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Assessing the impact of individual exposure to air pollution via biomarkers in sputum of children in Ho Chi Minh city, Vietnam☆

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ABSTRACT

Understanding the impact of exposure to air pollution on children's health is critical for developing effective child health protection policies. Alveolar macrophage black carbon (AMBC) provides an indicator of personal exposure to air pollution. Hence, we aimed to examine air pollution exposure and its effects on children by measuring AMBC area and inflammatory cytokines in sputum. Sputum induction was attempted in 120 children aged 13–14 years, but samples of sufficient volume and quality for analysis were only obtained from 47 (39.2%). Alveolar macrophages (AMs) were visualized, and black carbon (BC) area quantified by microscopy. Participants completed questionnaires, recording air pollution exposure and respiratory symptoms. Univariable associations between AMBC area and respiratory symptoms, exposure variables, cytokines, and pulmonary function were examined. Multivariable regression was conducted, adjusting for potential confounders. The median AMBC area was 0.23 μm^2 (range: 0.09–0.77 μm^2). Most participants (87%) reported a history of respiratory symptoms. AMBC area was related to the distance from home to school, living on the main road, and the habit of opening windows for ventilation. No significant associations were seen between AMBC area and respiratory symptoms, lung function, or inflammatory markers (IL-8, TNF-α, IFN-α). Our findings support the use of AMBC area as a biomarker of individual air pollution exposure. The lack of associations between AMBC area and health outcomes is likely due to a lack of study power, indicating more extensive studies are required.

1. Introduction

Air pollution imposes a substantial global health burden, particularly in low- and middle-income countries (LMICs) in Southeast Asia (World Health Organization, 2022). In addition to household air pollution, ambient air pollution, especially particulate matter (PM_{10}, $PM_{2.5}$, i.e. particulate matter with aerodynamic diameters of 10 μm or less, and of 2.5 μm or less, respectively) from traffic-related air pollution (TRAP), has adverse effects on human health at all life stages, negatively influencing the respiratory, cardiovascular, and neurological systems (Holgate et al., 2016; Newby et al., 2015). Air pollution is especially harmful to children due to their immature respiratory, immune, and metabolic systems (Bateson and Schwartz, 2008).

TRAP results largely from the combustion of fuels, which generates particulate matter (PM_{10}, $PM_{2.5}$), black carbon (BC), and gaseous pollutants. Individual exposure to TRAP is difficult to quantify, however BC has been used to indicate the extent of exposure (Quang et al., 2021; Romshoo et al., 2023). Long-term exposure to BC has been linked to increased risk of mortality and morbidity, reinforcing the notion that BC is a harmful component of $PM_{2.5}$ (Nilsson Sommar et al., 2021; Strak et al., 2021; Wen et al., 2023; Yang et al., 2021).

A primary role of alveolar macrophages (AMs) is to provide defense

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against inhaled xenobiotics, including PM, microbial species, and other foreign substances via phagocytosis and initiation of protective inflammatory responses. Alveolar macrophage black carbon (AMBC) has served as an indicator of both external exposure to air pollution and health responses (Bai et al., 2015). Consequently, measuring AMBC area has been suggested as a potential biomarker for assessing individual TRAP exposure (Bai et al., 2015; Terashima et al., 1997). In fact, the AMBC area has been used to investigate associations between air pollution exposure and lung function in children (Bai et al., 2015; Kulkarni et al., 2006). However, most previous studies have not been conducted in heavily polluted cities in LMICs where higher ambient BC concentrations are likely to result in greater AMBC area (Bai et al., 2018; Ji et al., 2020; Kurth, 2013). Additionally, factors influencing exposure are likely to differ between LMICs and more affluent settings. For example, research in Vietnam revealed that motorbike commuters experienced substantially higher ambient BC (up to three times) compared to passengers on buses or cars (Quang et al., 2021). A recent study, carried out in Ho Chi Minh City (HCMC), Vietnam, showed that the majority of residents use motorcycles for regular transportation (Ho et al., 2023). Riding motorcycles in heavily polluted cities is likely to result in higher BC exposure and higher AMBC area.

The current study aims to utilize the measurement of AMBC as a quantitative assessment of air pollution exposure to examine the impact of pollution exposure on the respiratory health of middle school children. This is the first study to assess the effects of air pollution on individual children in Vietnam using these quantified biomarkers of exposure. HCMC was chosen as the study location due to its status as one of Vietnam's most polluted cities. The annual mean $PM_{2.5}$ concentrations in HCMC have consistently exceeded both the air quality guidelines established by the World Health Organization (WHO) and the thresholds proposed by the National Technical Regulation on Ambient Air Quality in Vietnam (Nhung et al., 2022; World Health Organization, 2021).

2. Methods

2.1. Study setting

The study was conducted in HCMC, one of the most densely populated cities in Vietnam, with an estimated population of 10 million (General Statistics Office, 2022). Fig. S1 displays the study area location, with red dots indicating participant locations.

2.2. Study population and recruitment

Participants were recruited from two randomly selected secondary schools in HCMC. Eligibility criteria included children aged 13–14 who had been residing in HCMC for at least the preceding six months to allow for the range of AMBC area mean half-lives of 53 and 116 days, depending on the BC load (Bai et al., 2018). Exclusion criteria included children suffering from chronic respiratory conditions or exhibiting symptoms indicative of respiratory, gastrointestinal, or urinary infections in the three months leading up to the study. Forty children from one middle school (An Lac school) in the inner suburb and 40 children from Le Minh Xuan school in the outer suburb of the city met the study criteria and agreed to participate. An additional 40 children, who also met the selection criteria, were selected through word-of-mouth recruitment.

2.3. Study procedures

Questionnaires: Upon arrival at the clinic, the children and their guardians completed a self-administered questionnaire designed to obtain detailed information on general health, respiratory symptoms, mask usage habits, and both indoor and outdoor exposure to air pollution. It also incorporated the validated Vietnamese version of the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire to determine the children's respiratory health status (Le et al., 2021). *Height and weight* were measured, and body mass index (BMI) was calculated.

Lung function testing: After explaining the procedures to the parents, the children underwent spirometry at the clinic. A CareFusion SN73335 device was calibrated before each measurement and used to measure spirometry. Spirometry outcomes were forced vital capacity (FVC, L), forced expiratory volume in 1 s (FEV_1, L), and forced expiratory flow between 25% and 75% of FVC ($FEF_{25–75}$, L/s). These variables were expressed in absolute values and as percent predicted using the National Health and Nutrition Examination Survey (NHANES) III. The FEV_1/FVC ratio was determined by dividing the value of FEV_1 (L) by FVC (L). The results adhered to the technical standards recommended by the American Thoracic Society and the European Respiratory Society, which were adjusted for characteristics such as race, height, gender, and age (Graham et al., 2019).

2.4. Induced sputum collection

Subsequently, children underwent sputum induction using a standardized approach (Bai et al., 2018), wherein nebulized hypertonic saline (3%) was administered over three consecutive, 5-min inhalation periods. To mitigate the risk of bronchospasm induced by the hypertonic saline, 400 mcg of salbutamol was administered via a metered-dose inhaler and spacer prior to sputum induction. Participants were encouraged to attempt sputum expectoration; this entailed deep coughing accompanied by intermittent huffing and hacking, culminating in the expulsion of sputum. The process was repeated at 5-min intervals for up to a maximum of 20 min; the procedure was terminated if the participant exhibited any signs of wheezing or discomfort (Paggiaro et al., 2002).

In healthy children, the consistency of sputum is frequently watery and saliva-like. However, the presence of cellular plugs within the saliva is indicative of a viable sample. A minimum of 2 mL of sputum per sample was deemed sufficient for analysis. From 120 initial participants, we were able to collect 47 samples of sufficient volume and good quality for AMBC analysis (Fig. S2).

2.5. Sputum specimen processing

Sputum samples were processed within 2–4 h of collection to ensure optimal cell counting and effective staining (Pizzichini et al., 1996). The samples were stored refrigerated (temperatures 2–8 °C) between collection and processing. To break down the sputum plugs, 0.1% dithiothreitol (Thermo Fisher, USA) was added to sputum in a 1:1 vol ratio (Fahy et al., 1993; Gershman et al., 1996). Sputum samples were then cooled to 4 °C and homogenised on a horizontal shaker for 15 min. Subsequently, mucus and debris were removed by passing the samples through a 40 μm cell strainer (SPL Life Sciences, South) (Efthimiadis et al., 2002). The supernatant was aspirated and stored at −80 °C for future cytokine analysis. Additionally, cell pellets were washed by adding 2 mL of phosphate-buffered saline (PBS) (Sigma-Aldrich, UK), followed by another round of centrifugation under the same conditions to remove the supernatant. The remaining cell pellet was then resuspended in 600 μL of PBS.

2.6. Differential cell count and evaluation of AMBC area

The protocol was adapted from a previous study (Jary et al., 2015). Initially, cell pellets were evenly distributed onto microscopic slides (Greetmed, China) and allowed to dry at room temperature for 10 min. The slides were then stained using Hemacolor Rapid staining (Merck Millipore, Germany), following the manufacturer's instructions.

AMs were visualized using light microscopy. Digital color images of 50 randomly chosen AMs from each participant were obtained with an

Olympus BX 43 microscope, utilizing a 100x objective lens and immersion oil for clarity. These images were then uploaded for analysis into ImageJ 1.50i (National Institutes of Health, USA).

Deposits of BC within each AM were differentiated from other dark substances, such as nuclei and bacteria (which stain dark purple) that were either adhering to or being ingested by the AMs. The analysis provided AMBC area, measured in μm^2, with mean (SD) calculated from 50 AMs for each participant (Kulkarni et al., 2005).

2.7. Sputum cytokines measurement

Cytokine concentrations in all collected sputum samples were quantified using a commercially-available phase sandwich enzyme-linked immunosorbent assay (ELISA) (IL-8, TNF-α, and INF-α). These samples were analysed without dilution. Specifically, the concentrations of Interleukin 8 (IL-8), Tumor Necrosis Factor-alpha (TNF-α), and Interferon-alpha (IFN-α) were determined in the supernatants preserved from the samples. The sensitivity thresholds for these kits were <5 pg/mL for IL-8, 1.7 pg/mL for TNF-α, and 3.2 pg/mL for IFN-α, respectively. This measurement was performed using a Varioskan™ LUX multimode microplate reader (Thermo Fisher, USA) equipped with SkanIt RE 6.1.1 software.

2.8. Statistical analysis

Continuous variables were described using means and standard deviations (SD) or medians and interquartile ranges (IQR), as appropriate. Categorical variables were described using frequencies and percentages. Chi-square tests or Fisher's exact tests were used to compare categorical variables between groups. To assess the relationship between respiratory symptoms and AMBC area, logistic regression was used. The Mann-Whitney *U* test was utilized to evaluate differences between AMBC area and exposure variables. The effect size for the Mann-Whitney *U* test was calculated by dividing the z value by the square root of N (Rosenthal, 1994). To examine the bivariate correlations between AMBC area and variables such as biomarkers indicative of exposure to air pollution, the length of exposure, pulmonary function, and travel distances, Spearman's correlation coefficients were determined. Linear regression analyses were used to evaluate the relationship between respiratory outcomes (inflammatory cytokines and lung function indicators) and AMBC area. Multivariable regression models were applied to adjust for potential confounders, including sex, weight, and height. A significance level of $p < 0.05$ was considered significant for all assessments. Data analyses were performed using Stata software for Windows, version 14.0 (StataCorp LP, College Station, TX, USA).

2.9. Ethical approvals

The study protocol was approved by the Institutional Review Board of the Research Ethics Committee at the University of Medicine and Pharmacy at HCMC (789/UMP-BOARD). Written consent was obtained from the parents of the participating children.

3. Results

A total of 120 children were assessed in the initial evaluation. Of these, 90 participants provided sufficient sputum for analysis, and 47 (24 males and 23 females) had sufficient AMs for further analysis. Fig. 1 shows AMs without and with phagocytose BC. A sputum sample with 50 AMs is shown in Fig. S3.

Table S1 compares the characteristics of those participants who did and did not provide sufficient sputum samples for analysis. No significant differences were found between the groups. Additionally, AMBC area was found to be 0.23 μm^2 with an IQR of 0.09–0.77 μm^2.

No significant associations were seen between the history of respiratory symptoms (wheezing, sneezing, and rash) and AMBC area among healthy children ($p > 0.05$) (Table 1). The impact of exposure variables on AMBC area are shown in Table 2. Those associated with an increased AMBC area were opening windows for ventilation ($p = 0.039$), distance travelled from house to school ($p < 0.001$), and living on a main road ($p = 0.01$). We did not show an association between exposure to tobacco smoke and AMBC area, however, only two subjects with sufficient sputum were exposed to smoke.

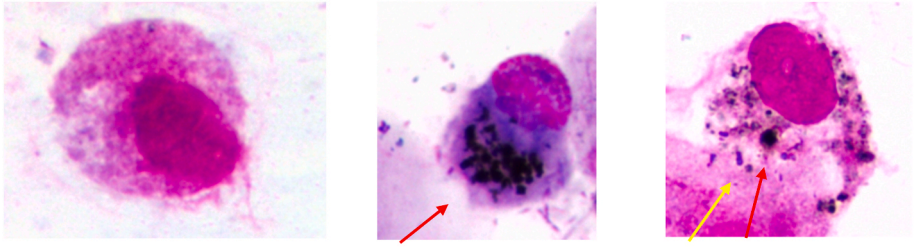
Table 3 shows associations between AMBC area and pulmonary function and inflammatory cytokines. Linear regression analysis showed a statistically significant association between FEV_1/FVC ratio and AMBC area ($\beta = 0.02$, 95% CI: −0.001 to −0.05, $p = 0.04$). However, a detailed analysis of the individual spirometric components indicated that this seemingly significant association was likely spurious. Both FVC [$\beta = -3.81$, 95% CI: −9.94 to 1.33] and FEV_1 [$\beta = -1.39$, 95% CI: −7.29 to 4.51] showed negative coefficients, suggesting that the significant FEV1/FVC ratio finding was an artifact of parallel decreases in both measurements rather than a genuine physiological relationship. Our investigation found no convincing evidence for a relationship between respiratory function and AMBC area (Figs. 2 and 3, and Table 3).

4. Discussion

This is the first study in Vietnam exploring the impact of air pollution on individual children using AMBC area derived from sputum samples as a biomarker of exposure. The findings provide evidence of an association between AMBC area and conditions likely to increase exposure,

Table 1
The relationship between history of respiratory symptoms and AMBC (n = 47).

Character	History of wheezing		History of sneezing		History of rash	
	OR	p	OR	p	OR	p
AMBC area (μm^2)	0.22 (−0.63 to 1.06)	0.61	4.71 (−1.24 to 10.66)	0.12	−21.83 (−57.67 to 14.03)	0.23



(a) (b) (c)

Fig. 1. AMs with phagocytosed BC: (a) Normal AM, (b) AM with phagocytosed BC (red arrow) from a sufficient sample, (c) AM with phagocytosed BC (red arrow) and bacteria (yellow arrow).

Table 2
The relationship between other exposure factors and AMBC (n = 47).

Character	n	AMBC area (μm²) Median [IQR]	p	Effect Size
Mode of transportation				
Private transport (motorbike, bike, and walking)	44	0.21 [0.08 to 0.78]	0.63	0.07
Public transport (bus, car, and taxi)	3	0.59 [0.13 to 0.72]		
Open door for ventilation				
Yes	28	0.25 [0.06 to 0.96]	0.88	0.02
No	19	0.19 [0.11 to 0.77]		
Open window for ventilation				
Yes	15	0.59 [0.17 to 1.13]	**0.03**	**0.30**
No	32	0.15 [0.06 to 0.55]		
Smoking				
Yes	2	0.42 [0.33 to 0.50]	0.56	0.08
No	45	0.19 [0.09 to 0.77]		
Family smoking				
Yes	23	0.26 [0.09 to 0.79]	0.82	0.03
No	24	0.21 [0.08 to 0.69]		
Wearing mask outdoors				
Always	42	0.21 [0.09 to 0.72]	0.55	0.09
Not always	5	0.33 [0.14 to 1.19]		
Type of mask used				
Nylon, paper mask	38	0.19 [0.09 to 0.72]	0.55	0.09
Others (N95, N97, 3D, 4D)	9	0.26 [0.16 to 0.97]		
House on main road				
Yes	10	1.20 [0.33 to 1.49]	**0.01**	**0.37**
No	37	0.20 [0.06 to 0.59]		
Travel distances (meters)				
From home to school		1650.00 [800.00 to 3500.00]	**< 0.001**	**0.59**
From home to the main road		190.00 [77.00 to 650.00]	0.72	0.05
Time of exposure to TRAP (min/day)		38.00 [20.00 to 60.00]	0.46	0.11

namely open windows for ventilation (p = 0.03), living on a main road (p = 0.01) and distance travelled to school (p < 0.001), but was not associated with increased respiratory symptoms (wheeze p = 0.61, sneezing 0.12). The small sample size likely compromised our ability to link AMBC area to health outcomes.

Median AMBC area in our study was 0.23 (0.09–0.77) μm², consistent with levels in previous studies conducted in Leicestershire, England [0.41 (0.03–1.14) μm²] (Kulkarni et al., 2006) and London [0.30 (0.20–0.52) μm²] (Liu et al., 2021) in healthy children. We had anticipated a higher AMBC area, given the high levels of air pollution in HCMC. Studies in adults have produced variable results. A recent study conducted on pregnant individuals revealed a decrease in AMBC area with a median (IQR) value of 0.12 (0.30) μm² (Miri et al., 2022), whereas another study found higher AMBC area in adults suffering from chronic obstructive pulmonary disease (COPD), with the mean value of 4.87 ± 4.41 μm² (Yin et al., 2021). While it is possible that different immune responses in children, pregnant women, and adults may contribute to different AMBC area, this is more likely to vary with air pollution concentrations across different regions (Bai et al., 2018).

Another possible explanation for the lower than anticipated AMBC area in our study, was the high level of face mask wearing by the children we studied. Face masks have been shown to lower BC exposure by 6%–61% and $PM_{2.5}$ exposure by 14%–96%, depending on mask type (Pacitto et al., 2019). A previous study in HCMC found that the use of a facemask by motorcyclists consistently reduced PM exposure, with surgical masks exhibiting significantly higher protective efficiency compared to cloth masks (Huy et al., 2022). The practice of mask-wearing among Vietnamese children is growing as a protective measure against air pollution and respiratory diseases post-COVID-19 (Le et al., 2023). In the present study almost 90% of children regularly wore masks for protection, yet there was no significant difference in AMBC area between those who wore masks and those who did not. However, as only five non-mask wearing children produced sufficient sputum for analysis, these results need to be treated with caution.

Table 3
Association between respiratory outcomes (inflammatory cytokines and lung function) and AMBC area (n = 47).

Character	Linear regression		Multiple linear regression	
	Coefficient (95% CI)	p	Coefficient (95% CI)	p
IL-8 (pg/mL)	0.25 (−0.04 to 0.53)	0.08	0.25 (−0.05 to 0.56)	0.09
TNF-α (pg/mL)	0.05 (−0.14 to 0.23)	0.61	0.02 (−0.50 to 0.01)	0.79
IFN-α (pg/mL)	0.04 (−0.11 to 0.20)	0.57	0.05 (−0.11 to 0.22)	0.51
FVC (% predicted)	−3.81 (−9.94 to 1.33)	0.14	−2.85 (−8.13 to 2.43)	0.28
FEV_1 (% predicted)	−1.39 (−7.29 to 4.51)	0.63	−0.10 (−6.04 to 5.85)	0.97
FEV_1/FVC	0.02 (−0.001 to −0.05)	**0.04**	0.02 (−0.004 to −0.05)	**0.02**
FEF_{25-75} (% predicted)	4.35 (−5.53 to 14.24)	0.38	6.02 (−4.35 to 16.40)	0.24

* The recorded parameters, including FVC, FEV_1, and FEF_{25-75} (as percent predicted using NHANES III), were adjusted for race, height, and gender. Data on inflammatory cytokines were log-transformed to address positive skewness.

The data in the present study are consistent with the notion that individual exposure to air pollution (i.e., PM) is associated with an increase in inhaled BC, and hence AMBC area. In Vietnam, the lack of public transport infrastructure and local habits means that people prefer private over public transportation for school commutes, which increases the exposure to ambient BC (Quang et al., 2021). Our data show that the distance from home to school and proximity to main roads were associated with an increase in AMBC area, consistent with previous studies (Bunn et al., 2001; Jacobs et al., 2010). In the present study, we found AMBC obtained from children residing in HCMC. None of the children in our study used charcoal stoves in their homes, minimizing the possibility that AMBC area was influenced by indoor air pollution. Thus, AMBC is highly likely to have come from TRAP exposure. Our results suggest that opening windows for ventilation increased AMBC area, likely due to the infiltration of outside PM into the inside space, as previously observed (Quang et al., 2017). Taken together, these data give confidence that AMBC area in our study was a reasonable biomarker of individual TRAP exposure.

Increased atmospheric concentrations of PM resulting from the combustion of fossil fuels and indoor air pollution has been associated with a higher prevalence of respiratory and allergic symptoms in children (Endaryanto et al., 2023; Le et al., 2024). However, in our study, we did not find a significant association between respiratory symptoms and AMBC area, despite nearly 87% of participants reporting respiratory symptoms in the past. Previous research indicates that AMBC area may not consistently differ between asthmatic and non-asthmatic children; children with asthma were found to have lower AMBC than healthy children (Kulkarni et al., 2006). The lack of association may be due to the small sample size.

Previous studies also show increases in inflammatory cytokines with

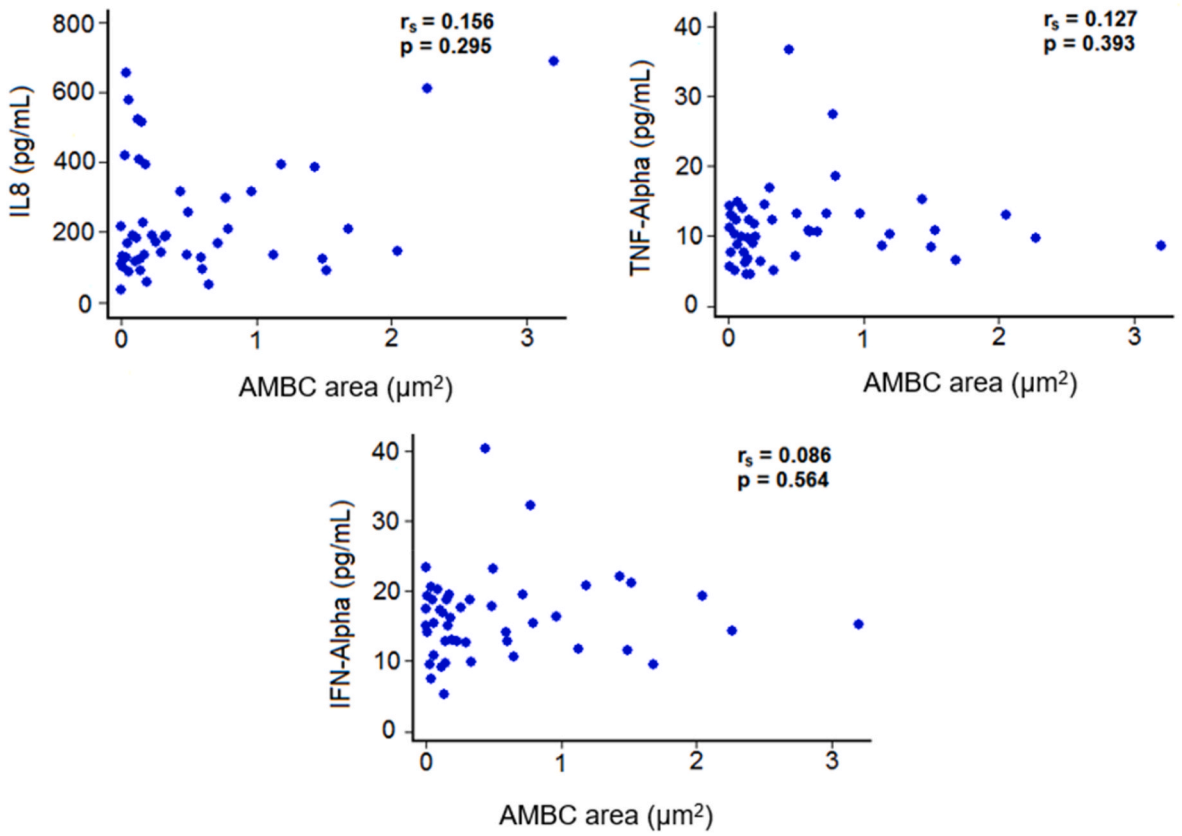


Fig. 2. Bivariate correlations between biomarkers for exposure to air pollution and AMBC area. IL-8 denotes Interleukin-8, TNF-α denotes Tumor Necrosis Factor-alpha (TNF-α), and IFN-α denotes Interferon-alpha.

TRAP exposure. One study showed a slight elevation in serum TNF-α concentrations among cyclists, suggesting a potential systemic effect of inhaled BC (David et al., 2011). Similarly, a study conducted in London showed that the concentration of TNF-α was higher in cyclists during their commutes compared to those who do not cycle (Chinedu et al., 2012). In the present study we did not show significant relationships between AMBC area and sputum concentrations of IL-8, TNF-α, and IFN-α. Similarly, a study in London found no correlation between TRAP exposure and the responsiveness of whole blood cytokines in the sample population (Gruzieva et al., 2017; Klümper et al., 2015). The airway epithelium plays a critical role in immune defense by acting as a physical barrier, facilitating ciliary action, and promoting mucus clearance, in addition to its involvement in inflammatory and antimicrobial responses through cytokine production (Calvén et al., 2020). Previous studies demonstrated that diesel exhaust particles not only increase cytokine production in human bronchial epithelial cells but also compromise the rigidity of the cytoskeleton and the adhesion of cell-matrix molecules (Doornaert et al., 2003), underscoring the complex interplay between air pollutants and the pulmonary immune response. In the present study, cytokine concentrations were measured in sputum samples, in contrast to the serum samples typically used in other investigations. A previous study demonstrated that sputum samples offer greater sensitivity for examining airway inflammatory responses in patients with COPD or asthma compared to serum samples (Cao et al., 2012). The small sample size in the present study is likely to mean that we were underpowered to determine the impact of AMBC area on cytokine secretion. Larger studies will be needed to address this.

5. Limitations

Some limitations of our study should be noted. Out of 120 healthy children recruited, 47 were able to provide adequate sputum samples. The success rate of sputum induction in this study was 39.16%, notably lower than the success rates reported in healthy children in prior studies (Gibson et al., 2003; Kulkarni et al., 2006; Liu, 2019). Although sputum induction is non-invasive and generally well tolerated, this technique is variability successful in children, leading to inconsistent yields of AMs. This variability was exacerbated by several children struggling to expectorate the sputum produced and others inadvertently swallowing it. We were unable to ensure the cytokines we measured in sputum were induced by air pollution exposure. Sputum contamination with saliva and respiratory secretions may have affected the cytokine values. Furthermore, the use of a questionnaire to collect information on respiratory symptoms and exposure to cigarette smoke may introduce recall bias among participants. Future studies should incorporate cotinine measurements in biosamples to better assess the impact of passive smoke exposure on AMBC area. It is also important to acknowledge that the black substance observed in macrophages may not necessarily be carbon. To account for this uncertainty, images showing characteristic features of bacteria in macrophages were excluded during image analysis, and carbon within macrophages was recorded as black. Lastly, due to the lack of prior data, we could not conduct a power analysis to estimate the number of participants required to achieve the distinction in exposure between sub-groups (e.g., mask-wearing vs. no mask-wearing). The small number of samples analysed (47) almost certainly mean our study was underpowered to detect associates with AMBC area. Finally, there remains uncertainty regarding the relative importance of various indices of TRAP exposure, such as PM_{10}, $PM_{2.5,}$ and traffic volume in determining AMBC area. Different fuels burnt (e.g. diesel, petroleum, kerosene, etc) may result in differing amounts of BC in PM.

6. Conclusion

This study is the first to assess the effects of air pollution on

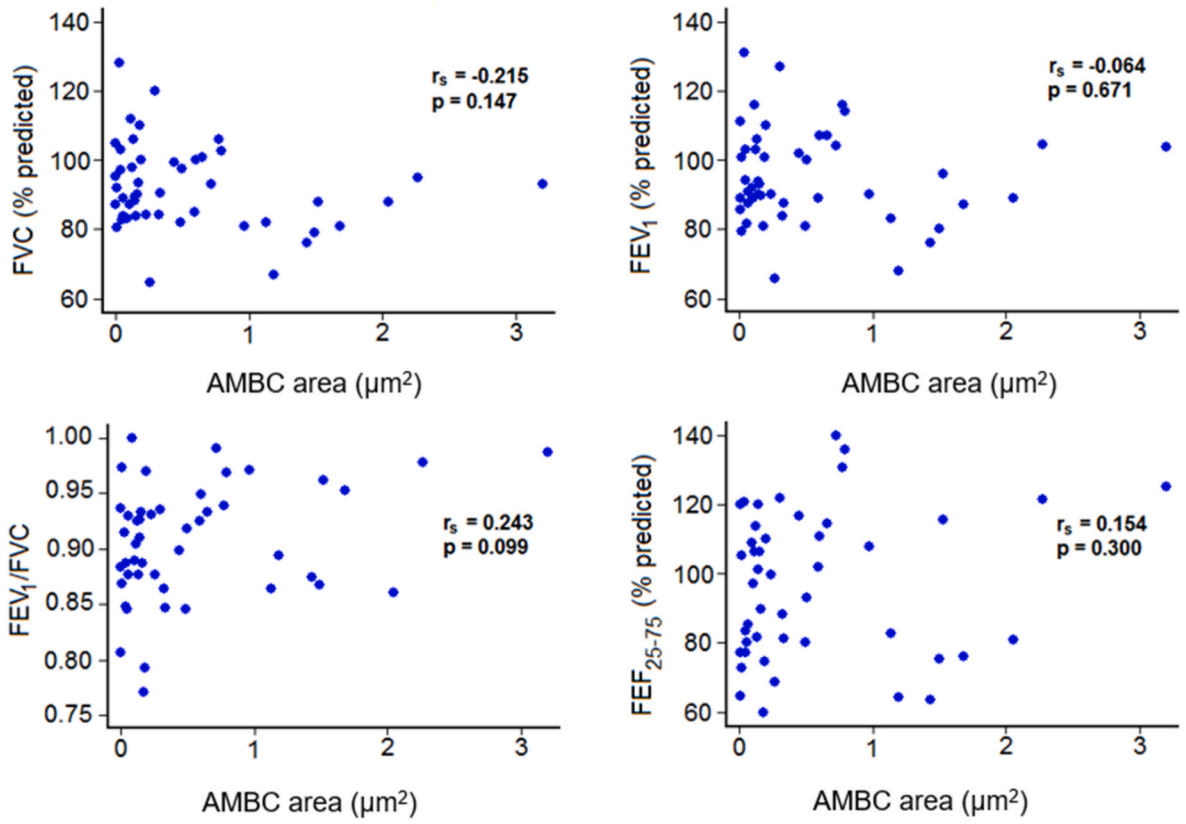


Fig. 3. Bivariate correlations between pulmonary ventilatory function and AMBC area. FVC denotes forced vital capacity, FEV_1 denotes forced expiratory volume in 1 s, and FEF_{25-75} denotes forced expiratory flow between 25 and 75 percent of the FVC.

Vietnamese children using AMBC area, obtained through sputum induction, as a biomarker of individual TRAP exposure. Our study did not observe any significant associations between respiratory symptoms and AMBC area, which might be explained by the small sample size and the widespread use of face masks among children when outdoors. Additionally, exposure to air pollution, as measured by AMBC, was not associated with increased inflammatory markers or changes in lung function in healthy children. However, all sputum samples that contained BC exhibited elevated cytokine concentrations, indicating that particulate air pollution might still trigger an inflammatory response, even if it does not directly impair lung function in this population. Additionally, we identified a correlation between increased AMBC and the habit of keeping windows open for better ventilation, distance from home to school, and proximity to the main road. These observations underscore the importance of proper ventilation and indoor air quality management to prevent or reduce health problems related to air pollution. Although our study could not definitively confirm that the black substance in AMBC was exclusively carbon, our findings provide more evidence supporting this identification and underscore its potential as a biomarker for individual exposure in future studies. Further studies with larger sample sizes and extended monitoring periods are necessary to explore the long-term impact of air pollution on children's respiratory health.

CRediT authorship contribution statement

Linh Le Tran: Writing – original draft, Investigation, Formal analysis, Data curation. **An Le Pham:** Writing – review & editing, Supervision. **Minh Duc Do:** Writing – review & editing, Supervision. **Quynh Nhat Nguyen:** Writing – review & editing, Investigation. **Hieu K.T. Ngo:** Writing – review & editing. **Hong H.T.C. Le:** Writing – review & editing, Formal analysis. **Vinh Nhu Nguyen:** Writing – review & editing. **Dung Phung:** Writing – review & editing. **Peter D. Sly:** Writing – review & editing, Conceptualization. **Phong K. Thai:** Writing – review & editing, Supervision.

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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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2024.125544.

Data availability

The data that has been used is confidential.

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