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Bioaerosol exposure and circulating biomarkers in a panel of elderly subjects and healthy young adults

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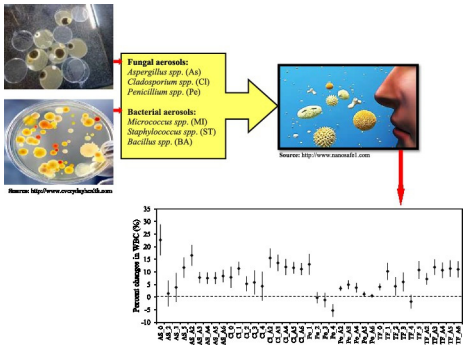
HIGHLIGHTS

- Exposure to bioaerosols increases the level of biomarkers both in elderly and healthy young subjects.
- Associations were stronger in the healthy young adults.
- Pooled results confirm the hypothesis that bioaerosols induced blood markers.

GRAPHICAL ABSTRACT



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ABSTRACT

Numerous studies have found that risk of cardiovascular diseases is associated with increased blood levels of circulating markers of systemic inflammation. We investigated associations of acute exposure to bioaerosols (bacteria and fungi) with blood markers of inflammation and coagulation using panels of elderly subjects and healthy young adults. We conducted a panel study of 44 nonsmoker elderly subjects in a retirement communities and a panel study of 40 healthy young adults living in a school dormitory within Tehran city, Iran. Blood sample biomarkers were measured weekly over 6 weeks and including high sensitive C-reactive protein (hsCRP), tumor necrosis factor-soluble receptor-II (sTNF-RII), von Willebrand factor (vWF), white blood cells (WBC) count and interleukin-6 (IL-6). We found significant positive associations for IL-6 and WBC with exposure to *Aspergillus* spp. (As), *Cladosporium* spp. (Cl), *Penicillium* spp. (Pe), total fungi (TF) and *Micrococcus* spp. (MI); vWF with Cl and MI; sTNF-RII with *Staphylococcus* spp. (ST) in healthy young adults from the current-day and multiday averages. For elderly subjects, we observed significant positive associations for hsCRP, sTNF-RII and WBC with exposure to MI, but not with ST and total bacteria (TB). Our results showed the strongest significant positive associations for IL-6 with MI, ST and TB in elderly people. In addition, IL-6 was also positively associated with

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As, Cl and Pe in elderly. Also, the results showed that increase of vWF was significantly associated with bacterial and fungal aerosols, except *Bacillus* spp. (BA) at some lags in elderly subjects. Pooled results support the pivotal role of bioaerosols in increasing the level of some of inflammatory biomarkers, especially IL-6 and WBC in healthy young adults but possibly also in elderly people.

1. Introduction

Human exposure to particulate matter (PM) is one of the most pressing topics in modern-day public health (Nelin et al., 2012). In addition, extensive epidemiological research links ambient air pollution, especially PM with increased cardiovascular events such as cardiovascular mortality and morbidity (Araujo and Nel, 2009; Bräuner et al., 2008; Brook et al., 2010; Huttunen et al., 2012; Nelin et al., 2012; Pelucchi et al., 2009; Valentino et al., 2016). These harmful health effects are suggested to be mediated by systemic inflammation (Huttunen et al., 2012; Nelin et al., 2012; Thompson et al., 2010). Several epidemiological and experimental studies have shown that exposure to PM increases levels of inflammatory markers circulating in blood, especially CRP, IL-6, and sTNF-RII (Delfino et al., 2010; Delfino et al., 2009; Hampel et al., 2015; Huttunen et al., 2012; Langrish et al., 2012; Rückerl et al., 2007; Rückerl et al., 2014). CRP, IL-6 and sTNF-RII act as predictors of cardiovascular events (Pai et al., 2004). PM is divided into three groups, such as: coarse (aerodynamic diameter between 2.5 and 10 μm), fine (particles with an aerodynamic diameter smaller than 2.5 μm), and ultrafine particles (have a diameter <0.1 μm) (Brook et al., 2010; Nelin et al., 2012). Bioaerosols, as a class of airborne pollutants, are particulate matter that contain one or more compounds of biological origin and comprise 5–50 (30% on average) of the aerosols larger than 0.2 μm (Blais-Lecours et al., 2015; Faridi et al., 2015; Ghosh et al., 2015; Perrino and Marcovecchio, 2016; Walser et al., 2015). This includes viable and non-viable bacterial and fungal spores, bacterial and fungal products (endotoxins, mycotoxins, peptidoglycans, β (1, 3)-glucans), viruses, pollen grains, fur fibres, airborne algae, plant debris, and fragments of microbial insects, animal and human skin (Blais-Lecours et al., 2015; Ghosh et al., 2015; Perrino and Marcovecchio, 2016; Walser et al., 2015). Bacterial and fungal aerosols, as the most important bioaerosols, are an inseparable part of the human societies and mainly present in most enclosed environments (Brook et al., 2004; Faridi et al., 2015; Ghosh et al., 2015; Goudarzi et al., 2016; Jones and Harrison, 2004). Bacterial and fungal aerosols have an aerodynamic diameter of 2.5 (Brook et al., 2004) and 1–30 μm (Faridi et al., 2015; Ghosh et al., 2015), respectively (Ghosh et al., 2015). In reality, bacteria are $PM_{2.5}$ and a part of fungal spores are PM_{10} (Blais-Lecours et al., 2015; Brook et al., 2004; Després et al., 2012). Both the upper and lower respiratory systems are affected by bacterial and fungal aerosols (Blais-Lecours et al., 2015; Després et al., 2012; Faridi et al., 2015; Pastuszka et al., 2000), hence their health assessment is highly essential. The occurrence of bioaerosols had been recognized in atmospheric PM samples since the second half of 19th century, but these parts of PM received less attention than others (Després et al., 2012; Perrino and Marcovecchio, 2016). These parts of PM not only in the atmospheric PM samples have presence, but also in about 5 to 34% of indoor PM can be attributed (Ghosh et al., 2015; Mandal and Brandl, 2011; Perrino and Marcovecchio, 2016). Health hazards of bioaerosols are divided into two categories, including: non-infectious diseases (e.g. hypersensitivity, allergies, and asthma) and infectious diseases (e.g. legionellosis, tuberculosis and anthrax) (Ghosh et al., 2015). Although numerous studies have been performed to assess the association of the physical and chemical characteristics of PM (Brucker et al., 2013; Delfino et al., 2010; Delfino et al., 2009; Delfino et al., 2008; Rückerl et al., 2007; Ruckerl et al., 2006; Steinvil et al., 2008) and pollutant gases (Delfino et al., 2009; Rückerl et al., 2007; Rückerl et al., 2014; Ruckerl et al., 2006; Steinvil et al., 2008; Thompson et al., 2010) with circulating biomarkers, few of those have been evaluated the association between bioaerosols and circulating biomarkers (Purokivi et al., 2001).

In this study, we hypothesized that exposure to bioaerosols (bacteria and fungi) would be associated with increased circulating biomarkers in the healthy young adults and the elderly subjects. To assess these acute responses, we carried out a study involving repeated measurements of bioaerosol exposures and circulating biomarkers (hsCRP, sTNF-RII, vWF, WBC and IL-6) in a panel of healthy young adults living in a school dormitory and elderly subjects living within a retirement home in Tehran, the capital of Iran. Tehran is the largest city of Iran with a population of approximately nine million people and has one of the most polluted atmosphere in the world (Hassanvand et al., 2014; Hassanvand et al., 2015; Hassanvand et al., 2017; Hoseini et al., 2016). In recent years, residents of this city have been exposed to severe air pollutant, especially PM and composition of PM, frequently exceeding the ambient air quality standards. Despite the significance of PM in Tehran, there is no information on effects of bioaerosols, as a class of PM, on human health.

2. Methods

2.1. Study participants and design

Two-prospective panel study in Tehran, Iran, including healthy young adults and the elderly subjects (>65 years of age) were recruited from May 2012 to May 2013. In the repeated measurement design, each subject serves as her or his own control. Elderly subjects (44 non-smokers) lived in a retirement home and healthy young adults (40 non-smokers, high school students, and 15–18 years of age) were living within a school dormitory in the city of Tehran, Iran.

Detailed information on the study sites could be found in our previous publication (Faridi et al., 2015). Briefly, the retirement home and school dormitory were located in central urban area of Tehran. Of 60 elderlies, 16 ones were excluded (10 were not eligible, 3 died, and 3 had insufficient biomarker data due to frequent infections), leaving 44 subjects. Of 45 healthy young volunteers, 5 ones were excluded (3 were not eligible, and 2 had insufficient biomarker data due to frequent infections), leaving 40 subjects. Table 1 provides summary data on the blood markers by study and descriptive characteristics of the subjects of each panel study. Over a 12-month period, participants were followed to take part in up to six blood withdrawals scheduled every seven to eight weeks on the same weekday (Wednesday afternoons) and the same time (13:00–15:00) to control circadian variation and weekly effects. Each participant contributed six blood withdrawals ($n = 240$ (6×40) and 264 (6×44) total samples for the healthy young and the elderly subjects, respectively). This approach was intended to increase the chance of having more variability in bioaerosol concentration. At each step of blood withdrawal, participants were visited by a physician and data on health status, medication use and disease was collected and subsequently venous blood samples were drawn. Over this study, blood markers of subjects with acute infectious illnesses were excluded.

2.2. Measurement of blood biomarkers

We took 10-ml blood sample from each subject in each time and kept on ice packs before centrifugation. Samples of venous blood were centrifuged by a refrigerated centrifuge for 15 min and then immediately stored at −70 °C before assay. Biomarkers of hsCRP, sTNF-RII, vWF and IL-6, were determined in plasma and WBC were measured in

Table 1
Characteristics of participants (demographic and clinical) and blood biomarkers.

Subjects	Variable	Mean ± SD or number (%)
Elderly (n = 44)	Sex	
	Male	19 (43.2)
	Female	25 (56.8)
	Age (years)	75.4 ± 5.8
	History of	
	Myocardia infructuous	14 (31.8)
	Positive angiogram or stress test	14 (31.8)
	Hypertension	13 (29.5)
	Hypercholesterolemia	8 (18.2)
	Type 2 diabetes mellitus	8 (18.2)
	Transient ischemic attack	9 (20.5)
	Hay fever	1 (2.2)
	Chronic kidney disease	2 (4.5)
	Stroke	5 (11.4)
	Medication use	
	ACE inhibitors	12 (27.3)
	HMG CoA reductase inhibitors (statins)	10 (22.8)
	Aspirin	26 (59.1)
	Antihyperlipidemic medication	10 (22.8)
	Antihypertensive and cardiac medication	9 (20.5)
	Beta-blockers	4 (9.1)
	Blood markers	
	hsCRP (ng/ml)	16,405.62 ± 956,715
	WBC (k/μl)	6.43 ± 1.61
	IL-6 (pg/ml)	18.95 ± 30.28
	sTNF-RII (pg/ml)	4828.26 ± 2664.23
	vWF (ng/ml)	833.16 ± 351.60
Healthy young adults (n = 40)	Sex	
	Male	40 (100)
	Female	0 (0)
	Age (years)	16.2 ± 0.5
	Blood markers	
	hsCRP (ng/ml)	6842.73 ± 3106.86
	WBC (k/μl)	6.38 ± 1.45
	IL-6 (pg/ml)	15.61 ± 26.32
	sTNF-RII (pg/ml)	1979.33 ± 846.51
	vWF (ng/ml)	1220.01 ± 451.21

whole blood. IL-6, sTNF-RII and vWF markers were measured with enzyme linked immunosorbent assay (Quantikine, R&D Systems) at Immunology, Asthma and Allergy Research center, Tehran University of Medical Sciences (Tehran, Iran). The minimum limits of detection (LoD) for each biomarker were set as the concentration above two times of the average optical densities of 20 replicates of the blank values (zero standard). WBC and hsCRP were measured in the Fajr Hospital Services (Tehran, Iran). hsCRP was measured with an immune turbidimetric method (Sentinel CRP Vario List No. 6K26-02) and WBC was counted using an automatic hematological analyzer (CellDyn 4000, Abbott). All samples were measured in duplicate to ensure reproducibility.

2.3. Bioaerosol measurement

Details of the bioaerosol measurement are described in our previous publication (Faridi et al., 2015). Simultaneous indoor and outdoor air sampling was done in the school dormitory and retirement home. Six days before each blood draw, we measured indoor and outdoor bioaerosols. Indoor/outdoor bioaerosol concentrations were measured using QuickTake® 30 (The QuickTake® 30 Sample Pump is a portable battery-powered air sampling pump that maintains constant airflow from 10 to 30 l/min for use with impactors, spore trap cassettes such as VersaTrap®, asbestos cassettes, microvacuum cassettes, or other samplers requiring flows up to 30 l/min). The indoor bioaerosol monitoring station in the retirement home was located in a common room of the building that the elderly spent >90% of their time there. The indoor bioaerosol sampling station in the school dormitory was installed in a multipurpose room that was used by students for resting, sleeping, studying, and occasionally as a classroom. The indoor bioaerosol measurement instruments were placed at breathing height in the middle of the rooms. Outdoor bioaerosol monitoring stations were installed on the rooftop of the retirement and the school buildings.

2.4. Time spent indoors and outdoors

To ultimately assess the individual exposure to bioaerosols, we used a time-budget survey (TBS) as a tool to assess time spent by each individual in different microenvironments (Almeida-Silva et al., 2015). We designed a data collection form, including information about environments (indoor/outdoor) where participants spend their time (hourly) in each environment. 6 days before blood sampling, the data collection form was distributed among participants and the forms have been collected at the end of each day. In the school dormitory, the data collection forms were completed by participants, but in the retirement home, the forms were completed with the help of the elderly's nurses. Finally, according to the time-budget data, participant's hourly pattern in indoor and outdoor was achieved.

2.5. Exposure assessment

Daily average exposure for each participants (EX_i) was assessed by integrating the results obtained from the time-budget survey with the bioaerosol concentrations measured in indoor and outdoor environments. Hence, the following Eq.uation (1) was applied:

$$EX_i = \frac{\sum_{j=1}^{En} Bio_{ij}{}^{*} t_{ij}}{\sum_{j=1}^{En} t_{ij}} \quad (1)$$

where Bio_{ij} is the concentration of bioaerosols (bacterial and fungal spores) measured in the *j*th environment (*En*), indoor/outdoor, of the *i*th participant, *tij* is the time spent by the ith participant in the *j*th environment, $\sum_{j=1}^{En} t_{ij} = 24$ h.

We used the 24 h immediately preceding blood withdrawal; and also in periods of up to 5 days preceding the blood sampling (lag 0: 0 to 23 h, lag 1: 24 to 47 h, lag 2: 48 to 71 h, lag 3: 72 to 95 h, lag 4: 96 to 119 h, lag 5: 120 to 143 h), and the 2-day average (0 to 47 h), the 3-day average (0 to 71 h), the 4-day average (0 to 95 h), the 5-day average (0 to 120 h), the 6-day average exposure (0 to 143 h) before blood withdrawal.

2.6. Statistical analysis

The individuals' demographic and clinical characteristics were expressed as mean ± standard deviation (SD) for continuous variables and frequencies in count and percentage for categorical variables. The association between the bacterial and fungal aerosols with the blood biomarkers (WBC, hsCRP, IL6, vWF, sTNF-RII) was assessed, separately for the elderly and young adults, using the linear mixed-effects model. As in this panel study, each subject was considered as his/her own control over the measurements in different time periods, the fixed attributes of the study participants cannot be considered as potential confounders. On the other hand, we had no time-dependent covariate for adjustment. Therefore, PROC MIXED in SAS 9.2 software (SAS institute, Cary, NC, USA) was used including random intercept term to consider the correlation between the repeated measurements of each subject. Some kinds of fungal aerosols were not detectable in all study periods. Those (including *Rhodotorula*, *Monilia*, *Alternaria*, *Ulocladium*, *Paecilomyces*, *Mycelium*, *Acremonium*, *Fusarium*, *Chrysosporium*, *Rhizopus*, *Saprophyte* and *Syncephalastrum*) were detected in <4

measurements discarded from the analyses. For the purpose of assessing the delayed and cumulative effect of exposure to bioaerosols, different lag times as well as averages preceding the blood draw were analyzed. Based on investigating the residuals for deviations from the linear mixed-model assumptions, we found that the distribution of hsCRP, IL6, vWF and sTNF-RII were skewed for the elderly and young adults. So, the logarithm transformation was applied for them. Finally, the exposure effects were expressed as percent change (with 95% confidence interval (CI)) in biomarker levels with interquartile range (IQR) increase in the bioaerosol concentrations. These were computed as $[10(\beta * IQR) - 1] * 100\%$ for log10-transformed biomarkers and $[(\beta * IQR) / \text{mean of biomarker}] * 100\%$ for other biomarkers where β is the estimated regression coefficient. $P < 0.05$ was regarded as statistically significant.

3. Results

The mean age of the elderly and healthy young adults was 75.4 and 16.2 years, respectively. The summary information on blood markers and participants' demographic and clinical characteristics are displayed in Table 1. A total of 264 samples of the elderly subjects and 240 samples of the healthy young subjects for each blood markers were available for analysis.

3.1. Bioaerosol concentrations

Fig. 1 displays the indoor and outdoor bioaerosol concentrations in both the retirement home and the school dormitory. In the retirement home, BA, ST, MI and TB indoor concentrations ranged from not

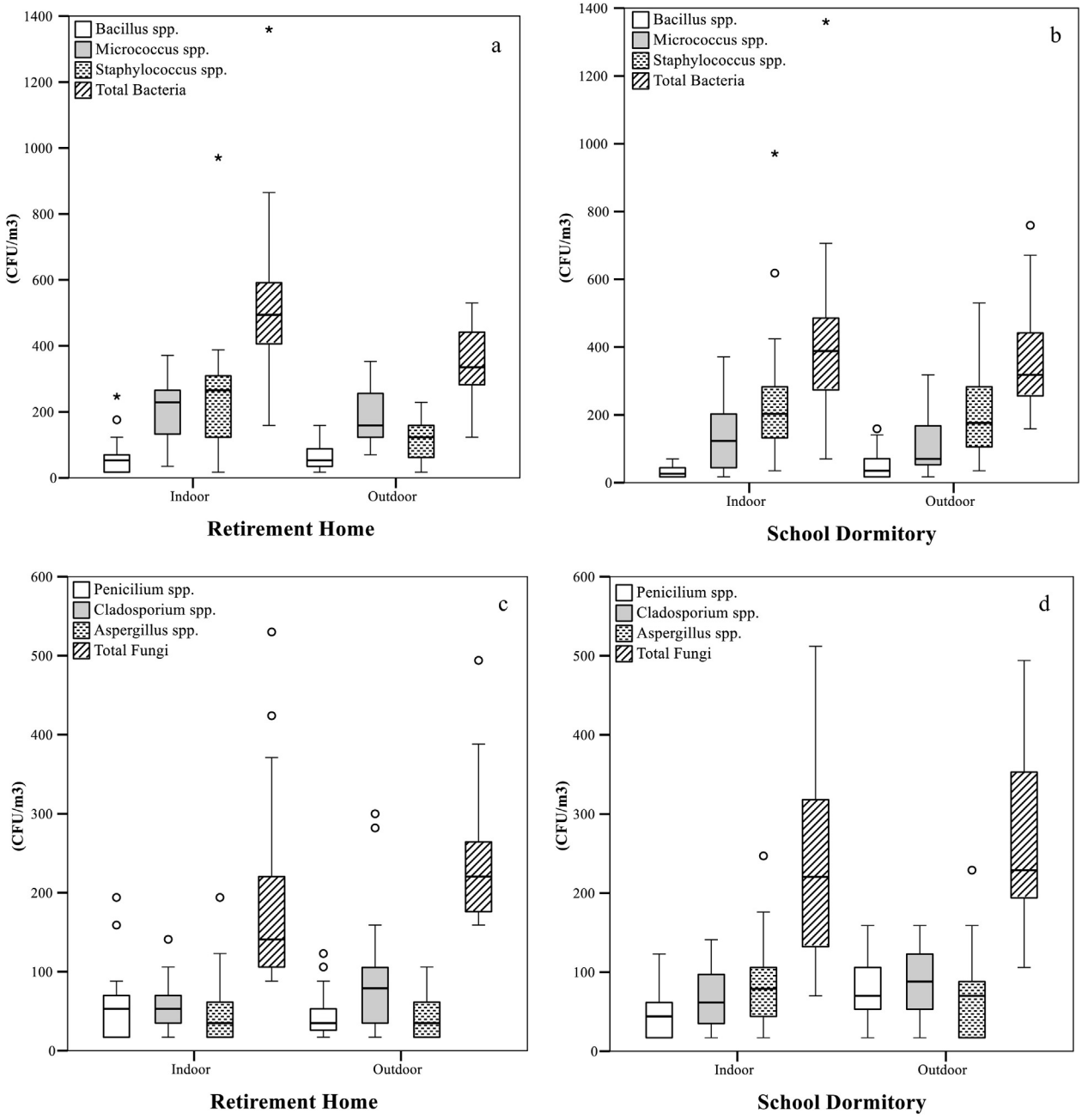

Fig. 1. Boxplot for indoor and outdoor bacterial (a and b) and fungal (c and d) aerosol concentrations during the study period (May 2012 to May 2013) in the retirement home and the school dormitory.

Table 2
Interquartile range (IQR) of bioaerosols at different lag times in the healthy young adults and elderly panel.

Subjects	Lag times	IQR of bacterial and fungal aerosol							
		BA	MI	ST	TB	As	Cl	Pe	TF
Healthy young adults	Lag 0	–	141	124	177	230	106	–	159
	Lag 1	–	177	194	124	–	71	292	318
	Lag 2	–	177	141	318	124	35	35	336
	Lag 3	18	159	177	353	71	76	18	212
	Lag 4	–	141	442	389	–	141	35	71
	Lag 5	451	53	53	124	53	–	–	141
	2-day-ave	15	71	168	168	188	88	35	238
	3-day-ave	9	53	259	277	88	59	35	224
	4-day-ave	–	27	163	172	62	57	31	203
	5-day-ave	12	11	230	254	62	36	11	170
	6-day-ave	19	15	203	233	53	29	6	159
Elderly panel	Lag 0	199	124	565	777	124	101	71	406
	Lag 1	–	71	177	212	44	0	35	71
	Lag 2	44	88	71	212	88	79	124	159
	Lag 3	35	35	336	795	–	35	44	88
	Lag 4	71	318	18	530	–	35	35	18
	Lag 5	–	181	42	336	–	194	212	371
	2-day-ave	185	35	371	574	62	26	35	238
	3-day-ave	71	35	236	353	41	18	29	177
	4-day-ave	53	40	119	106	41	6	25	124
	5-day-ave	60	46	102	184	35	13	24	99
	6-day-ave	93	32	94	79	25	29	39	124

detectable (ND)-1237, ND-972, ND-477 and 88–1360 CFU m^{-3}, respectively. The corresponding outdoor concentrations ranged from ND-159, ND-230, 18–353 and 18–530 CFU m^{-3} (Fig. 1a). In the school dormitory, the indoor concentrations of BA, ST, MI and total bacteria were in the range of ND-618, ND-1767, ND-495 and 18–1943 CFU m^{-3}, respectively, while the corresponding outdoor levels ranged from ND-159, ND-848, ND-318 and 53–1042 CFU m^{-3} (Fig. 1b). As shown in Fig. 1(c and d), the indoor concentrations of Pe, Cl, As and TF were in the range of ND-1060, ND-265, ND-194 and 18–1343 CFU m^{-3} in the retirement home and ND–424, ND-535, ND-247 and 18–601 CFU m^{-3} in the school dormitory, respectively, while the corresponding outdoor concentrations ranged from ND-177, ND-300, ND-141 and 53–495 CFU m^{-3} in the retirement home and ND-159, ND-353, ND-230 and 35–495 CFU m^{-3} in the school dormitory.

3.2. Bioaerosols and biomarkers

Table 2 presents IQR of bioaerosols at different lag times in the healthy young adults and elderly panel. Associations between circulating markers and fungal aerosols for all lags in healthy young adults are given in the Table 3. We observed consistent and clear increases in IL-6 and WBC in association with fungal aerosols in nearly all lag times. IL-6 and WBC were somewhat more strongly associated with As, Cl and TF than was Pe aerosol. Small but statistically significant positive

Table 3
Effect estimates [% (95% CI)] for differences in circulating biomarkers in healthy young adults (n = 40) per IQR (Table 2) change in fungal spore concentration.

Bioaerosols/biomarkers	Lag times	WBC (k/μl)	hsCRP (ng/ml)	IL-6 (pg/ml)	sTNF-RII (pg/ml)	vWF (ng/ml)
As	Lag 0	22.7 (16.5 to 28.8)	−13.8 (−26.8 to 2.0)	389.2 (201.5 to 694.1)	−8.6 (−21.2 to 6.3)	15.7 (−2.1 to 36.3)
	Lag 1	–	–	–	–	–
	Lag 2	1.4 (−3.7 to 6.6)	−12.8 (−23.9 to −0.2)	−23.9 (−45.9 to 6.8)	−16.9 (−24.4 to −8.5)	−0.9 (−10.3 to 9.7)
	Lag 3	3.8 (−1.9 to 9.6)	−10.5 (−22.8 to 4.0)	3.7 (−27.2 to 47.7)	−10.0 (−19.1 to 0.1)	−3.5 (−12.9 to 6.7)
	Lag 4	–	–	–	–	–
	Lag 5	11.7 (7.7 to 15.8)	−12.5 (−23.2 to −0.1)	93.1 (51.6 to 145.9)	−15.8 (−22.3 to −8.9)	4.1 (−6.1 to 15.6)
	2-day-ave	16.6 (12.5 to 20.7)	−6.3 (−16.6 to 5.0)	249.5 (157.0 to 375.5)	−3.4 (−12.5 to 6.9)	9.3 (−1.3 to 21.2)
	3-day-ave	7.8 (5.5 to 10.0)	−6.1 (−11.8 to −0.2)	60.7 (36.6 to 89.1)	−6.1 (−10.6 to −1.6)	3.6 (−0.6 to 8.2)
	4-day-ave	7.5 (5.2 to 9.8)	−6.2 (−11.9 to −0.1)	52.7 (29.1 to 80.7)	−6.2 (−10.8 to −1.6)	2.9 (−1.4 to 7.6)
	5-day-ave	7.6 (5.2 to 9.9)	−5.8 (−11.7 to 0.4)	55.5 (31.0 to 84.7)	−6.1 (−10.6 to −1.3)	2.9 (−1.7 to 7.6)
	6-day-ave	8.4 (5.9 to 10.8)	−6.4 (−12.6 to 0.2)	62.7 (35.7 to 95.0)	−6.8 (−11.6 to −1.7)	3.4 (−1.5 to 8.4)
Cl	Lag 0	7.9 (3.7 to 12.1)	0.1 (−10.8 to 12.5)	152.9 (101.3 to 217.9)	2.2 (−6.1 to 11.1)	11.4 (0.6 to 23.4)
	Lag 1	11.4 (8.7 to 14.0)	−7.8 (−14.7 to −0.2)	114.1 (75.7 to 160.8)	−7.4 (−12.8 to −1.5)	7.9 (2.2 to 14.0)
	Lag 2	5.3 (2.3 to 8.3)	1.5 (−6.1 to 9.7)	63.2 (32.4 to 101.3)	2.1 (−3.9 to 8.6)	7.5 (1.9 to 13.4)
	Lag 3	5.8 (1.1 to 10.6)	−11.8 (−21.2 to −1.6)	28.0 (−11.7 to 85.6)	−8.8 (−17.2 to 0.4)	9.3 (−3.1 to 23.3)
	Lag 4	4.3 (−1.4 to 10.1)	9.6 (−3.5 to 24.6)	30.3 (−14.7 to 99.4)	5.9 (−6.3 to 19.4)	78.1 (−4.5 to 231.8)
	Lag 5	–	–	–	–	–
	2-day-ave	15.5 (11.7 to 19.3)	−6.9 (−16.6 to 4.1)	247.0 (167.2 to 350.5)	−5.0 (−12.9 to 3.6)	14.9 (6.1 to 24.4)
	3-day-ave	13.6 (10.3 to 16.8)	−4.8 (−13.5 to 4.8)	206.5 (145.1 to 283.3)	−3.2 (−10.3 to 4.3)	14.0 (6.5 to 22.1)
	4-day-ave	11.9 (8.7 to 15.1)	−4.8 (−13.2 to 4.3)	167.4 (114.2 to 233.9)	−3.3 (−9.9 to 4.0)	13.8 (6.4 to 21.8)
	5-day-ave	11.6 (9.3 to 13.9)	−3.6 (−10.3 to 3.6)	150.3 (113.3 to 193.8)	−2.6 (−7.9 to 3.1)	9.5 (4.3 to 14.9)
	6-day-ave	11.1 (8.7 to 13.5)	−4.0 (−10.7 to 3.3)	138.7 (101.5 to 182.7)	−2.3 (−7.7 to 3.5)	9.2 (4.0 to 14.8)
Pe	Lag 0	–	–	–	–	–
	Lag 1	13.0 (8.9 to 17.2)	−5.9 (−15.4 to 4.9)	181.1 (107.6 to 280.5)	−2.6 (−11.4 to 6.9)	8.0 (−2.0 to 19.1)
	Lag 2	−0.3 (−2.5 to 1.9)	−3.0 (−7.7 to 1.9)	−7.5 (−21.5 to 9.0)	−2.4 (−6.5 to 1.9)	1.2 (−3.5 to 6.2)
	Lag 3	−1.2 (−4.0 to 1.7)	−4.9 (−11.8 to 2.6)	−11.5 (−25.8 to 5.5)	−2.1 (−7.4 to 3.4)	−4.4 (−10.6 to 2.2)
	Lag 4	−5.3 (−7.8 to −2.8)	7.3 (0.1 to 15.1)	−25.9 (−36.8 to −13.2)	10.2 (4.9 to 15.7)	−3.3 (−8.4 to 2.2)
	Lag 5	–	–	–	–	–
	2-day-ave	3.5 (2.5 to 4.5)	−2.4 (−5.4 to 0.6)	26.1 (17.2 to 35.6)	−2.6 (−4.8 to −0.4)	1.5 (−0.5 to 3.6)
	3-day-ave	4.9 (3.2 to 6.6)	−4.7 (−9.0 to −0.2)	32.8 (17.2 to 50.5)	−4.9 (−8.3 to −1.5)	1.9 (−1.3 to 5.3)
	4-day-ave	3.7 (1.8 to 5.7)	−4.9 (−9.6 to −0.1)	20.2 (4.7 to 38.1)	−4.9 (−8.6 to −1.2)	0.7 (−2.7 to 4.3)
	5-day-ave	1.2 (0.3 to 2.1)	−1.9 (−4.2 to 0.4)	5.2 (−1.6 to 12.4)	−1.6 (−3.4 to 0.2)	−0.1 (−1.8 to 1.5)
	6-day-ave	0.5 (−0.1 to 1.2)	−1.1 (−2.7 to 0.5)	1.4 (−3.1 to 6.1)	−0.9 (−2.1 to 0.4)	−0.3 (−1.5 to 0.8)
TF	Lag 0	4.1 (2.7 to 5.4)	−2.5 (−6.0 to 1.1)	30.3 (18.4 to 43.3)	−2.2 (−5.0 to 0.7)	1.6 (−0.7 to 4.2)
	Lag 1	10.2 (6.8 to 13.6)	−7.7 (−16.1 to 0.9)	69.5 (31.9 to 117.9)	−9.1 (−15.5 to −2.2)	3.3 (−3.6 to 10.7)
	Lag 2	4.3 (0.6 to 7.9)	−5.3 (−13.7 to 4.3)	17.5 (−9.6 to 53.1)	−6.7 (−13.0 to 0.5)	4.6 (−2.3 to 11.7)
	Lag 3	6.0 (2.3 to 9.7)	−9.7 (−18.1 to −0.5)	34.7 (3.0 to 76.2)	−7.5 (−14.5 to −0.4)	2.6 (−4.8 to 10.3)
	Lag 4	−1.8 (−4.5 to 0.9)	2.1 (−4.6 to 9.4)	−24.4 (−37.5 to −8.6)	0.3 (−4.9 to 5.8)	−4.9 (−11.3 to 2.0)
	Lag 5	10.8 (7.2 to 14.3)	−8.7 (−16.9 to 0.6)	69.4 (30.0 to 120.7)	−9.6 (−16.1 to −2.6)	3.7 (−3.5 to 11.6)
	2-day-ave	7.2 (4.9 to 9.5)	−4.8 (−10.9 to 1.2)	53.1 (29.5 to 80.9)	−4.8 (−9.4 to −0.1)	2.6 (−1.6 to 7.3)
	3-day-ave	11.9 (8.8 to 15.0)	−8.9 (−16.5 to −0.5)	96.7 (56.0 to 148.0)	−9.8 (−15.6 to −3.0)	5.8 (−0.5 to 12.5)
	4-day-ave	10.7 (7.6 to 13.7)	−9.4 (−16.7 to −0.9)	81.3 (44.1 to 128.2)	−9.4 (−15.1 to −2.8)	4.9 (−1.4 to 11.3)
	5-day-ave	11.3 (8.1 to 14.6)	−9.7 (−17.7 to −1.2)	84.0 (43.7 to 135.5)	−10.0 (−16.1 to −3.5)	5.0 (−1.5 to 12.0)
	6-day-ave	11.0 (7.8 to 14.2)	−9.4 (−17.0 to −1.1)	79.6 (41.0 to 128.8)	−9.7 (−15.8 to −3.2)	4.8 (−1.5 to 11.5)

associations were found between vWF and Cl at lags 0, 1, 2, 2-, 3- and 4-day-average, whereas no associations were seen for As, Pe and TF in this panel. There was an unexpected inverse association of sTNF-RII and hsCRP with fungal aerosols in some lags, whereas only a positive association was found for sTNF-RII and hsCRP with Pe 7.3% (95% CI: 0.1 to 15.1) and 10.2% (95% CI: 4.9 to 15.7) at lag 4 per IQR increase (Table 2), respectively. In addition, the highest level of IL-6 was observed with As 389.2% (95% CI: 201.5 to 694.1) at lag 0, Cl 247.0% (95% CI: 167.2 to 350.5) at 2-day-average, Pe 181.1% (95% CI: 107.6 to 280.5) at lag 1 and TF 96.7% (95% CI: 56.0 to 148.0) at 3-day-average for healthy young adults. Similar to IL-6, the highest level of WBC was showed with As 22.69% (95% CI: 16.54 to 28.84) at lag 0, Pe 13% (95% CI: 8.9 to 17.2) at lag 1, Cl 15.5% (95% CI: 11.7 to 19.3) at 2-day-average, and TF 11.9% (95% CI: 8.8 to 15) at 3-day-average in the healthy young panel. Eventually, our data display more and stronger associations for IL-6 and WBC than for sTNF-RII, vWF and hsCRP with fungal aerosols in the healthy young adults.

Table 4 shows the results for percent changes in circulating biomarkers per an IQR (Table 2) increase in fungal spore concentrations in the panel of elderly subjects. We found clear increases in vWF, sTNF-RII, IL-6, hsCRP and WBC in association with fungal aerosols at some lags. The strongest association was observed at lag 0 with 20.3% (95% CI: 15.0 to 25.7) increasing in WBC per IQR change in Cl concentration. In the elderly subjects, in some lags exposure to fungal aerosols were not associated with IL-6 and sTNF-RII. Nonetheless, the highest level of association was observed between IL-6 and As 137.6% (95% CI: 74.0 to 224.5), Pe 137.9% (95% CI: 59.8 to 254.4) and Cl 92.7% (95% CI: 38.5 to 168.1) at lag 2-day-average, lag 3 and lag 0, respectively. There was an unexpected inverse association of vWF, sTNF-RII, IL-6, hsCRP and WBC with As at lag 2. The most robust association was observed between vWF and Pe in almost all lag times.

Associations between bacterial aerosols and circulating markers in the healthy young adults shown in the Table 5. vWF, IL-6 and WBC were positively associated with MI at lag 0 and 1 and TB at lag 5, whereas sTNF-RII significantly associated with ST at lag 2, 4 and 5 and TB at lag 4. hsCRP was not significantly associated with almost any of the bacterial aerosols in this investigation. As shown in Table 5, unexpected inverse associations of vWF, IL-6 and WBC observed at some lags. Our data show the strongest association of IL-6 was seen with BA 205.7% (95% CI: 122.3 to 320.4) at lag 5 per an IQR increase. Generally, sTNF-RII can be regarded as a specific biomarker for exposure to ST in all lag times; and CRP was not associated with none of the bacteria evaluated.

Table 6 displays the relationships of bacterial aerosol exposures with vWF, sTNF-RII, IL-6, hsCRP and WBC in the panel of elderly subjects. The strongest significant positive associations with bacterial aerosols were seen for IL-6 and MI, ST and TB but not with BA. As displayed in Table 6, in the elderly subjects IL-6 increased with MI 155.2% (95% CI: 100.1 to 225.4) and 71.5% (95% CI: 32.3 to 122.3) at lag 0 and 4, and with ST

Table 4
Effect estimates [% (95% CI)] for differences in circulating biomarkers in a panel of elderly subjects (n = 44) per IQR (Table 2) change in fungal spore concentration.

Bioaerosols/biomarkers	Lag times	WBC (k/μl)	hsCRP (ng/ml)	IL-6 (pg/ml)	sTNF-RII (pg/ml)	vWF (ng/ml)
As	Lag 0	5.2 (−1.0 to 11.4)	26.3 (8.4 to 47.1)	98.1 (44.5 to 171.6)	12.5 (0.7 to 25.7)	−7.1 (−14.0 to −0.4)
	Lag 1	12.9 (7.5 to 18.4)	10.2 (−5.2 to 28.1)	41.7 (3.6 to 93.9)	8.6 (−2.4 to 20.9)	11.4 (2.9 to 20.7)
	Lag 2	−29.3 (−35.5 to −23.1)	−26.4 (−38.1 to −12.6)	−74.7 (−83.0 to −62.5)	−26.9 (−37.2 to −14.8)	−50.0 (−72.9 to −7.8)
	Lag 3	–	–	–	–	–
	Lag 4	–	–	–	–	–
	Lag 5	–	–	–	–	–
	2-day-ave	10.9 (4.8 to 17.0)	33.3 (14.2 to 55.6)	137.6 (74.0 to 224.5)	17.2 (4.7 to 31.2)	−4.5 (−12.2 to 4.0)
	3-day-ave	−4.5 (−10.5 to 1.4)	17.6 (1.1 to 36.7)	21.0 (−12.5 to 67.4)	0.4 (−10.0 to 12.0)	−4.9 (−12.0 to 2.7)
	4-day-ave	–	–	–	–	–
	5-day-ave	–	–	–	–	–
	6-day-ave	–	–	–	–	–
Cl	Lag 0	20.3 (15.0 to 25.7)	4.6 (−11.2 to 23.4)	92.7 (38.5 to 168.1)	19.5 (6.6 to 34.0)	13.5 (−1.6 to 30.9)
	Lag 1	0	0	0	0	0
	Lag 2	−15.5 (−22.0 to −9.1)	−20.6 (−32.5 to −6.5)	−34.5 (−57.9 to 2.0)	−16.6 (−25.8 to −6.4)	19.0 (6.7 to 32.8)
	Lag 3	1.8 (−1.0 to 4.5)	−5.4 (−11.9 to 1.6)	6.3 (−10.6 to 26.5)	1.9 (−2.6 to 6.6)	−4.1 (−7.4 to −0.8)
	Lag 4	6.4 (1.2 to 11.6)	−6.6 (−17.6 to 5.8)	24.1 (−5.7 to 63.3)	2.3 (−6.5 to 12.0)	3.3 (−3.7 to 10.8)
	Lag 5	10.4 (5.7 to 15.0)	17.4 (4.8 to 31.5)	3.7 (−20.0 to 34.7)	7.8 (−0.9 to 17.1)	9.8 (3.5 to 16.4)
	2-day-ave	5.9 (2.0 to 9.8)	−1.4 (−11.2 to −8.7)	7.1 (−16.7 to 37.7)	5.0 (−1.6 to 12.1)	−5.3 (−10.4 to 0.0)
	3-day-ave	−2.0 (−5.4 to 1.4)	−13.6 (−20.7 to −2.7)	−3.1 (−21.8 to 20.1)	−4.1 (−9.2 to 1.3)	1.5 (−2.4 to 5.6)
	4-day-ave	−0.4 (−1.2 to 0.4)	−2.9 (−4.8 to −0.8)	−0.3 (−5.1 to 4.8)	−0.6 (−1.9 to 0.7)	−0.5 (−1.4 to 0.4)
	5-day-ave	−0.1 (−2.0 to 1.7)	−6.3 (−10.6 to 8.3)	7.5 (−4.5 to 20.9)	−0.9 (−3.9 to 2.1)	−0.4 (−2.5 to 1.8)
	6-day-ave	10.2 (6.2 to 14.2)	7.4 (−3.8 to 8.6)	−10.5 (−31.6 to 17.3)	6.7 (−0.4 to 14.3)	6.9 (1.4 to 12.7)
Pe	Lag 0	3.5 (0.3 to 6.7)	12.1 (3.1 to 41.4)	−6.0 (−23.3 to 15.2)	2.9 (−2.4 to 8.5)	1.4 (−2.3 to 5.4)
	Lag 1	11.0 (7.2 to 14.8)	6.8 (−4.0 to −2.5)	93.4 (51.8 to 146.4)	12.9 (5.7 to 20.5)	−11.1 (−17.3 to −4.3)
	Lag 2	−14.3 (−19.1 to −9.4)	−19.9 (−30.0 to 19.4)	−12.8 (−37.7 to 21.6)	−15.9 (−22.6 to −8.7)	16.6 (7.8 to 26.0)
	Lag 3	5.5 (−0.9 to 12.0)	−8.6 (−21.6 to −0.7)	137.9 (59.8 to 254.4)	1.6 (−9.1 to 13.5)	15.6 (7.7 to 24.0)
	Lag 4	−7.4 (−10.2 to −4.6)	−8.4 (−15.6 to 1.3)	−13.6 (−28.8 to 4.7)	−7.4 (−11.9 to −2.6)	5.4 (0.3 to 10.7)
	Lag 5	3.6 (2.3 to 4.9)	4.7 (1.4 to 22.1)	1.3 (−6.1 to 9.5)	3.2 (0.8 to 5.7)	2.4 (0.6 to 4.2)
	2-day-ave	6.0 (3.1 to 8.9)	12.4 (4.1 to 10.7)	13.0 (−6.4 to 36.4)	5.9 (0.9 to 11.2)	−0.1 (−3.8 to 3.8)
	3-day-ave	−3.0 (−6.8 to 0.9)	−1.6 (−11.1 to 9.7)	6.3 (−16.8 to 35.8)	−5.7 (−11.4 to 0.5)	7.3 (2.5 to 12.4)
	4-day-ave	−0.3 (−4.9 to 4.2)	−3.7 (−14.3 to 3.0)	23.7 (−6.7 to 64.0)	−4.9 (−11.6 to 2.3)	12.9 (7.3 to 18.7)
	5-day-ave	−5.3 (−9.8 to −0.9)	−8.4 (−18.4 to 3.5)	8.1 (−18.6 to 43.6)	−8.8 (−15.1 to −2.0)	16.5 (10.3 to 23.1)
	6-day-ave	2.0 (1.0 to 2.9)	3.1 (0.4 to 1.9)	−4.3 (−10.5 to 2.4)	1.7 (0.0 to 3.4)	1.2 (0.0 to 2.4)
TF	Lag 0	−2.9 (−7.6 to 1.7)	7.5 (−4.6 to 41.1)	−19.4 (−39.7 to 8.4)	−0.1 (−7.2 to 7.9)	−8.9 (−13.9 to −4.6)
	Lag 1	1.0 (−0.9 to 2.9)	1.0 (−4.0 to −0.8)	1.5 (−10.0 to 14.6)	0.4 (−2.7 to 3.6)	−1.0 (−3.2 to 1.4)
	Lag 2	−9.5 (−11.5 to −7.6)	−7.4 (−13.0 to 8.8)	−25.4 (−35.1 to −13.9)	−9.4 (−12.7 to 3.6)	6.4 (−0.4 to 13.8)
	Lag 3	5.8 (1.3 to 10.2)	−6.7 (−16.9 to −7.6)	4.7 (−21.0 to 38.7)	2.3 (−12.7 to −6.0)	0.9 (−4.8 to 6.9)
	Lag 4	−2.1 (−3.1 to −1.1)	−4.1 (−6.6 to 0.6)	2.3 (−4.4 to 9.5)	−2.2 (−3.8 to −0.5)	−0.5 (−1.7 to 0.8)
	Lag 5	5.2 (3.3 to 7.1)	7.3 (2.3 to 28.2)	2.2 (−8.2 to 14.2)	4.5 (0.9 to 8.2)	3.6 (1.0 to 6.3)
	2-day-ave	−1.2 (−5.3 to 2.8)	5.5 (−5.3 to 11.7)	−12.3 (−32.3 to 13.7)	0.2 (−6.4 to 7.2)	−6.9 (−10.9 to −2.2)
	3-day-ave	−9.6 (−13.4 to −5.8)	−2.0 (−11.9 to 10.1)	−31.3 (−46.6 to −11.9)	−8.2 (−14.0 to −2.0)	−5.5 (−10.1 to −0.8)
	4-day-ave	−8.0 (−11.5 to −4.5)	−2.8 (−11.8 to 6.2)	−28.5 (−43.3 to −10.0)	−7.1 (−12.5 to −1.4)	−4.7 (−9.0 to −0.6)
	5-day-ave	−7.9 (−11.2 to −4.6)	−4.0 (−12.4 to 20.3)	−25.3 (−40.0 to −7.0)	−7.2 (−12.2 to −1.8)	−4.5 (−8.3 to −0.5)
	6-day-ave	1.2 (−3.1 to 5.6)	12.6 (0.6 to 26.7)	−41.5 (−55.2 to −23.5)	0.5 (−6.3 to 8.0)	−0.6 (−5.3 to 4.8)

Table 5
Effect estimates [% (95% CI)] for differences in circulating biomarkers in healthy young adults (n = 40) per IQR (Table 2) change in bacteria concentration.

Bioaerosols/biomarkers	Lag times	WBC (k/μl)	hsCRP (ng/ml)	IL-6 (pg/ml)	sTNF-RII (pg/ml)	vWF (ng/ml)
BA	Lag 0	–	–	–	–	–
	Lag 1	–	–	–	–	–
	Lag 2	–	–	–	–	–
	Lag 3	−5.4 (−8.1 to −2.6)	1.9 (−5.3 to 9.8)	−23.8 (−38.7 to −5.3)	0.1 (−5.7 to 6.1)	−2.8 (−7.4 to 2.1)
	Lag 4	–	–	–	–	–
	Lag 5	10.9 (6.7 to 15.1)	1.7 (−8.0 to 12.6)	205.7 (122.3 to 320.4)	2.7 (−6.0 to 12.8)	15.8 (6.4 to 26.0)
	2-day-ave	−16.7 (−21.8 to −11.6)	4.1 (−10.3 to 20.7)	−62.0 (−74.9 to −42.5)	10.0 (−2.1 to 23.6)	−28.2 (−40.7 to −13.1)
	3-day-ave	−18.7 (−23.2 to −14.2)	1.2 (−12.1 to 16.7)	−77.4 (−83.7 to −68.3)	−1.0 (−11.5 to 10.8)	−14.5 (−22.0 to −6.4)
	4-day-ave	−2.5 (−3.4 to −1.5)	1.3 (−1.3 to 4.0)	−13.1 (−19.4 to −6.3)	0.8 (−1.2 to 3.0)	−1.6 (−3.2 to 0.0)
	5-day-ave	−3.8 (−6.3 to −1.3)	3.2 (−3.3 to 10.3)	−18.7 (−33.0 to −1.2)	0.8 (−4.4 to 6.2)	−3.1 (−7.0 to 0.9)
	6-day-ave	2.5 (1.4 to 3.5)	0.1 (−2.7 to 3.0)	27.9 (18.5 to 37.9)	−0.4 (−2.6 to 1.9)	3.0 (1.3 to 4.9)
MI	Lag 0	8.1 (4.6 to 11.7)	5.8 (−3.8 to 16.2)	97.8 (53.9 to 154.2)	6.2 (−1.3 to 14.3)	10.9 (1.4 to 21.2)
	Lag 1	9.6 (6.0 to 13.2)	−10.0 (−18.1 to −0.8)	68.5 (28.8 to 120.3)	−11.1 (−17.4 to −4.0)	7.1 (0.0 to 14.7)
	Lag 2	1.4 (−2.0 to 4.8)	3.3 (−5.2 to 12.8)	4.9 (−18.1 to 34.5)	6.3 (−0.8 to 13.8)	0.8 (−5.5 to 7.7)
	Lag 3	−5.7 (−8.5 to −2.9)	2.1 (−5.3 to 10.1)	−23.7 (−39.0 to −5.0)	0.8 (−5.0 to 7.0)	−2.9 (−7.7 to 2.4)
	Lag 4	−12.2 (−16.6 to −7.8)	0.1 (−11.1 to 12.9)	−55.1 (−67.4 to −38.0)	5.1 (−4.5 to 15.4)	−13.1 (−21.4 to −3.8)
	Lag 5	9.4 (6.9 to 11.9)	−3.0 (−10.3 to 4.9)	69.5 (39.7 to 105.7)	−5.7 (−11.1 to 0.0)	9.7 (−0.6 to 21.1)
	2-day-ave	11.8 (9.4 to 14.2)	−4.0 (−10.8 to 3.5)	124.4 (87.5 to 168.6)	−4.6 (−9.9 to 1.1)	9.7 (4.0 to 15.6)
	3-day-ave	5.3 (3.5 to 7.1)	−0.4 (−5.3 to 4.7)	41.3 (23.5 to 61.8)	0.4 (−3.5 to 4.4)	4.6 (0.6 to 8.7)
	4-day-ave	8.4 (5.9 to 11.0)	1.6 (−5.5 to 9.1)	98.4 (65.7 to 137.6)	2.2 (−3.4 to 8.1)	8.6 (3.2 to 14.2)
	5-day-ave	3.0 (1.0 to 4.9)	1.6 (−3.5 to 7.0)	38.0 (19.9 to 58.7)	4.8 (0.7 to 9.0)	3.5 (−0.2 to 7.3)
	6-day-ave	8.0 (5.9 to 10.1)	1.1 (−4.8 to 7.4)	100.1 (73.5 to 130.8)	2.2 (−2.5 to 7.2)	7.5 (3.2 to 12.0)
ST	Lag 0	0.1 (−0.9 to 1.1)	1.0 (−1.4 to 3.5)	−3.1 (−9.5 to 4.0)	0.0 (−2.0 to 2.0)	−6.3 (−11.8 to −0.6)
	Lag 1	−5.2 (−9.3 to −1.1)	−2.6 (−12.2 to 8.4)	−49.1 (−61.8 to −32.2)	−8.2 (−15.6 to −0.3)	−6.9 (−14.1 to 1.1)
	Lag 2	−1.9 (−2.8 to −0.9)	2.1 (−0.3 to 4.6)	−9.6 (−15.6 to −3.2)	3.3 (1.4 to 5.3)	−1.0 (−2.6 to 0.8)
	Lag 3	4.6 (2.1 to 7.1)	3.0 (−3.6 to 10.0)	35.4 (12.7 to 62.7)	0.7 (−4.4 to 6.1)	14.1 (3.0 to 26.4)
	Lag 4	−1.5 (−3.1 to 0.1)	3.4 (−0.7 to 7.8)	−0.9 (−11.5 to 11.5)	5.9 (2.6 to 9.3)	0.2 (−3.0 to 3.3)
	Lag 5	−0.1 (−2.6 to 2.3)	6.7 (−0.2 to 14.1)	11.9 (−6.6 to 34.0)	9.6 (4.3 to 15.1)	0.5 (−4.2 to 5.3)
	2-day-ave	−0.6 (−2.7 to 1.6)	1.4 (−3.8 to 7.0)	−14.3 (−26.3 to −0.2)	−1.2 (−5.3 to 3.0)	−8.5 (−16.0 to −0.8)
	3-day-ave	−6.5 (−10.3 to −2.6)	8.5 (−1.8 to 19.7)	−40.8 (−54.8 to −22.2)	8.3 (0.2 to 16.9)	−9.1 (−16.9 to −0.4)
	4-day-ave	−2.3 (−4.9 to 0.4)	5.5 (−1.5 to 12.9)	−18.7 (−32.6 to −1.5)	4.7 (−0.7 to 10.4)	−5.5 (−12.3 to 1.7)
	5-day-ave	−3.1 (−5.8 to −0.3)	7.3 (−0.1 to 15.1)	−12.8 (−28.3 to 6.4)	9.6 (3.9 to 15.7)	−1.1 (−6.1 to 3.9)
	6-day-ave	−2.8 (−5.6 to 0.0)	7.5 (0.1 to 15.5)	−10.2 (−26.6 to 10.2)	9.9 (4.1 to 16.2)	−0.9 (−5.9 to 4.4)
TB	Lag 0	0.6 (−0.6 to 1.7)	1.6 (−1.2 to 4.7)	1.4 (−6.7 to 10.3)	0.7 (−1.6 to 3.1)	−2.0 (−8.6 to 5.0)
	Lag 1	1.3 (−0.3 to 3.0)	−3.4 (−7.4 to 0.9)	−2.8 (−14.3 to 9.8)	−5.3 (−8.5 to −2.3)	0.4 (−2.5 to 3.5)
	Lag 2	−2.2 (−3.9 to −0.6)	3.3 (−0.7 to 7.8)	−11.7 (−21.5 to −0.3)	5.5 (2.1 to 8.9)	−1.5 (−4.3 to 1.9)
	Lag 3	−2.7 (−6.7 to 1.2)	8.3 (−2.4 to 19.6)	−8.6 (−31.2 to 21.7)	3.6 (−4.0 to 12.1)	−0.3 (−7.8 to 7.8)
	Lag 4	−1.7 (−3.1 to −0.3)	3.1 (−0.6 to 6.8)	−3.5 (−12.6 to 6.8)	5.1 (2.4 to 8.0)	−0.2 (−2.6 to 2.4)
	Lag 5	4.6 (2.9 to 6.2)	1.3 (−3.1 to 6.0)	52.4 (36.1 to 70.7)	0.7 (−2.8 to 4.4)	5.0 (1.8 to 8.2)
	2-day-ave	1.5 (−0.5 to 3.5)	0.7 (−4.2 to 5.9)	0.6 (−12.6 to 15.9)	−1.5 (−5.6 to 2.2)	−0.8 (−9.2 to 8.7)
	3-day-ave	−1.7 (−5.1 to 1.7)	6.2 (−2.5 to 15.8)	−16.9 (−34.8 to 6.0)	6.5 (−0.4 to 13.9)	−3.8 (−11.4 to 4.9)
	4-day-ave	−1.6 (−4.0 to 0.9)	5.3 (−0.8 to 11.9)	−11.9 (−25.7 to 4.5)	4.6 (−0.3 to 9.7)	−2.4 (−8.4 to 3.8)
	5-day-ave	−3.2 (−6.0 to −0.4)	7.7 (0.4 to 15.6)	−12.6 (−28.8 to 6.6)	9.8 (4.0 to 16.0)	−1.2 (−6.2 to 4.1)
	6-day-ave	−2.2 (−5.1 to 0.8)	8.2 (0.5 to 16.7)	−3.7 (−21.8 to 19.4)	10.3 (4.1 to 16.8)	0.2 (−5.2 to 5.8)

54.5% (95% CI: 14.8 to 107.8) and 53.7% (95% CI: 25.7 to 87.9) at lag 0 and 1, and with TB 56.2% (95% CI: 28.0 to 90.1) and 41.1% (95% CI: 18.7 to 67.7) and 69.6% (95% CI: 33.1 to 116.5) at lag 0, 1 and 4 per an IQR increase, respectively. We found that the association of vWF with bacterial aerosol was weaker than the other circulating biomarkers in the elderly subjects. sTNF-RII was positively associated with MI at lag 0, 3, 5 and 2-, 3-, 4- and 6-day-average, but not BA, ST and TB. Similar to sTNF-RII, for the panel of elderly subjects hsCRP and WBC showed significant positive associations with MI at many lags. We observed inverse associations of ST with WBC, sTNF-RII and hsCRP. In sum, MI was associated with all biomarkers except vWF, in the elderly people.

4. Discussion

We examined the association between bioaerosol (bacteria and fungi) concentrations and circulating biomarkers in a panel of elderly subjects and healthy young adults. We hypothesized that circulating biomarkers would increase with increased bacterial and fungal concentrations. In these two panel studies, we found increased IL-6 and WBC after exposure to high fungal aerosol concentrations. Our results observed that levels of vWF were related to increased fungal aerosol concentrations in two groups. We also found significant relationship between IL-6, WBC, sTNF-RII and vWF in association with bacterial aerosol concentrations at healthy young adults in some lag times. For the panel of elderly subjects, blood biomarkers of IL-6, WBC, hsCRP and sTNF-RII were increased in association with bacterial aerosol concentrations.

4.1. Bacterial and fungal aerosols

Our data demonstrate that in two panels, the dominant bacterial aerosols were ST, MI, and BA. All the bacterial aerosols in our study were gram-positive. Pe, As, and Cl were the most observed fungal aerosols detected in the panel of elderly subjects and healthy young adults. ST, MI, BA, Pe, Cl and As have an aerodynamic diameter 0.866, 1, 0.8–1.3, 2–3, 1.1–1.9 and 1.4–1.9 μm, respectively (Jan, 2006; Kulkarni et al., 2011; Ruzer and Harley, 2012). Generally, bacterial and fungal aerosols observed in our panel study were fine PM ($PM_{2.5}$), and as such they may reach the alveoli (Brook et al., 2004; Ghosh et al., 2015). In these two panels, bacterial and fungal aerosols were found both indoors and outdoors. Our results showed that in both the retirement home and the school dormitory, the bacterial aerosols were higher than the fungal aerosols.

4.2. Bacterial/fungal aerosols and biomarkers

Though several epidemiological studies have been shown that PM and constituents of PM can affect the cardiovascular system (Brook

Table 6
Effect estimates [% (95% CI)] for differences in circulating biomarkers in a panel of elderly subjects (n = 44) per IQR (Table 2) change in bacteria concentration.

Bioaerosols/biomarkers	Lag times	WBC (k/μl)	hsCRP (ng/ml)	IL-6 (pg/ml)	sTNF-RII (pg/ml)	vWF (ng/ml)
BA	Lag 0	2.0 (−3.1 to 7.2)	22.3 (2.2 to 58.3)	−61.6 (−72.4 to −46.3)	3.1 (−5.8 to 13.0)	−3.6 (−9.6 to 2.9)
	Lag 1	–	–	–	–	–
	Lag 2	−1.2 (−6.0 to 3.7)	−4.6 (−17.7 to 42.7)	−2.3 (−25.6 to 28.4)	−5.5 (−14.2 to 4.1)	4.9 (−2.3 to 12.7)
	Lag 3	5.6 (−0.8 to 12.1)	7.8 (−12.6 to 28.7)	−29.2 (−45.0 to −8.9)	7.8 (−4.8 to 22.0)	2.3 (−7.0 to 12.6)
	Lag 4	8.8 (2.8 to 14.8)	37.2 (13.7 to 14.6)	12.9 (−12.1 to 44.8)	10.0 (−2.3 to 23.8)	7.5 (−1.8 to 17.7)
	Lag 5	–	–	–	–	–
	2-day-ave	3.6 (−1.2 to 8.4)	20.8 (2.1 to 67.8)	−53.6 (−66.5 to −35.6)	5.0 (−3.8 to 14.5)	−1.3 (−7.4 to 4.8)
	3-day-ave	2.7 (−0.7 to 6.1)	8.9 (−2.6 to 28.5)	−43.4 (55.0 to −29.1)	0.9 (−5.2 to 7.5)	2.9 (−1.8 to 7.9)
	4-day-ave	2.5 (−0.8 to 5.9)	8.1 (−3.2 to 19.1)	−44.5 (−55.6 to 30.7)	0.7 (−5.3 to 7.2)	2.7 (−1.9 to 7.6)
	5-day-ave	2.6 (−1.0 to 6.1)	8.4 (−3.7 to −0.3)	−46.9 (−58.1 to −32.8)	0.5 (−5.9 to 7.4)	2.9 (−2.1 to 8.1)
	6-day-ave	−1.9 (−4.3 to 0.5)	−7.8 (−14.8 to 21.6)	−36.7 (−45.6 to −26.2)	−3.4 (−7.4 to 1.1)	−1.3 (−4.6 to 2.1)
MI	Lag 0	9.9 (5.8 to 14.1)	16.0 (3.8 to 27.2)	155.2 (100.1 to 225.4)	14.7 (7.1 to 22.9)	−2.8 (−8.2 to 2.7)
	Lag 1	3.2 (−1.3 to 7.7)	2.0 (−9.3 to 18.4)	−49.2 (−61.3 to −33.2)	0.8 (−6.3 to 8.6)	−1.3 (−6.5 to 4.2)
	Lag 2	12.3 (7.5 to 17.1)	8.0 (−5.5 to −2.4)	−10.8 (−35.6 to 23.5)	13.3 (4.4 to 23.0)	−5.7 (−12.4 to 1.2)
	Lag 3	−0.9 (−2.4 to 0.6)	−4.8 (−8.4 to 1.0)	−16.8 (−24.1 to −8.8)	−1.5 (−3.9 to 1.0)	−0.9 (−2.6 to 0.9)
	Lag 4	−4.3 (−8.5 to 0.0)	−1.5 (−12.3 to 57.1)	71.5 (32.3 to 122.3)	−2.2 (−8.4 to 5.0)	1.8 (−3.6 to 7.2)
	Lag 5	17.2 (11.1 to 23.3)	14.3 (0.9 to 248.1)	27.9 (−11.0 to 84.1)	12.3 (0.1 to 26.0)	15.7 (6.3 to 25.9)
	2-day-ave	11.5 (8.6 to 14.4)	17.4 (8.0 to 24.0)	80.1 (48.6 to 118.4)	15.3 (9.6 to 21.2)	−6.1 (−11.2 to −0.9)
	3-day-ave	11.7 (8.8 to 14.5)	14.1 (4.9 to 14.5)	42.5 (16.3 to 74.4)	14.6 (9.0 to 20.5)	−8.4 (−13.7 to −2.7)
	4-day-ave	9.6 (6.0 to 13.3)	5.3 (−4.8 to 10.4)	6.0 (−17.0 to 35.4)	11.0 (4.4 to 18.1)	−7.3 (−12.5 to −1.8)
	5-day-ave	1.8 (−2.0 to 5.7)	1.5 (−8.1 to 17.9)	87.9 (50.1 to 135.1)	4.9 (−1.5 to 11.6)	−1.3 (−5.5 to 3.3)
	6-day-ave	8.5 (5.3 to 11.6)	2.9 (−5.7 to 0.3)	140.3 (101.5 to 186.7)	9.2 (3.4 to 15.2)	4.4 (0.0 to 9.0)
ST	Lag 0	−8.0 (−12.7 to −3.4)	−6.3 (−17.7 to 16.0)	54.5 (14.8 to 107.8)	−6.3 (−13.3 to 0.9)	4.9 (−1.3 to 11.7)
	Lag 1	−2.5 (−5.8 to 0.8)	−4.0 (−11.8 to 80.0)	53.7 (25.7 to 87.9)	−1.2 (−6.3 to 4.3)	1.8 (−2.0 to 5.9)
	Lag 2	−2.8 (−7.2 to 1.6)	12.9 (0.8 to 4.7)	−46.5 (−58.9 to −30.2)	−1.5 (−8.3 to 6.0)	−4.3 (−9.0 to 0.8)
	Lag 3	5.6 (0.3 to 10.9)	8.5 (−6.0 to −68.1)	−46.5 (−61.7 to −25.5)	2.1 (−6.7 to 11.4)	2.4 (−3.8 to 9.1)
	Lag 4	−4.5 (−6.9 to −2.1)	−11.5 (−16.8 to −4.7)	−4.7 (−18.6 to 11.6)	−5.3 (−9.0 to −1.5)	0.1 (−2.8 to 3.1)
	Lag 5	−26.1 (−31.7 to −20.6)	−21.9 (−31.2 to 0.7)	−46.1 (−18.6 to 11.6)	−21.3 (−30.0 to −11.5)	−18.3 (−27.6 to −7.7)
	2-day-ave	−6.3 (−10.6 to −2.0)	−5.8 (−15.7 to 10.6)	59.3 (21.3 to 109.1)	−5.0 (−11.3 to 2.2)	3.8 (−1.7 to 9.9)
	3-day-ave	−6.8 (−11.1 to −2.5)	−4.8 (−15.0 to 16.3)	51.8 (15.8 to 99.2)	−5.3 (−11.7 to 1.9)	3.6 (−2.1 to 9.6)
	4-day-ave	−6.8 (−10.9 to −2.7)	−3.2 (−13.1 to 8.2)	34.6 (3.7 to 74.8)	−6.1 (−12.3 to 0.5)	6.8 (1.0 to 12.9)
	5-day-ave	−7.8 (−12.1 to −3.4)	−4.8 (−15.2 to 4.6)	36.8 (3.4 to 81.0)	−7.1 (−13.6 to −0.1)	7.5 (1.2 to 14.1)
	6-day-ave	−4.4 (−8.4 to −0.4)	−6.3 (−15.6 to 1.0)	63.9 (27.7 to 110.4)	−4.7 (−10.7 to 1.9)	7.4 (2.2 to 12.9)
TB	Lag 0	−2.4 (−5.7 to 0.9)	−0.1 (−8.6 to 11.1)	56.2 (28.0 to 90.1)	−1.2 (−6.9 to 4.2)	2.9 (−1.1 to 7.0)
	Lag 1	−2.1 (−5.0 to 0.7)	−4.3 (−11.1 to 24.4)	41.1 (18.7 to 67.7)	−1.5 (−5.7 to 3.5)	1.8 (−1.5 to 5.3)
	Lag 2	5.8 (1.9 to 9.7)	12.3 (1.4 to 4.2)	−34.3 (−48.8 to −15.7)	6.6 (−0.1 to 13.6)	−5.2 (−10.2 to −0.5)
	Lag 3	4.2 (−1.1 to 9.4)	1.8 (−12.0 to 13.0)	−51.9 (−65.4 to −34.4)	0.5 (−7.1 to 9.6)	1.1 (−5.3 to 7.6)
	Lag 4	−3.9 (−7.8 to 0.1)	−2.4 (−11.5 to 0.2)	69.6 (33.1 to 116.5)	−2.4 (−8.2 to 4.6)	2.2 (−2.4 to 7.3)
	Lag 5	2.7 (0.4 to 5.1)	−5.3 (−10.3 to 4.9)	0.8 (−10.9 to 14.0)	0.9 (−3.0 to 5.1)	1.4 (−1.5 to 4.6)
	2-day-ave	−2.9 (−6.6 to 0.9)	−1.3 (−11.2 to 19.5)	67.2 (33.2 to 110.2)	−1.3 (−7.6 to 4.7)	3.2 (−1.3 to 8.0)
	3-day-ave	−2.4 (−6.4 to 1.7)	0.4 (−9.3 to 42.2)	75.0 (37.0 to 123.8)	−0.5 (−7.1 to 6.3)	2.9 (−2.4 to 8.0)
	4-day-ave	−0.8 (−4.1 to 2.5)	2.0 (−6.4 to 5.6)	46.8 (20.0 to 79.5)	−0.5 (−5.7 to 5.0)	5.7 (1.6 to 10.0)
	5-day-ave	−2.3 (−5.9 to 1.3)	0.0 (−8.9 to 5.6)	59.6 (27.9 to 99.1)	−1.3 (−6.9 to 4.8)	4.3 (−0.2 to 9.1)
	6-day-ave	1.1 (−0.6 to 2.7)	−2.0 (−6.2 to 2.4)	40.0 (27.1 to 54.1)	0.6 (−2.2 to 3.3)	3.2 (1.3 to 5.2)

and Rajagopalan, 2010; Martinelli et al., 2013), few of those have been assessed the association between bacterial/fungal aerosols and cardiovascular system. In this study, we measured systemic inflammation including cytokines (e.g. IL-6, vWF, sTNF-RII and hsCRP) and white blood cell counts and one of PM constitutes (bacterial/fungal aerosols). Overall, new studies propose the idea that exposure to PM and PM constituents may effects on the cardiovascular system by three main intermediary biopathways (Boritz et al., 2008; Brook and Rajagopalan, 2010; Chuang et al., 2007; Martinelli et al., 2013; Nelin et al., 2012; Polichetti et al., 2009; Zuurbier et al., 2011), including: I) release of cytokines, activated white blood cells/platelets as pro-inflammatory mediators and endothelial-1, possibly histamine, or microparticles as vasoactive mediators from lung-based cells; II) Imbalance of autonomic nervous system (e.g., heart rate variability) resulting activation of lung autonomic nervous system induced by PM and constituents of PM interactions with lung receptors and III) direct translocation of PM and PM constituents, especially ultrafine particles, enter the circulatory system and/or be carried into circulating cells. Therefore, inflammatory biomarkers may produce by the first and second above mechanisms (Brook et al., 2004; Brook and Rajagopalan, 2010; Martinelli et al., 2013), because of the fact that bacterial and fungal aerosols detected in the present study are classified as a fine PM ($PM_{2.5}$). This study presented the first experimental data linking inflammatory markers to short-term bioaerosol exposure. Our results support evidence that some of the measured circulating biomarkers are affected by bacterial and fungal aerosols, as one of PM constitutes. For example, IL-6 and WBC were significantly associated with fungal aerosols, particularly Cl and total fungi in healthy young adults. Our findings observe that only Cl, among fungal aerosols was significantly associated with vWF in healthy young adults. hsCRP was not positive association with any of the bacterial and fungal aerosols in this healthy young people. In our study of young healthy subjects, we observed that sTNF-RII was associated with ST and TB, but not with any of the fungal aerosols. For bacterial aerosols, MI was positively associated with WBC, sTNF-RII and hsCRP biomarkers, but not with vWF in our panel study of elderly people. In addition, IL-6 was positively associated with each of the bacterial aerosols investigated, except for BA in elderly subjects. All biomarkers were positively associated with fungal aerosols at some lags in elderly panel. For instance, WBC was associated with As at lag 1, Cl at lag 0, 4 and 5, Pe at 0, 1 and 5 and TF at lag 3 and 5; hsCRP with As at lag 0, Cl at lag 5, Pe at 0 and 5 and TF at lag 5; IL-6 with As at lag 0 and 1, Cl at lag 0, Pe at lag 1 and 3; sTNF-RII with As at lag 0, Pe at 1 and 5 and total fungi at lag 5 and vWF with As at lag 1, Cl at lag 2 and 5, Pe at 2, 3, 4 and 5 and TF at lag 5. Healthy young subjects showed a stronger IL-6 and WBC response to increased levels of fungal aerosols than elderly people. In the panel of healthy people, there was an unexpected inverse association of WBC and IL-6 with Pe at lag 4. For sTNF-RII and hsCRP there were unexpected inverse associations with any of the

fungal aerosols, whereas only an association with Pe at lag 4 could be detected for these biomarkers at healthy young people. There was an unexpected inverse association of circulating biomarkers with As at lag 2 in the panel of elderly subjects. Cl was inversely associated with WBC, sTNF-RII and hsCRP at lag 2 and vWF at lag 3 in elderly panel. For elderly subjects, all of biomarkers (except for IL-6) showed unexpected inverse association with Pe at some lags. For elderly panel, WBC, sTNF-RII and hsCRP were inversely associated with ST of bacterial aerosols, whereas no inverse associations were found for these biomarkers with other bacterial aerosols. In the panel of elderly subjects, IL-6 was inversely associated with each of the bacterial aerosols investigated at some lags. On the other hand, found IL-6 to be strongly inversely associated with BA at lag 0 (−61.6%) and 3 (−29.2%); MI lag 1 (−49.2%); ST at lag 2 and 3 (−46.5%) and TB at lag 2 (−34.3%) and 3 (−51.9%). The levels of vWF were inversely associated with MI at lag 2-, 3-, 4-day average, ST at lag 5 and total bacteria at lag 2 in elderly people. For the panel of healthy young people, we found inverse associations of all biomarkers with the same bacterial aerosols. For example, MI was inversely associated with WBC and IL-6 at lag 3 and 4, sTNF-RII and hsCRP at lag 1 and vWF at lag 4. In addition, we also observed significant inverse association for WBC and IL-6 at lag 1 and 2, sTNF-RII at lag 1 and vWF at lag 0 with ST. Our results found that TB were inversely correlated with WBC at lag 2 and 4, IL-6 at lag 2 and sTNF-RII at lag 1. To date, no studies explore whether systemic inflammation including cytokines (e.g. IL-6, vWF, sTNF-RII and hsCRP) and white blood cell counts were associated with bacterial and fungal aerosols, so we could not compare our findings to those of other researches.

5. Conclusions

To our knowledge, this is the first panel study to show associations of circulating biomarkers in elderly subjects and healthy young adults to bacterial and fungal aerosols exposure. In summary, our results indicate the evidence that the biological composition of PM, bacterial and fungal aerosols, may cause systemic inflammatory. Such adverse health outcomes were confirmed by the results of correlation analysis between circulating biomarkers, especially IL-6 and WBC, and bacterial and fungal aerosols in the elderly subjects and in healthy young adults. Increased level of circulating biomarkers after exposure to high levels of bacterial and fungal aerosol concentrations may cause of cardiovascular diseases.

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