. Tissue processing, histochemistry and morphological analysis

Cardiac samples (approximately 1.5 cm^3) were collected from the left ventricle wall, specifically to the left of the anterior interventricular artery, for histopathological analysis. These samples were fixed in 4 % formalin for 24 h and then routinely processed for histology. Sections of each paraffin block (5 μm in thickness) were obtained and stained with Picrosirius Red to analyze fibrosis. The stained sections were visualized and images were captured using a Nikon Eclipse E200 microscope coupled to the camera. Images from ten fields of view (20x magnification) of the left ventricle per section were captured for quantification of fibrosis. The collagen volume fraction (%), i.e., the fibrosis fraction, was calculated by determining the area stained for collagen as a percentage of the total area of the sampled tissue per field of view. All measurements were conducted in a blinded manner for all biopsies.

2.6. Statistical analysis

The primary objective of the statistical analysis is to evaluate not only the association between the fraction of cardiac fibrosis and FBC but also to verify if this association is significant in non-smokers, while controlling for hypertension, age, and gender. In the initial stage of the analysis, we assessed the existence of an association between the fraction of fibrosis and FBC, alongside each of the control variables, as well as with the variables: years living in São Paulo, daily commuting (number of hours from home to work), GeoSES, myocardial thickness, diabetes, environmental tobacco smoking, previous history of cardiovascular diseases (previous CVD), circulatory diseases as the cause of death, and cardiac ischemia. The Mann-Whitney test (Altman, 1991) was applied to evaluate the association of fibrosis fraction with each of the qualitative variables, while the Spearman's correlation coefficient was calculated to measure the correlation between cardiac fibrosis and quantitative variables. Subsequently, a multiple linear regression model was fitted to assess the association between the fibrosis fraction and FBC, sex, age, tobacco, and hypertension. Variables with a p-value smaller than 0.20 in association tests with the fibrosis fraction were also considered as predictors (Hosmer et al., 2013). The model was specified to allow different FBC coefficients in each combination of hypertension and tobacco categories, with the category composed of hypertensive and smokers considered as the reference. The model specification is illustrated in the supplementary material. The least squares method was used in the fitting of the model, and the logit transformation (Altman, 1991) was applied to the fibrosis fraction, i.e., $\log_e$ (fibrosis fraction/(1-fibrosis fraction)), in order to satisfy the assumptions of constancy of the error variance and normality of the error distributions. In the fitting of the models, a backward elimination procedure was adopted, in which the variables with non-significant coefficients were eliminated, one by one, at each step of the fitting process. Hypertension, tobacco, and the terms involving the FBC were kept in the model at all stages of the variable selection process. The goodness of fit was evaluated through residual analysis (Kutner et al., 2005). As an illustration, the associations between FBC and sex, hypertension, diabetes, tobacco, environmental tobacco smoking, previous CVD, circulatory death, and cardiac ischemia, were assessed individually by means of the Mann-Whitney test. Associations between various cardiovascular-related variables were evaluated using the Phi correlation coefficient.

3. Results

3.1. Study population characteristics

We have collected data and samples from 238 individuals. After analyzing the data and following the inclusion criteria, ten cases were excluded. Thereby, the study included a total of 228 participants and their main descriptive characteristics for quantitative variables are presented in Table 1. The mean [± standard deviation (SD)] age of the participants was 70.02 ± 14.52 years old. Eighty-four individuals (36.84 %) lived in Sao Paulo for their entire life and 50 % lived in the city for at least 79 % of their lives. The individuals spent an average of 2.12 ± 2.93 h in daily commuting. Of note, 69 (30.26 %) participants worked from home and were supposed to be less exposed to traffic-related air pollution, compared to the 25 individuals who had outdoor occupations and were more exposed to traffic (data not shown). The average value ± SD of FBC observed on the pleura surface was 0.22 ± 0.14. We also measured the myocardium thickness of the left ventricle, with a mean of 16.55 mm ± 4.25. Other evaluated quantitative results included the amount of fibrosis in the myocardium.

In terms of qualitative parameters, 116 (50.88 %) individuals were women, and a significant number of individuals (151 individuals or 66.23 %) had at least one cardiovascular disease reported on the death certificate, which was considered an event that led to death. Among them, 140 (61.40 %) had circulatory diseases as the underlying cause of death and 71 (31.14 %) had cardiac ischemia. The ischemic event was acute myocardial infarction for 31 individuals. The number of individuals with hypertension was 134 (58.77 %). In relation to other cardiovascular risk factors, 82 individuals (35.96 %) were diabetics, 125 (54.82 %) were smokers or former smokers, and 63 (27.63 %)

Table 1
Descriptive summary of the individuals included in the study.

Variable	N	Mean	StDev	Minimum	Median	Maximum	IQR
Age	228	70.02	14.52	19	70	110	20.80
Years living in São Paulo	228	52.20	20.37	0	54	98	23.80
Proportion of lifespan living in Sao Paulo	228	0.76	0.27	0.00	0.79	1.00	0.37
Socioeconomic index (GeoSES)	202	−0.21	0.38	−0.82	−0.28	1.00	0.49
Daily commuting (hours)	207	2.12	2.93	0.00	0.83	11.00	3.00
Myocardial thickness (mm)	212	16.55	4.25	7.00	16.00	35.00	6.00
Black carbon fraction	228	0.22	0.14	0.00	0.20	0.60	0.19
Fibrosis fraction	228	0.11	0.07	0.01	0.10	0.40	0.09

StDev: standard deviation; IQR: interquartile range.

individuals were environmental tobacco smokers. The number of participants according to qualitative variables can be observed in Tables 2 and 3.

The percentage of hypertensive patients in diabetics (78.05 %) is higher than in non-diabetics (47.94 %) (p-value<0.001) (data not shown). Additionally, as expected, we found a thicker left ventricle myocardium in hypertensive individuals (p-value = 0.024) (Table S1). We have also observed that cardiovascular-related variables such as hypertension, diabetes, previous CVD, circulatory death, and cardiac ischemia are positively associated and demonstrated in Table S2.

3.2. FBC and individual characteristics

A thorough analysis of the FBC (or also described as a fraction of anthracosis) has been previously published by our research group (Takano et al., 2019). In the present study, we compared the distributions of FBC in the categories of each qualitative variable considered in the study. Interestingly, the results evidenced a significant association between FBC and sex, with higher values observed in men (p-value<0.001). Higher deposition of black carbon in smokers (p-value<0.001) and in environmental tobacco smokers (p-value = 0.023) were also noted, as expected. There is no significant association of FBC with the variables hypertension, diabetes, CVD, circulatory cause of death and cardiac ischemia. The results are summarized in Table 2.

3.3. Association of cardiac fibrosis with FBC and individual characteristics

The scatterplots of cardiac fibrosis fraction and the FBC deposition in each combination of the categories hypertension and tobacco are depicted in Fig. 2. Smooth fits of the fibrosis fraction, obtained by the loess method (James et al., 2013), were added to the graphs to make it easier to identify possible trends. The results suggest that the association of these variables is dependent on categories of hypertension and tobacco. In non-smoker and non-hypertensive deceased, it suggests that there is no association between these variables. In fact, the Spearman correlation coefficient between them is 0.008 (p-value = 0.962).

The results obtained in the first step of the analysis of the association between the fibrosis fraction and each of the other variables considered in the study are presented in Tables 3 and 4. These tables show that there are no significant associations between cardiac fibrosis and the qualitative variables (Table 3) and most of the quantitative variables (Table 4) when considered individually. However, the most relevant finding indicates a positive correlation between cardiac fibrosis and FBC deposition (r = 0.24; p-value<0.001). The only variables for which a p-value smaller than 0.20 was obtained, and which are therefore candidates for predictors in the regression model, in addition to those already mentioned (age, gender, hypertension, tobacco and FBC) was the number of years living in São Paulo.

During the variable selection process, the interaction between hypertension and tobacco (p = 0.584) and the variables age (p-value = 0.707), number of years of residence in São Paulo (p-value = 0.534) and sex (p-value = 0.225) were excluded. The results obtained in the fitting of the final model are presented in Table 5 and permit the comparison of FBC coefficients in the combinations of hypertension and tobacco categories, with hypertensive individuals and smokers as the reference category. It is noteworthy that the FBC coefficient for hypertensive individuals who are smokers is higher than the FBC coefficient in other combinations of hypertension and tobacco categories.

The results in Table 5 permitted to estimate and test the significance of the FBC coefficients in the combinations of the categories of hypertension and tobacco as presented in Table 6 (refer to the supplementary material for details). It can be noted that the FBC coefficient is significant in hypertensive individuals, in both groups of smokers (p-value<0.001) and non-smokers (p-value = 0.034). In non-hypertensive individuals, there is an association between the FBC and cardiac

Table 2
Descriptive statistics and p-values obtained in the analysis of the association of the FBC with the categories of sex, hypertension, diabetes, previous history of cardiovascular diseases, circulatory diseases as the cause of death, tobacco, environmental tobacco smoking in residence, and cardiac ischemia.

Variable		N	Mean	StDev	Minimum	Median	Maximum	IQR	p-value (*)
Sex	Female	116	0.190	0.122	0.013	0.174	0.597	0.169	0.001
	Male	112	0.254	0.147	0.004	0.257	0.562	0.219	
Hypertension	No	94	0.211	0.149	0.004	0.193	0.562	0.218	0.265
	Yes	134	0.228	0.131	0.011	0.209	0.597	0.176	
Diabetes	No	146	0.216	0.141	0.004	0.199	0.597	0.196	0.366
	Yes	82	0.230	0.133	0.013	0.209	0.582	0.188	
Previous CVD	No	77	0.204	0.133	0.004	0.182	0.582	0.190	0.181
	Yes	151	0.230	0.141	0.011	0.211	0.597	0.200	
Circulatory death	No	88	0.212	0.138	0.004	0.181	0.582	0.183	0.358
	Yes	140	0.227	0.139	0.011	0.211	0.597	0.203	
Tobacco	No	103	0.181	0.125	0.004	0.170	0.597	0.181	<0.001
	Yes	125	0.254	0.141	0.011	0.248	0.562	0.206	
Environmental tobacco smoking	No	144	0.206	0.136	0.004	0.178	0.597	0.186	0.036
	Yes	63	0.249	0.142	0.018	0.247	0.561	0.178	
Cardiac Ischemia	No	157	0.216	0.136	0.004	0.193	0.582	0.173	0.413
	Yes	71	0.231	0.143	0.011	0.218	0.597	0.215	

StDev: standard deviation; IQR: interquartile range; CVD: cardiovascular diseases; (*) Mann-Whitney test.

Table 3
Descriptive statistics and p-values obtained in the analysis of the association of the cardiac fibrosis fraction with the categories of sex, hypertension, diabetes, previous history of cardiovascular diseases, circulatory diseases as the cause of death, tobacco, environmental tobacco smoking, environmental tobacco smoking in residence, and cardiac ischemia.

Variable		N	Mean	StDev	Minimum	Median	Maximum	IQR	p-value (*)
Sex	Female	116	0.107	0.070	0.012	0.088	0.353	0.081	0.065
	Male	112	0.122	0.073	0.013	0.106	0.401	0.100	
Hypertension	No	94	0.113	0.073	0.012	0.099	0.401	0.090	0.691
	Yes	134	0.116	0.070	0.013	0.097	0.329	0.100	
Diabetes	No	146	0.110	0.068	0.012	0.096	0.353	0.087	0.384
	Yes	82	0.122	0.078	0.013	0.100	0.401	0.111	
Previous CVD	No	77	0.113	0.074	0.013	0.100	0.353	0.083	0.748
	Yes	151	0.115	0.071	0.012	0.095	0.401	0.104	
Circulatory death	No	88	0.116	0.069	0.020	0.100	0.353	0.086	0.675
	Yes	140	0.114	0.073	0.012	0.094	0.401	0.100	
Tobacco	No	103	0.117	0.072	0.012	0.101	0.401	0.089	0.438
	Yes	125	0.112	0.072	0.013	0.094	0.329	0.104	
Environmental Tobacco smoking	No	144	0.116	0.072	0.013	0.102	0.401	0.108	0.493
	Yes	63	0.110	0.073	0.012	0.094	0.310	0.067	
Cardiac Ischemia	No	157	0.118	0.072	0.013	0.101	0.353	0.105	0.266
	Yes	71	0.107	0.069	0.012	0.089	0.401	0.074	

StDev: standard deviation; IQR: interquartile range; CVD: cardiovascular diseases; (*) Mann-Whitney test.

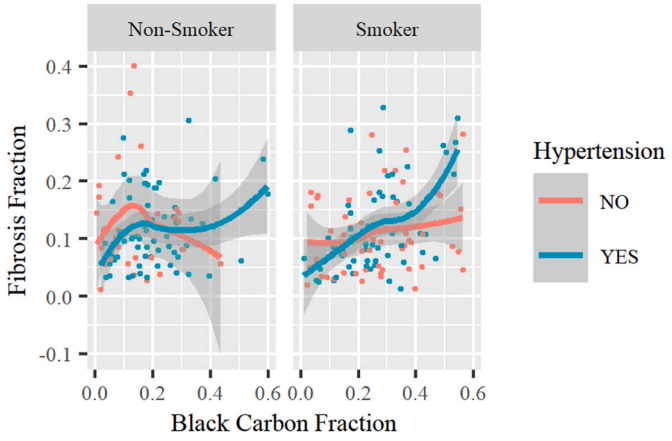

Fig. 2. Scatter plots between the fibrosis fraction and the black carbon fraction in the combinations of tobacco and hypertension. The lines in the graphs represent smooth fits of the fibrosis fraction, obtained by the loess method, to facilitate the identification of possible trends. The gray shaded areas represent the 95 % confidence intervals around these fits.

Table 4
Spearman's correlation coefficients of cardiac fibrosis and the variables age, years in the municipality, proportion of lifespan in São Paulo, hours of daily commuting in traffic, GeoSES, myocardial thickness and FBC.

Variable	r	p-value
Age	0.11	0.110
Years living in Sao Paulo	0.09	0.183
Proportion of lifespan living in Sao Paulo	0.06	0.365
Daily commuting (hours)	−0.01	0.860
GeoSES	−0.08	0.292
Myocardial thickness	0.04	0.559
FBC	0.24	<0.001

GeoSES: socioeconomic index; FBC: fraction of black carbon; r: Spearman correlation coefficient.

fibrosis only in smokers (p-value = 0.046). The residual analysis is presented in the supplementary material (Fig. S1).

4. Discussion

Our study investigated the association between a specific marker of chronic exposure to airborne particles, using black carbon as a proxy

Table 5
Results obtained in the fitting of the final regression model.

Term	Coefficient	Standard error	95 % CI	t-value	p-value
Constant	−2.16	0.17	(-2.48; −1.83)	−13.08	<0.001
FBC (reference category)	2.86	0.59	(1.69; 4.03)	4.82	<0.001
Tobacco *no*; Hypertension *yes*	−1.52	0.77	(-3.03; −0.01)	−1.98	0.049
Tobacco *yes*; Hypertension *no*	−1.75	0.70	(-3.14; −0.36)	−2.49	0.014
Tobacco *no*; Hypertension *no*	−2.94	1.35	(-5.61; −0.28)	−2.18	0.030
Hypertension *yes*	−0.33	0.18	(-0.69; 0.03)	−1.80	0.074
Tobacco *yes*	−0.46	0.18	(-0.82; −0.11)	−2.54	0.012

The lines corresponding to 'Tobacco *no*; Hypertension *yes*', 'Tobacco *yes*; Hypertension *no*' and 'Tobacco no; Hypertension *no*' present the values to be added to the FBC coefficient in the reference category 'Tobacco *yes*; Hypertension *yes*' (see Supplementary Material for details).

Table 6
FBC coefficients in the combinations of hypertension and tobacco categories.

Term	Coefficient	Standard error	95 % CI	t-value	p-value
Tobacco *yes*; Hypertension *yes*	2.86	0.59	(1.69; 4.03)	4.82	<0.001
Tobacco *no*; Hypertension *yes*	1.34	0.63	(0.10; 2.58)	2.13	0.034
Tobacco *yes*; Hypertension *no*	1.11	0.55	(0.02; 2.20)	2.01	0.046
Tobacco *no*; Hypertension *no*	−0.08	1.08	(-2.22; 2.05)	−0.08	0.937

CI: confidence intervals.

estimate, and an objective histological tracer of interstitial myocardial fibrosis. Considering this, the most significant finding of our study is that the association of FBC with cardiac fibrosis is dependent on an individual's smoking status and hypertension.

This association was notably most pronounced in hypertensive individuals who were smokers, suggesting a potential synergistic effect of

these factors on cardiac remodeling. The result also implies an increased susceptibility to particle-induced cardiovascular effects within this particular group. In fact, tobacco use was found to be associated with elevated FBC and cardiac fibrosis in both hypertensive and non-hypertensive individuals. These outcomes clearly evidence the detrimental impact of smoking habits. A prospective cohort study demonstrated an increased cardiovascular mortality risk at very low levels of cigarette smoking and a slight significant risk at lower exposure levels of secondhand smoke and ambient air pollution (Pope et al., 2009). The risks of cigarette smoke assuredly exceed those observed from exposure to ambient air pollution exposure in cardiovascular circumstances. Our study also explored the influence of both conditions, by analyzing the particles trapped in the lungs of smokers and non-smokers residents of Sao Paulo. In stratified analyses, we noted stronger effects among current or former smokers than among non-smokers.

Nevertheless, it is important to highlight that we have detected an association between BC deposition and cardiac fibrosis that is dependent on the occurrence of hypertension in non-smokers participants. This finding strongly suggests the relevant contribution of urban air pollution exposure to the cardiovascular outcomes evaluated. Hypertension is well known to promote adverse cardiac remodeling by inducing cardiac hypertrophy, systolic and diastolic dysfunction (Berk et al., 2007; Santos and Shah, 2014). Our data confirmed that the study participants with hypertension have an increased thickness of the left ventricular wall compared to non-hypertensive subjects. Hypertension is highly correlated with other cardiovascular-related variables such as death by circulatory causes, other previous CVD, and diabetes. Therefore, all those variables might contribute for the effects on cardiac fibrosis.

Based on personal monitoring approaches, some controlled-exposure studies have evaluated the acute effects of exposure to ambient particles of non-smokers and have detected an increase in systolic blood pressure associated with an increase in fine particle or BC concentration (Brook et al., 2011; Bellavia et al., 2013; Santos et al., 2019; Rabito et al., 2020). Moreover, extensive literature has shown the association between chronic exposure to BC, particulate matter, and gaseous pollutants with hypertension (Fuks et al., 2011, 2017; Schwartz et al., 2012; Chen et al., 2014; Liang et al., 2014; Rajagopalan et al., 2018; Yang et al., 2018; Zhao et al., 2022; Lv et al., 2023). Experimental studies have also demonstrated that exposure to $PM_{2.5}$ leads to cardiac fibrosis and significant increase in systolic blood pressure in mice (Wold et al., 2012; Wu et al., 2022). Then, particulate air pollution can lead to adverse cardiac remodeling through indirect mechanisms by exacerbating processes known to promote myocardium damage, such as hypertension (Liu et al., 2015). Our findings also imply that hypertensive individuals may be more sensitive to the effects of air pollution exposure, which certainly contributes to cardiovascular consequences, such as cardiac remodeling and interstitial fibrosis. The unique approach of our study provided biological validation for findings that are relatively well-known.

Possible biological mechanisms of inhaled particles triggering cardiovascular events involve the release of pro-inflammatory mediators into the bloodstream and the activation of lung sensory receptors, affecting the autonomic nervous system. Nanoparticles or soluble particles may translocate to the cardiovascular system directly after inhalation, potentially impacting cardiovascular tissues (Brook, 2008; Liu et al., 2015). In fact, both particles from air pollution and particles associated with cigarette smoking share these basic mechanisms of injury (Ambrose and Barua, 2004; Sangani and Ghio, 2011). Thereby, exposure to particles exacerbates pre-existing heart conditions and appears to have a role in disease development (Hamanaka and Mutlu, 2018). Experimental studies may contribute with more detailed and enlightening data regarding the cellular signaling and molecular mechanisms triggered to understand the interconnection between hypertension and myocardial interstitial fibrosis after exposure to environmental particles or those originating from cigarette smoke.

The use of biomarkers is a noteworthy method employed to enhance epidemiological studies by establishing the linkages between health effects and environmental pollutants. However, there are a limited number of studies that consider the use of exposure biomarkers for monitoring the short or long-term exposure to ambient particles. Short-term exposure to air pollution might be estimated by exhaled carbon monoxide (Lawin et al., 2017), but this and other combustion-derived chemicals biomarker measurements have limitations due to their rapid elimination kinetics, therefore reflecting very recent exposures (Bai et al., 2015). Only a few studies have directly measured biomarkers in respiratory airways as indicators of personal long-term exposure to ambient particles. Studies conducted by Bai et al., (2015, 2018) proposed the measurement of carbon load in airway macrophages obtained by either bronchoalveolar lavage or sputum samples to assess chronic exposure to ambient pollutant particles. Indeed, the carbon particles inside macrophages in any part of the respiratory airways, even within or on the surface of the lung, could serve as a marker to reflect an individual's exposure to particulate air pollution.

Our previous research has demonstrated that the macroscopic quantification of carbon pigment deposition on the lung surface is a valuable tool for estimating individual exposure to environmental particles throughout their lifetime in a megacity (Takano et al., 2019). It is important to note that in that study, we conducted analysis of FBC as the primary outcome of the study and examined categories of variables such as smoking amount in pack years and occupational exposure/commuting time. In the present study, we used the same approach to estimate exposure to carbon-based particles in both non-smoker and smoker participants, but without considering the categorization of variables.

An increased amount of black carbon was expected to be found in the lungs of smokers. Most of them were male, who also had the highest average commuting time compared to women. Among all individuals included in the study, we observed a positive correlation between FBC and age, as well as years lived in Sao Paulo (data not shown). An additional result, not included in this manuscript, is the temporal evolution of FBC among 103 non-smokers in our study, showing that black carbon accumulation progressively increases with age. However, as ages progresses, black carbon levels do not consistently increase, which may be attributed not only to physiological factors but also to the influence of time spent at home. Our results are, in part, comparable with an earlier study that also reported an association of BC (mentioned as anthracosis) with age and time of residence in Nashville, through microscopic evaluation of lung specimens obtained from autopsy procedures (Zeidberg and Prindle, 1963). Of note, the same study did not find an association between anthracosis and cardiorespiratory symptoms manifested during life of the individuals.

The term anthracosis refers to black patches that are macroscopically identified on the lungs. The accumulated black pigment is clearly evident in the visceral pleura, where the lymphatic drainage is reduced (Takeda et al., 2022). The duration of black carbon (BC) permanence in the lungs has not yet been precisely defined. Initially, these particles can be eliminated by the lymphatic system toward the thoracic lymph nodes, but this process can become overwhelmed with increased inhaled doses. The duration of this overload depends on various factors. For example, coal miners might experience a rapid evolution (coal workers' pneumoconiosis), while accumulation may proceed more gradually in smokers, depending on the number of years of smoking. Additionally, the capacity for pulmonary clearance of inhaled particles might be influenced by systemic functional deterioration in different organs and systems due to aging. Thus, accurately determining the residence time of black carbon in the lungs is challenging due to various factors such as individual exposure patterns, environmental conditions, and the complex interactions within the respiratory system. To our knowledge, the natural progression of black carbon accumulation due to exposure to urban particulates has not been fully elucidated. The complexity of studying potential determinants of black carbon accumulation is significant and cannot be thoroughly addressed in our study.

We must also stress that the purpose of the present study was neither

to measure the complex mixture of urban pollution nor to verify the composition of the black patches on the lung surface of participants. Previous studies have already detected and quantified the chemical element profile of anthracosis in lungs of Sao Paulo dwellers (Saieg et al., 2011; Dos Santos et al., 2022). The elements detected in particles retained in the lung are associated with carbonaceous substances derived from different sources. In urban environments of megacities, the population is exposed to exceeding levels of soot or atmospheric BC (Xu et al., 2020; Popovicheva et al., 2021), an undesired byproduct arising from incomplete combustion of fossil fuels (Watson and Valberg, 2001; Long et al., 2013). Previous data have indicated that a large amount of cardiovascular disease mortality burden over the megacity is substantially attributable to BC in air pollution exposures, which could be potentially reduced to save about fifty thousand lives in megacities each year (Verma et al., 2022). Then we have considered that BC could serve as the primary constituent in the black patches (or anthracosis) observed in our lung samples and may be a proxy estimate of the complex mixture of airborne contaminants present in the urban atmosphere.

Independent of the composition of the retained particles, two interesting systematic reviews indicated similar associations for both BC and fine particulate matter (PM2.5) with cardiovascular effects (Kirrane et al., 2019) and more severe consequences, such as hospital admissions and mortality from cardiovascular causes (Luben et al., 2017). These findings reinforce the importance of understanding the contribution of exposure and deposition of airborne particles in the respiratory tract to human health, particularly in cardiovascular outcomes.

The implications of our findings underscore the potential impact of chronic exposure to urban air pollution, represented by FBC, on cardiac remodeling, especially in individuals with established cardiovascular risk factors such as hypertension and smoking. To our knowledge, this is the first study to explore such relevant associations using an autopsy-based approach. In fact, we combined epidemiological aspects and biomarkers in sampling of post-mortem human tissue to provide a comprehensive assessment of individual responses to inhaled particles and evidence of health damage, such as cardiac remodeling outcomes.

The strengths of this study encompass a substantial number of participants, and the samples underwent comprehensive evaluation. The sampling strategy spanned nearly two years, mitigating biases related to seasonal prevalence in disease-caused deaths. Furthermore, our sample aligns with global parameters concerning deaths due to circulatory causes, representing most individuals in the study and standing as the primary cause of mortality among the elderly worldwide (World Health Organization, 2021). A significant portion of the individuals in the study lived in São Paulo for almost their entire lives, suggesting they were exposed to the city's polluted air (to a greater or lesser extent) for a long period of their lives. Linked to this, another notable strength lies in the study's design, which explored the intersection between lifelong biomarkers of exposure and predictive markers of cardiac disease. Our method for assessing exposure received strong support from data obtained through detailed interviews with family members. These interviews covered a wide range of topics, including habits, activities, and potential occupational exposures throughout an individual's lifetime. Moreover, the wealth of information on cardiovascular pathologies, acquired from both family members and autopsy reports, significantly fortified the dataset. This comprehensive data collection approach rendered our dataset robust and sufficiently reliable for conducting the study's analyses.

Nevertheless, we acknowledge that the study has potential limitations. We have quantified only one biological marker to predict exposure from different sources, rather than utilize complementary measurements of other biomarkers or integrating with other exposure assessment method. Furthermore, it is valid to note that some information, such as the number of cigarettes smoked, duration of smoking, and the use of anti-hypertensive medications by the participants, was not widely collected. The commuting time was only considered for the individual's most recent occupation, but this approach has been shown to provide a robust estimate of the individual's life course exposure to air pollution (Takano et al., 2019). The study design did not intend to obtain a representative sample of Sao Paulo's population, due to a well-known bias in cases that require autopsy to define the cause of death. Consequently, our sample exhibits a bias toward the low-income and underprivileged segments of Sao Paulo's population. Additionally, since our service deals with deaths caused by natural diseases (violent and accidental deaths are under the jurisdiction of the police coroner), there is a notable bias towards elderly individuals. We assert that our sample is representative of one of the world's largest autopsy services handling natural causes of death in Sao Paulo.

Although a wide range of modern techniques have emerged for the study of human pathophysiological processes aggravated by exposure to urban ambient particles, the autopsy has proven to be a very rich source of material for the advancement of scientific knowledge. Our study has contributed to clearly evidence the association between a biomarker of exposure to particles derived from air pollution and tobacco smoking, and cardiac fibrosis, which is an important underlying consequence of cardiovascular diseases. In fact, we aimed to highlight the effect of chronic exposure to air pollution particles on the outcome of cardiac fibrosis. We can conclude that smoking and pre-existing hypertension appear to be important risk factors for the onset of cardiac remodeling in individuals exposed to atmospheric pollutants in the megacity of Sao Paulo. These findings may be considered in global efforts devoted to curtailing the tobacco epidemic and supporting policies to control anthropogenic pollution sources from a cardiorespiratory health perspective.

CRediT authorship contribution statement

Ana Paula Cremasco Takano: Writing – original draft, Methodology, Investigation, Conceptualization. **Carmen Diva Saldiva de André:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Raquel de Almeida:** Investigation. **Dunia Waked:** Investigation. **Mariana Matera Veras:** Resources, Methodology. **Paulo Hilário Nascimento Saldiva:** Writing – review & editing, Resources, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2024.118380.

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