Association of short-term air pollution with systemic inflammatory biomarkers in routine blood test: a longitudinal study

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Abstract

The biological mechanisms behind health effects of air pollution have not been well known. Inflammation plays an important role in occurrence and development of a wide range of diseases. In this study, we assessed the effects of short-term exposure to ambient air pollution on systemic inflammatory biomarkers among 12,508 participants who underwent routine physical examination annually at the Hebei General Hospital in Shijiazhuang, China. For each participant, white blood cell count (WBC), lymphocytes, neutrophils and eosinophils were measured for two or three times during September 2016 to December 2018. Daily concentrations of nitrogen dioxide (NO$_2$), sulfur dioxide (SO$_2$), ozone (O$_3$) and particulate matter less than 2.5 \(\mu\text{m}\) in aerodynamic diameter (PM$_{2.5}$) were interpolated to each district, where the participants worked. The linear mixed-effects regression with a constrained distributed lag model was applied to examine the associations between air pollution and inflammatory biomarkers during lag 0–14 d. It was observed that WBC, neutrophils and eosinophils [percent change (Δ%)] and 95% confidence interval (95%CI)] significantly decreased by \(-0.07\) (\(-0.11, -0.04\)), \(-0.08\) (\(-0.12, -0.03\)) and \(-0.15\) (\(-0.25, -0.05\)) at lag 14 d, associated with per 10 \(\mu\text{g}\text{m}^{-3}\) increase in O$_3$. WBC, lymphocytes and eosinophils (Δ% and 95%CI) significantly elevated by \(0.08\) (\(0.04, 0.12\)), \(0.16\) (\(0.11, 0.21\)) and \(0.22\) (\(0.10, 0.35\)) at lag 0 d, associated with per 10 \(\mu\text{g}\text{m}^{-3}\) increase in PM$_{2.5}$. This study reveals short-term effects of air pollution on systemic inflammatory biomarkers in routine blood test, which is helpful for further study to explore the biological mechanisms.

1. Introduction

A growing body of studies have revealed that ambient air pollution was associated with a wide range of adverse health outcomes, such as cardiopulmonary and autoimmune diseases, thrombosis and cognitive impairment [1–5]. Air pollution is one of leading causes of global burden of disease [6, 7]. Despite the growing epidemiological evidence, the biological mechanisms by which air pollution causes specific diseases remain unclear.

Inflammation plays an important role in occurrence and development of a wide range of diseases [8, 9]. Investigating the inflammatory response can help to reveal the pathogenesis of diseases induced by air pollution [9–12]. According to a cross-sectional study in the U.S., exposures to higher levels of particulate matter $\leq 10$ \(\mu\text{m}\) in aerodynamic diameter
tries translation are different from those of high-income countries, where levels of air pollution may cause changed levels of inflammatory biomarkers (WBC, lymphocytes, neutrophils and eosinophils) among hospital visits for routine physical examination in Shijiazhuang, China during 2016–2018.

2. Methods

2.1. Data collection

The participants included in this study were urban residents or office workers, who visited the Hebei General Hospital for annual physical examination during September 2016 to December 2018. In total, 12,508 participants were recruited who provided complete blood samples and finished all items of physical examination, among which, 11,885 underwent physical examination twice and 623 for three times during the study period. We excluded the participants who only underwent physical examination once. The locations of Shijiazhuang and the hospital are shown in figure S1 (available online at stacks.iop.org/ERL/16/035007/mmedia) in the supplementary material. The study has been approved by the scientific review and ethics committee of the Hebei General Hospital.

2.2. Inflammatory biomarkers

Four inflammatory biomarkers in peripheral blood were measured for all participants at each hospital visit, including WBC, lymphocytes, neutrophils and eosinophils. Specifically, 2 ml overnight fasting blood samples were extracted from the antecubital vein and were put in EDTA tubes, and then they were stored in refrigerator (2 °C–8 °C) until sent to analytical laboratories for testing. Blood routine examination was performed using a flow cytometry in automated

| Table 1. Basic characteristics of all participants (N = 12,508) in this study. |
|---------------------------------|
| Factor                        | n (%)   |
| Age (years)                    |         |
| <40                           | 2510 (20.1) |
| 40–60                         | 6130 (49.0) |
| >60                           | 3867 (30.9) |
| Gender                        |         |
| Male                          | 6989 (55.9) |
| Female                        | 5519 (44.1) |
| BMI (kg m⁻²)                  |         |
| <24                           | 5714 (45.7) |
| 24–28                         | 4716 (37.7) |
| >28                           | 1627 (13.0) |
| Smoking status                |         |
| Non-smoker                    | 9379 (75.0) |
| Ex-smoker                     | 694 (5.5) |
| Current smoker                | 1991 (15.9) |
| Drinking status               |         |
| Non-drinker                   | 7540 (60.3) |
| Ex-drinker                    | 362 (2.9) |
| Current drinker               | 4163 (33.3) |
| In total                      | 12,508 (100) |

Abbreviation: BMI, body mass index.

(PM₁₀), nitrogen dioxide (NO₂) and sulfur dioxide (SO₂) pollution were associated with significant changes in levels of white blood cell count (WBC) and fibrinogen. The relative odds (95%CI) of WBC and fibrinogen were associated with an interquartile range (IQR) increase in PM₁₀ [1.77 (2.26, 2.49) and 1.64 (1.17, 2.30), respectively]. These changes were relevant to cardiovascular effects of air pollution [10]. A case crossover study in the U.K. illustrated that exposure to ozone (O₃) caused a 39% increase in sputum neutrophils in normal subjects, and neutrophils remained at high levels over a longer time period. This change further led to a neutrophil inflammatory response which induced reduced lung function [11]. Similar findings were reported by a study in northern France that levels of O₃, NO₂ and PM₁₀ were associated with pulmonary inflammation markers like eosinophils and high-sensitivity C-reactive protein (hs-CRP). An increment in NO₂ level was significantly associated with a higher level of hs-CRP [5.03 (0.36, 9.91)] on the day of the examination. These inflammatory reactions were one of the pathways leading to subclinical decrease in distal lung function [9]. In summary, exposure to particulate matter and gaseous pollutants may cause changed levels of inflammatory biomarkers, which further triggers inflammatory responses.

According to previous studies, alterations in inflammatory pathways have been proposed to be important mechanisms by which air pollution induces cardiovascular disease, reduced lung function and diabetes mellitus [13]. Meanwhile, the expression of pro-inflammatory cytokines by air pollution may lead to the anomalous change of inflammatory biomarkers, such as WBC, lymphocytes, neutrophils and eosinophils [9–11]. These four biomarkers are different types of peripheral white blood cells which can reflect the level of inflammation in human body at the early stage. Furthermore, abnormal changes in these four biomarkers in lung and other parts of the body are associated with increased risk of relevant diseases.

Although existing evidence have showed the associations of air pollution with inflammatory markers, however, the majority of the evidence is from cross-sectional and case–control studies [14–16]. Evidence from large-scale prospective studies is very limited. In addition, most of previous studies have been conducted in high-income countries, but it is uncertain whether the conclusions also apply to low- and middle-income countries, where levels of air pollution, types of pollutants and characteristics of population are different from those of high-income countries [17–19].

Shijiazhuang is almost the most polluted city in China located on the North China Plain, due to rapid industrial development and high population density [20]. This study aims to examine the short-term effects of air pollution on four inflammatory
complete blood count analyzer (Sysmex XN-B4), through which the total WBC ($10^9$ l$^{-1}$), lymphocytes ($10^9$ l$^{-1}$), neutrophils ($10^9$ l$^{-1}$) and eosinophils ($10^9$ l$^{-1}$) were determined.

### 2.3. Data on air pollutants and weather conditions

Daily mean levels of NO$_2$, SO$_2$, O$_3$ and particulate matter $\leq 2.5 \mu m$ in aerodynamic diameter (PM$_{2.5}$) were obtained from 15 monitoring stations of the China National Environmental Monitoring Center in Shijiazhuang and surrounding cities during the study period. The measurements of air pollutants have been reported in details previously [21, 22]. The level of pollutant was interpolated to each district where the participants worked using the inverse distance weighted (IDW) method [23, 24]. The interpolation of air pollutants was validated using a leave-one-out cross-validation method [25]. The results of cross-validation are shown in table S1. Meteorological data in Shijiazhuang and surrounding cities were obtained from six weather stations of the China Meteorological Data Sharing Service System. Daily mean temperature ($^\circ C$) and relative humidity (%) were estimated using the same IDW method as the air pollution data during the same exposure period.

### 2.4. Statistical analysis

To examine the associations between air pollution and inflammatory biomarkers, a linear mixed-effects regression model was used in this study. A range of potential confounders were controlled as fixed-effect terms in the model, including age (‘$\leq 60$ years’ and ‘$>60$ years’), gender (‘Male’ and ‘Female’), body mass index (BMI, ‘$\leq 24$ kg m$^{-2}$’ and ‘$>24$ kg m$^{-2}$’), smoking and drinking status (‘Current smoker/drinker’, ‘Ex-smoker/drinker’ and ‘Non-smoker/drinker’) [26–28]. Each participant (personal ID) was also included as the random-effect term in the model. In order to test both the current-day and lag-effect of air pollutants, concentrations of each pollutant on the current day and previous 14 d (lag 0–14 d) were fitted using the constrained distributed lag model (CDLM) (natural cubic spline given three degrees of freedom for lag time) [29], according to our previous works [30, 31]. Moreover, meteorological variables (including daily mean temperature and relative humidity) during lag 0–14 d were also controlled in the model using natural cubic splines given three degrees of freedom [32]. Both the single-day and cumulative estimated effects of air pollution were examined during lag 0–14 d [14, 18, 33].

Based on the overall estimated effects of air pollutants during lag 0–14 d, multi-pollutant models were developed to examine the joint effects of different air pollutants on biomarkers. In addition, a series of analyses were also performed stratified by age (‘$\leq 60$ years’ and ‘$>60$ years’), gender (‘Male’ and ‘Female’), BMI (‘$\leq 24$ kg m$^{-2}$’ and ‘$>24$ kg m$^{-2}$’). A two-sample test was performed to examine the statistically difference between different subgroups [30, 34]. As data on WBC, lymphocytes, neutrophils and eosinophils were not normally distributed, levels of these three biomarkers were log transformed in the model [19, 35, 36], and their results were showed as percent change ($\%\Delta$) and corresponding 95% confidence interval (95%CI), in relation to per 10 $\mu g$ m$^{-3}$ increment in each pollutant. Percent change ($\%\Delta$) was back-transformed using the formula [100 $\times$ (exp$^\beta$) – 1]) ($\beta$ was coefficient from the linear regression model) [36, 37]. As some previous studies considered the maximum lag time for short-term effects of air pollution as 10 d [38, 39], we have performed sensitivity analyses by shortening the lag period from lag 0–14 d to 0–10 d. As the current day exposure of each participant is the 24 h average level of pollutant, which may overlap with the time when the participant underwent examination, we performed sensitivity analyses by only considering the exposure during lag 1–14 d (excluding the current day exposure).

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### Table 2. A summary of levels inflammatory biomarkers for all participants.

| Biomarkers ($\times 10^9$ l$^{-1}$) | Mean (SD) | Median (P25–P75) | Range |
|-----------------------------------|-----------|------------------|-------|
| WBC                               | 6.33 (1.52) | 6.17 (5.27–7.20) | 2.49–18.79 |
| Lymphocytes                       | 2.14 (0.61) | 2.06 (1.71–2.48) | 0.43–6.27 |
| Neutrophils                       | 3.66 (1.14) | 3.51 (2.87–4.27) | 0.75–15.61 |
| Eosinophils                       | 0.15 (0.14) | 0.11 (0.07–0.18) | 0.01–2.58 |

These biomarkers have the same sample size ($n = 25,659$). Abbreviation: WBC, white blood cell count.

### Table 3. A summary of daily mean concentrations of air pollutants in Shijiazhuang City during 2016–2018.

| Pollutants ($\mu g$ m$^{-3}$) | Mean (SD) | Median (P25–P75) | Range |
|-------------------------------|-----------|------------------|-------|
| NO$_2$                        | 49.91 (23.23) | 45.86 (33.44–61.43) | 7.79–196.13 |
| SO$_2$                        | 28.44 (22.67) | 21.28 (13.19–36.08) | 1.77–159.09 |
| O$_3$                         | 61.06 (39.66) | 53.81 (27.95–88.46) | 2.16–200.83 |
| PM$_{2.5}$                     | 87.29 (75.19) | 61.27 (40.18–106.75) | 10.07–686.53 |

Abbreviations: NO$_2$, nitrogen dioxide; SO$_2$, sulfur dioxide; O$_3$, ozone; PM$_{2.5}$, particulate matter less than 2.5 $\mu m$ in aerodynamic diameter.
Figure 1. Changes in four inflammatory biomarkers associated with single-day effects of air pollutants (per 10 µg m$^{-3}$ increase) during lag 0–14 d. WBC, lymphocytes, neutrophils and eosinophils were expressed as percent change (%) and 95%CI. We used a linear mixed-effects regression model in this study. The lag time for daily mean temperature and relative humidity was 0–14 d. The random-effect term was ID. Models adjusted for age, gender, BMI, smoking status, drinking status, mean daily temperature and relative humidity.

3. Results

A summary of participants’ basic information is shown in table 1. The mean (SD) age of all participants was 53.08 (14.24) years, and the majority of them were men (55.9%). Most of them never smoke or drink (75.0% and 60.3%). The measurements of four inflammatory biomarkers are summarized in table 2. The mean (SD) of WBC, lymphocytes, neutrophils and eosinophils were $6.33 \times 10^9$ l$^{-1}$ (1.52), $2.14 \times 10^9$ l$^{-1}$ (0.61), $3.66 \times 10^9$ l$^{-1}$ (1.14) and $0.15 \times 10^9$ l$^{-1}$ (0.14), respectively. The mean levels of daily air pollution in Shijiazhuang City during the study period are summarized in table 3. The mean

All statistical analyses were performed using R software (version 3.6.2). The ‘dlmn’ and ‘nlme’ packages were used for the CDLM and linear-mixed model.
Table 4. Changes in four inflammatory biomarkers associated with cumulative effects of air pollutants (per 10 µg m⁻³ increase) during lag 0–14 d.

| Pollutants | WBC (%) | Lymphocytes (%) | Neutrophils (%) | Eosinophils (%) |
|------------|---------|-----------------|----------------|-----------------|
| NO₂        | 1.58 (1.32, 1.85) | 2.56 (2.24, 2.88) | 0.93 (0.58, 1.28) | 3.74 (2.92, 4.58) |
| SO₂        | 2.35 (1.97, 2.73) | 2.90 (2.44, 3.36) | 1.59 (1.07, 2.11) | 4.03 (2.86, 5.21) |
| O₃         | −0.63 (−0.79, −0.48) | −1.08 (−1.27, −0.89) | −0.34 (−0.55, −0.12) | −1.45 (−1.93, −0.98) |
| PM₂.₅      | 0.86 (0.73, 0.99) | 1.29 (1.14, 1.44) | 0.49 (0.32, 0.67) | 1.40 (1.02, 1.79) |

WBC, lymphocytes, neutrophils and eosinophils were expressed as percent change (%) and 95%CI. We used a linear mixed-effects regression model in this study. The lag time for daily mean temperature and relative humidity was 0–14 d. The random-effect term was ID. Models adjusted for age, gender, BMI, smoking status, drinking status, mean daily temperature and relative humidity.

levels of NO₂, SO₂, O₃ and PM₂.₅ were 49.91 µg m⁻³, 28.44 µg m⁻³, 61.06 µg m⁻³ and 87.29 µg m⁻³, respectively.

The results are shown in figure 1 for single-day estimated effects of air pollutants on inflammatory biomarkers during lag 0–14 d. It was observed that WBC was significantly associated with per 10 µg m⁻³ increase in PM₂.₅ during lag 0–13 d, with the maximum elevation on lag 0 d [∆% (95%CI): 0.08 (0.04, 0.12)] and the minimum elevation on lag 13 d [∆% (95%CI): 0.03 (0.01, 0.06)]. Significant higher level of lymphocytes was observed associated with increased SO₂ during lag 0–10 d, with the maximum elevation on lag 0 d [∆% (95%CI): 0.53 (0.35, 0.70)] and the minimum elevation on lag 10 d [∆% (95%CI): 0.07 (0.02, 0.12)]. Moreover, neutrophils were observed significantly associated with increased O₃ during lag 9–14 d, with the maximum decrease on lag 14 d [∆% (95%CI): −0.08 (−0.12, −0.03)] and the minimum decrease on lag 9 d [∆% (95%CI): −0.03 (−0.05, > −0.01)]. The remarkable change of eosinophils associated with NO₂ was observed during lag 0–10 d, with the maximum elevation on lag 0 d [%∆ (95%CI): 0.51 (0.20, 0.83)] and the minimum elevation on lag 10 d [%∆ (95%CI): 0.13 (0.02, 0.23)].

The results are shown in table 4 for overall estimated effects of air pollutants on inflammatory biomarkers during lag 0–14 d. WBC and lymphocytes were significantly associated with per 10 µg m⁻³ in SO₂ [%∆ (95%CI): 2.35 (1.97, 2.73) and 2.90 (2.44, 3.36)]. Neutrophils changed [%∆ (95%CI)] by −0.34 (−0.55, −0.12) and 0.49 (0.32, 0.67), associated with per 10 µg m⁻³ in O₃ and PM₂.₅ during lag 0–14 d. Eosinophils significantly changed associated with exposures to NO₂ and O₃ [%∆ (95%CI): 3.74 (2.92, 4.58) and −1.45 (−1.93, −0.98)].

With both PM₂.₅ and gaseous pollutants in the model, the estimated effects of air pollutants weakened, compared with the single-pollutant model (table 5). WBC changed [%∆ (95%CI)] by −0.48 (−0.64, −0.33) and 0.77 (0.64, 0.90), associated with per 10 µg m⁻³ increase in O₃ and PM₂.₅ in the ‘O₃ + PM₂.₅’ model. Lymphocytes and eosinophils increased [%∆ (95%CI)] by 0.27 (−0.35, 0.90) and 2.02 (0.42, 3.65), associated with per 10 µg m⁻³ increase in SO₂ in the ‘SO₂ + PM₂.₅’ model. Neutrophils changed [%∆ (95%CI)] by 0.16 (−0.36, 0.68), −0.21 (−0.46, 0.05) and 0.42 (0.20, 0.64), associated with per 10 µg m⁻³ increase in NO₂, O₃ and PM₂.₅ in the ‘NO₂ + O₃ + PM₂.₅’ model, respectively.

The results of stratified analyses for cumulative estimated effects of air pollutants are shown in figure 2. For the association between Lymphocytes and NO₂ or PM₂.₅, it was shown that older adults were more affected by pollutants. Compared with younger participants, more remarkable changes in lymphocytes were observed among older adults associated with per 10 µg m⁻³ increase in NO₂ [∆% (95%CI): 2.28 (1.93, 2.64) and 3.55 (2.83, 4.28), respectively], and PM₂.₅ [∆% (95%CI): 1.13 (0.97, 1.29) and 1.91 (1.54, 2.28), respectively]. In contrast, stronger estimated effects of PM₂.₅ on neutrophils were presented in the ‘≤60 years’ group than other age subgroups. No substantial interactions by gender and BMI were presented.

The results for overall estimated effects of air pollutants during lag 0–10 d were also similar to those during lag 0–14 d (table S2 in the supplementary material). Shortening maximum lag day from 14 d to 10 d did not substantially change the results. The results did not substantially change by only considering the exposure during lag 1–14 d (tables S3, S4 and figure S2). The Pearson correlation coefficients of air pollutants is shown in table S5. It showed O₃ was negatively associated with other pollutants.

4. Discussion

Our study has revealed the short-term effects of air pollution on inflammatory biomarkers. Higher levels of inflammatory biomarkers were significantly associated with increase in NO₂, SO₂ and PM₂.₅, while their lower levels were associated with increase in O₃.

Consistent with this study, previous studies also reported significant changes in inflammatory biomarkers induced by air pollution [18, 40–42]. A study conducted in Seoul, South Korea, reported that WBC in non-smokers was negatively associated with exposure to O₃ at lag 1–4 d, lag 6 d and lag 8 d [40]. A panel study in Germany stated that increased level of 24 h-lymphocytes was associated with nitrogen monoxide (NO) and carbon monoxide (CO) pollution, but such association was not observed for eosinophils [41]. A
Table 5. Changes in four inflammatory biomarkers associated with cumulative effects of air pollutants (per 10 µg m\(^{-3}\) increase) during lag 0–14 d in multi-pollutant models.

| Model          | Pollutants     | WBC (%)     | Lymphocytes (%) | Neutrophils (%) | Eosinophils (%) |
|----------------|----------------|-------------|-----------------|-----------------|-----------------|
| PM\(_{2.5}\) + NO\(_{2}\) | NO\(_{2}\)     | 0.66 (0.32, 0.99) | 1.29 (0.88, 1.69) | 0.39 (−0.06, 0.84) | 2.95 (1.89, 4.01) |
| PM\(_{2.5}\)   | PM\(_{2.5}\)   | 0.69 (0.53, 0.85) | 0.95 (0.76, 1.14) | 0.39 (0.17, 0.61) | 0.61 (0.12, 1.10) |
| PM\(_{2.5}\) + SO\(_{2}\) | SO\(_{2}\)     | 1.03 (0.50, 1.55) | 0.27 (−0.35, 0.90) | 1.10 (0.37, 1.82) | 2.02 (0.42, 3.65) |
| PM\(_{2.5}\)   | PM\(_{2.5}\)   | 0.65 (0.48, 0.82) | 1.28 (1.07, 1.48) | 0.25 (0.02, 0.48) | 1.03 (0.52, 1.55) |
| PM\(_{2.5}\) + O\(_{3}\) | O\(_{3}\)      | −0.48 (−0.64, −0.33) | −0.85 (−1.04, −0.66) | −0.26 (−0.48, −0.04) | −1.20 (−1.68, −0.71) |
| PM\(_{2.5}\)   | PM\(_{2.5}\)   | 0.77 (0.64, 0.90) | 1.14 (0.99, 1.29) | 0.44 (0.26, 0.62) | 1.20 (0.82, 1.59) |
| PM\(_{2.5}\) + NO\(_{2}\) + SO\(_{2}\) | NO\(_{2}\)     | 0.85 (0.49, 1.20) | 1.32 (0.90, 1.74) | 0.61 (0.14, 1.08) | 3.38 (2.27, 4.51) |
| PM\(_{2.5}\)   | PM\(_{2.5}\)   | 1.15 (0.60, 1.70) | 0.58 (−0.07, 1.23) | 1.15 (0.39, 1.91) | 2.88 (1.19, 4.60) |
| PM\(_{2.5}\) + NO\(_{2}\) + O\(_{3}\) | NO\(_{2}\)     | 0.38 (0.17, 0.60) | 0.83 (0.57, 1.09) | 0.07 (−0.22, 0.37) | −0.12 (−0.78, 0.54) |
| PM\(_{2.5}\)   | PM\(_{2.5}\)   | 0.22 (−0.16, 0.61) | 0.58 (0.12, 1.04) | 0.16 (−0.36, 0.68) | 2.29 (1.09, 3.50) |
| PM\(_{2.5}\) + SO\(_{2}\) + O\(_{3}\) | SO\(_{2}\)     | −0.40 (−0.59, −0.22) | −0.67 (−0.88, −0.45) | −0.21 (−0.46, 0.05) | −0.59 (−1.16, −0.03) |
| PM\(_{2.5}\)   | PM\(_{2.5}\)   | 0.74 (0.58, 0.90) | 1.04 (0.84, 1.23) | 0.42 (0.20, 0.64) | 0.69 (0.20, 1.19) |
|                  | PM\(_{2.5}\)   | 1.31 (0.76, 1.87) | 0.73 (0.08, 1.39) | 1.23 (0.46, 2.00) | 2.91 (1.21, 4.64) |

WBC, lymphocytes, neutrophils and eosinophils were expressed as percent change (%) and 95%CI. We used a linear mixed-effects regression model in this study. The lag time for daily mean temperature and relative humidity was 0–14 d. The random-effect term was ID. Models adjusted for age, gender, BMI, smoking status, drinking status, mean daily temperature and relative humidity. The bold values mean significant changes, and \(p\)-value < 0.05.
Figure 2. Changes in four inflammatory biomarkers associated with cumulative effects of air pollutants (per 10 µg m⁻³ increase) during lag 0–14 d modified by age, gender and BMI. WBC, lymphocytes, neutrophils and eosinophils were expressed as percent change (%) and 95%CI. We used a linear mixed-effects regression model in this study. The lag time for daily mean temperature and relative humidity was 0–14 d. The random-effect term was ID. Models adjusted for age, gender, BMI, smoking status, drinking status, mean daily temperature and relative humidity. * refers to \( p < 0.05 \), ** refers to \( p < 0.01 \), *** refers to \( p < 0.001 \).

A nested case–control study in Beijing among prediabetic and healthy individuals found that WBC and neutrophils were associated with PM\(_{2.5}\) at lag 1–3 d and lag 1–4 d, respectively, and the maximum elevations of WBC and neutrophils were both observed on lag 2 d [\%Δ (95%CI): 3.0 (1.5, 4.6) and 3.9 (1.6, 6.2) for per IQR increase] [18]. A longitudinal cohort study in Taiwan indicated that leukocytes and neutrophils elevated [\%Δ (95%CI)] by 3.51 (0.78, 6.23) and 3.45 (0.89, 6.01), associated with per IQR increase in PM\(_{2.5}\), and elevated [\%Δ (95%CI)] by 2.39 (0.13, 4.64) and 2.17 (0.10, 4.25), associated with O\(_3\) on the current day (lag 0 d) [42].

Changes in inflammatory biomarkers induced by air pollution may play an important part in the occurrence and development of various diseases, such as chronic obstructive pulmonary disease [43] and asthma [44]. However, their biological mechanisms are complex and have not been well understood. One potential pathway is that exposure to air pollutants triggers the release of pro-inflammatory mediators which further leads to the migration, infiltration, or extravasation of relevant inflammatory cells [45–53]. Evidence also indicated alveolar macrophages involved in changes of inflammatory markers that related to particulate matter exposure. An animal study revealed that phagocytosis of fine particles by rabbit alveolar macrophages could elevate white blood cells by releasing precursors from the bone marrow, and then white blood cells quickly entered into bloodstream [46]. Gaseous pollutants can also have an impact on inflammatory markers by facilitating the release of chemoattractant and stress hormones in vivo. After 5 h exposure to SO\(_2\), the rats showed increased neutrophil chemoattractant GRO/KC coinciding with early signs of acute airway inflammation with neutrophilic and macrophage infiltrates, followed by a shift from neutrophils to eosinophils [47]. The increase and extravasation of neutrophils in lungs were associated with the release of O\(_3\)-induced stress hormones [49]. Moreover, increased circulating epinephrine and corticosterone induced by O\(_3\) could trigger innate neutrophilic immune response [50]. Blocking the receptors of the stress hormones could minimize O\(_3\)-induced neutrophilic inflammatory, lymphopenia, and pulmonary inflammatory cytokine expression in rats [51].

Some results for single pollutant changed after adjusting for other pollutants, which suggested that these four pollutants were not associated with levels of biomarkers independently. According to the correlation table of pollutants (table S5), the correlation coefficients between O\(_3\) and NO\(_2\), SO\(_2\) and PM\(_{2.5}\) were −0.53, −0.36 and −0.33, respectively, which showed O\(_3\) was negatively associated with other air pollutants. This may be one of the reasons for the opposite association between O\(_3\) and inflammatory biomarkers compared with other pollutants [54]. Shijiazhuang is almost the most polluted city in China. Some studies indicated that the level of O\(_3\) was associated with different changes of biomarkers. Changes in biomarkers under high-level O\(_3\) exposure may be opposite to those under low-level O\(_3\) exposure. A multicenter crossover study found that with the increase in level of O\(_3\), nitrotyrosine increased when the level of O\(_3\) was low (0–70 ppb), but decreased when the level of O\(_3\) was high (70–120 ppb) [55]. Similar associations between O\(_3\) and

| Pollutant | Correlation Coefficient |
|-----------|-------------------------|
| O\(_3\)   | −0.53                   |
| NO\(_2\)  | −0.36                   |
| SO\(_2\)  | −0.33                   |
| PM\(_{2.5}\)|                     |
hs-CRP were also reported by a cross-sectional study in Germany [14]. In addition, it has been reported that the toll-like receptor 4 and the nuclear factor-kB pathway could be suppressed by O₃, followed by a reduction of inflammatory cytokines levels [36, 57].

Although our study also found the significant associations between air pollution and inflammatory biomarkers, inconsistency in effect estimate (e.g. percent change) and lag time remains comparing with previous studies. The inconsistency may be due to different study populations, designs and statistical approaches. For study population, most of the participants in our study were urban residents or office workers who visited hospital for routine health examination. They tended to have comparable occupation, living environment and working schedule, which helps to reduce the impact of some socioeconomic confounders on the results. Our study is a large-scale prospective study based on routine health examination. It takes into account the variabilities of measurements both between- and within-individual over time. Thus, the findings of our study can provide valuable information for the causal relationship of air pollution with health outcomes. For statistical approach, the CDLM we used facilitates users to model the complex exposure-responses association (linear or nonlinear) and its lag structure, and it provides the additional temporal dimension that helps to express the association [29].

In our stratified analyses, we found older adults were more vulnerable to air pollution, showing greater changes in inflammatory markers. Similar findings were also reported by several previous studies [58–60]. Inconsistent results were reported by previous studies regarding the interaction of gender and BMI in the association between air pollution and inflammatory biomarkers [61–63]. Therefore, further investigation is needed to identify the vulnerable population. Few parameters were statistically significant, indicating that no statistical differences in associations between air pollution and inflammatory biomarkers among different subgroups. Moreover, the smaller sample size in each stratum compared with main analyses resulted reduced statistical power.

Our study has several limitations. Due to the lack of individual’s home address, we interpolated level of air pollution to each district where the participants worked rather than individual’s home address [64]. This may bring some measurement errors for the assessment of exposure. Due to lack of data, we cannot consider participants’ hourly level of exposure, but it does not have substantial impacts on the mean level of exposure during lag period. Levels of air pollutants were calculated on an average of 0–24 h on any given day. The 24 h mean O₃ concentration was lower than the daily maximum 8 h O₃ concentration, but it had already reached the threshold value that induced abnormal changes of risk indicators of certain diseases, such as cardiovascular diseases [65]. In addition, some potential confounders were not controlled in our study such as household income and education, as the information was not collected at the hospital visit.

5. Conclusion
In this large-scale longitudinal study, short-term exposure to ambient air pollution showed significantly estimated effects on levels of WBC, lymphocytes, neutrophils and eosinophils. Considering the lag effects of air pollution and joint effects of different air pollutants on health, the government should take effective measures to curb air pollution and protect vulnerable population. Regarding the complex relationships between air pollution and various diseases or biomarkers, more researches should be conducted in future to explore the biological mechanisms for health effects of air pollution.

Data availability statement
All data that support the findings of this study are included within the article (and any supplementary files).

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Conflict of interest
The authors declare that they have no competing interests.

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References
[1] Vidale S and Campana C 2018 Ambient air pollution and cardiovascular diseases: from bench to bedside Eur. J. Prev. Cardiol. 25 818–25
[2] Chan Y L et al 2019 Pulmonary inflammation induced by low-dose particulate matter exposure in mice Am. J. Physiol. Lung Cell. Mol. Physiol. 317 L424–130
[3] Zhao C N et al 2019 Emerging role of air pollution in autoimmune diseases Autoimmun. Rev. 18 607–14
[4] Robertson S and Miller M R 2018 Ambient air pollution and thrombosis Part. Fibre Toxicol. 15 1
[5] Clifford A, Lang L, Chen R, Anstey K J and Seaton A 2016 Exposure to air pollution and cognitive functioning across
the life course—a systematic literature review. Environ. Res. 147 383–98

[6] Tong S 2019 Air pollution and disease burden. Lancet Planet. Health 3 e49–e50

[7] Forouzanfar M H et al 2016 Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet 388 1596–724

[8] Pope C A 3rd, Burnett R T, Thun M J, Calle E E, Krewski D and Godleski J J 2004 Cardiovascular mortality and long-term exposure to particulate air pollution: epidemiological evidence of general pathophysiological pathways of disease. Circulation 109 71–7

[9] Dauchet L, Hulo S, Cherot-Kornobis N, Matran R, Amouyel P, Edme J L and Giovannelli J 2018 Short-term exposure to air pollution: associations with lung function and inflammatory markers in non-smoking, healthy adults. Environ. Int. 121 610–9

[10] Schwartz J 2001 Air pollution and blood markers of cardiovascular risk. Environ. Health Perspect. 109 405–9

[11] Nightingale J A, Rogers D F and Barnes P J 1999 Effect of inhaled ozone on exhaled nitric oxide, pulmonary function, and induced sputum in normal and asthmatic subjects. Thorax 54 1061–9

[12] Ruckerl R et al 2014 Associations between ambient air pollution and blood markers of inflammation and coagulation/fibrinolysis in susceptible populations. Environ. Int. 70 32–49

[13] Feng S, Gao D, Liao F, Zhou F and Wang X 2016 The health effects of ambient PM2.5 and potential mechanisms. Ecotoxical. Environ. Saf. 128 67–74

[14] Zhao T Y, Markovlevich I, Standl M, Schikowski T, Berdel D, Koletzko S, Jörres R A, Nowak D and Heinrich J 2019 Short-term exposure to ambient ozone and inflammatory biomarkers in cross-sectional studies of children and adolescents: results of the GINIplus and LISA birth cohorts. Environ. Pollut. 255 113264

[15] Karotki D G et al 2014 Cardiovascular and lung function in relation to outdoor and indoor exposure to fine and ultrafine particulate matter in middle-aged subjects. Environ. Int. 73 372–81

[16] Panasevich S, Leander K, Rosenlund M, Ljungman P, Nightingale J A, Rogers D F and Barnes P J 1999 Effect of inhaled ozone on exhaled nitric oxide, pulmonary function, and induced sputum in normal and asthmatic subjects. Thorax 54 1061–9

[17] Rich D Q et al 2012 Association between changes in air pollution levels during the Beijing Olympics and biomarkers of inflammation and thrombosis in healthy young adults. Environ. Health 318 2068–78

[18] Han Y, Wang Y, Li W, Chen X, Xue T, Chen W, Fan Y, Qiu X and Zhu T 2019 Susceptibility of prediabetes to the health effect of air pollution: a community-based panel study with a nested case-control design. Environ. Health 18 65

[19] Li X et al 2019 Air pollution exposure and immunological and systemic inflammatory alterations among schoolchildren in China. Sci. Total Environ. 66 747–53

[20] Shi H, Crito A, Torresan S and Gao Q 2018 The temporal and spatial distribution characteristics of air pollution index and meteorological elements in Beijing, Tianjin, and Shijiazhuang, China. Integ. Environ. Assess. Manag. 14 710–21

[21] Cao J, Yang C, Li J, Chen R, Chen B, Gu D and Kan H 2011 Association between long-term exposure to outdoor air pollution and mortality in China: a cohort study. J. Hazard. Mater. 186 1594–600

[22] Zhou M, Liu Y, Wang L, Kuang X, Xu X and Kan H 2014 Particulate air pollution and mortality in a cohort of Chinese men. Environ. Pollut. 186 1–6

[23] Milillo T M and Gardella J A Jr 2008 Spatial analysis of time of flight-secondary ion mass spectrometric images by ordinary kriging and inverse distance weighted interpolation techniques. Anal. Chem. 80 4986–905

[24] Brauer M, Lencar C, Tamburic L, Kheoornm D, Demers P and Karr C 2008 A cohort study of traffic-related air pollution impacts on birth outcomes. Environ. Health Perspect. 116 680–6

[25] Shukla K, Kumar P, Mann G S and Khare M 2020 Mapping spatial distribution of particulate matter using Kriging and Inverse Distance Weighting at supersites of megacity Delhi. Sustain. Cities Soc. 54 101997

[26] Liao D, Heiss G, Chinchilli V M, Duan Y, Folsom A R, Lin H M and Salomaa V 2005 Association of criteria pollutants with plasma hemostatic/inflammatory markers: a population-based study. J. Expo. Anal. Environ. Epidemiol. 15 519–28

[27] Pekkanen J, Brunner E J, Anderson H R, Tödtmann P and Atkinson R W 2000 Daily concentrations of air pollution and plasma fibrinogen in London. Occup. Environ. Med. 57 818–22

[28] Bao S, Xia W, Xu S, Li Y, Lu B, Wu C, Liao J, Liu H, Sun X and Zhou A 2020 Multiple metal exposure and platelet counts during pregnancy: a repeated measure study. Int. Environ. Pollut. 260 113776

[29] Gasparini A 2014 Modeling exposure-lag-response associations with distributed lag non-linear models. Stat. Med. 33 881–99

[30] Chen L, Zhang Y, Zhang W, Chen G, Lu P, Guo Y and Li S 2019 Short-term effect of PM1 on hospital admission for ischemic stroke: a multi-city case-crossover study in China. Environ. Pollut. 260 113776

[31] Chen G et al 2017 Attributable risks of emergency hospital visits due to air pollutants in China: a multi-city study. Environ. Pollut. 228 43–59

[32] Guo Y, Li S, Tian Z, Pan X, Zhang J and Williams G 2013 The burden of air pollution on years of life lost in Beijing, China, 2004–08: retrospective regression analysis of daily deaths. BMJ 347 f7139

[33] Khafai M A, Salvi S S, Ojha A, Khafai B, Gore S S and Yakinik C S 2013 Systemic inflammation (C-reactive protein) in type 2 diabetic patients is associated with ambient air pollution in Pune City, India. Diabetes Care 36 625–30

[34] Di Q, Dui L, Wang X, Zamobetti A, Choifat C, Schwartz J D and Dominici F 2017 Association of short-term exposure to air pollution with mortality in older adults. JAMA 318 2456–56

[35] Li W et al 2017 Short-term exposure to ambient air pollution and biomarkers of systemic inflammation: the Framingham heart study. Arterioscler. Thromb. Vasc. Biol. 37 1793–800

[36] Li W et al 2019 Short-term exposure to ambient air pollution and circulating biomarkers of endothelial cell activation: the Framingham heart study. Environ. Res. 171 36–43

[37] Green R et al 2016 Long- and short-term exposure to air pollution and inflammatory/hemostatic markers in midlife women. Epidemiology 27 211–20

[38] Sullivan J H, Hubbard R, Liu S L, Shepherd K, Treng A C, Koenig J Q, Chandler W L and Kaufman J D 2007 A community-based study of the effect of particulate matter on blood measures of inflammation and thrombosis in an elderly population. Environ. Health 6 3

[39] Davdand P et al 2014 Air pollution and biomarkers of systemic inflammation and tissue repair in COPD patients. Eur. Respir. J. 44 603–13

[40] Lee H, Myung W, Jeong B H, Choi H, Ihun B W and Kim H 2018 Short- and long-term exposure to ambient air pollution and circulating biomarkers of inflammation in non-smokers: a hospital-based cohort study in South Korea. Environ. Int. 119 264–73

[41] Broske I, Mampel R, Socher M M, Ruckler R, Schneider A, Heinrich J, Oberdörster G, Wichmann H E and Peters A 2010 Impact of ambient air pollution on the differential white blood cell count in patients with chronic pulmonary disease. Inhal. Toxicol. 22 245–52
Chen B Y, Chan C C, Lee C T, Cheng T J, Huang W C, Jhou J C, Han Y-Y, Chen C-C and Guo Y L 2012 The association of ambient air pollution with airway inflammation in schoolchildren *Am. J. Epidemiol.* 175 764–74

Barnes P J 2016 Inflammatory mechanisms in patients with chronic obstructive pulmonary disease *J. Allergy Clin. Immunol.* 138 16–27

Holgate S T and Polosa R 2006 The mechanisms, diagnosis, and management of severe asthma in adults *Lancet* 368 780–93

Tan W C, Qiu D, Liam B L, Ng T P, Lee S H, van Eeden S F, Fujii T, Qui D, Vincent R and Hogg J 2000 The human bone marrow response to acute air pollution caused by forest fires *Am. J. Respir. Crit. Care Med.* 161 1213–7

Terasshima T, Wiggins B, English D, Hogg J C and van Eeden S F 1997 Phagocytosis of small carbon particles (PM(10)) by alveolar macrophages stimulates the release of polymorphonuclear leukocytes from bone marrow *Am. J. Respir. Crit. Care Med.* 155 1441–7

Wigenstam E, Elfmark L, Bucht A and Jonasson S 2016 Inhaled sulfur dioxide causes pulmonary and systemic inflammation leading to fibrotic respiratory disease in a rat model of chemical-induced lung injury *Toxicology* 368 28–36

van Eeden S F, Tan W C, Suwa T, Mukae H, Terasushima T, Fuji T, Qui D, Vincent R and Hogg J 2001 Cytokines involved in the systemic inflammatory response induced by exposure to particulate matter pollutants (PM(10)) by alveolar macrophages stimulates the release of polymorphonuclear leukocytes from bone marrow *Am. J. Respir. Crit. Care Med.* 164 826–30

Miller D B, Snow S J, Schladweiler M C, Richards J E, Ghiro A J, Ledbetter A D and Kodavanti U P 2016 Acute ozone-induced pulmonary and systemic metabolic effects are diminished in adrenallectomized rats *Toxicol. Sci.* 150 812–22

Dhabhar F S, Malarkey W B, Neri E and McEwen B S 2012 Stress-induced redistribution of immune cells–from barracks to boulevards to battlefields: a tale of three hormones–Curt Richter Award winner *Psychoneuroendocrinology* 37 1345–68

Henriquez A R, Snow S J, Schladweiler M C, Miller C N, Dye J A, Ledbetter A D, Richards J E, Mauge-Lewis K, McGee M A and Kodavanti U P 2018 Adrenergic and glucocorticoid receptor antagonists reduce ozone-induced lung injury and inflammation *Toxicol. Appl. Pharmacol.* 339 161–71

Wigenstam E, Elfmark L, Agren L, Akfur C, Bucht A and Jonasson S 2018 Anti-inflammatory and anti-fibrotic treatment in a rodent model of acute lung injury induced by sulfur dioxide *Clin. Toxicol.* 56 1185–94

Nobutomo K 1978 Air pollution and cytological changes in sputum *Lancet* 1 523–6

Sarrat J A, Schwartz J, Catalano P J and Sub H H 2001 Gaseous pollutants in particulate matter epidemiology: confounders or surrogates? *Environ. Health Perspect.* 109 1053–61

Balmes J R, Arjomandini M, Bromberg P A, Costantini M G, Dagincourt N, Hazucha M J, Hollenbeck-Pringle D, Rich D Q, Stark P and Frampton M W 2019 Ozone effects on blood biomarkers of systemic inflammation, oxidative stress, endothelial function, and thrombosis: the multicenter ozone study in older subjects (MOSES) *PLoS One* 14 e0222601

Yu G et al 2016 Ozone therapy could attenuate tubulointerstitial injury in adenine-induced CKD rats by mediating Nrf2 and NF-κB *Iran. J. Basic Med. Sci.* 19 1136–43 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5110663/)

Chen Z, Liu X, Yu G, Chen H, Wang L, Wang Z, Qiu T and Weng X 2016 Ozone therapy ameliorates tubulointerstitial inflammation by regulating TLR4 in adenine-induced CKD rats *Ren. Fail.* 38 822–30

Fischer P, Hoek G, Brunekreef B, Verhoeff A and Balmes J R, Arjomandi M, Bromberg P A, Costantini M G, Henriquez A R, Snow S J, Schladweiler M C, Miller C N, Yu G 2016 Inflammatory mechanisms in patients with chronic obstructive pulmonary disease *Int. J. Epidemiol.* 35 1347–54

Zeka A, Sullivan J R, Volkonas P S, Sparrow D and Schwartz J 2006 Inflammatory markers and particulate air pollution: characterizing the pathway to disease *Int. J. Epidemiol.* 35 1347–54

Dixon J B and O’Brien P E 2006 Obesity and the white blood cell count: changes with sustained weight loss *Obes. Surg.* 16 251–7

Zeka A, Sullivan J R, Volkanas P S, Sparrow D and Schwartz J 2006 Inflammatory markers and particulate air pollution: characterizing the pathway to disease *Int. J. Epidemiol.* 35 1347–54

Cabello N, Mishra V, Sinha U, DiAngelos S L, Chrones Z C, Ekpa N A, Cooper T K, Caruso C R and Silveya P 2015 Sex differences in the expression of lung inflammatory mediators in response to ozone *Am. J. Physiol. Lung Cell. Mol. Physiol.* 309 L1150–63

Lin H et al 2016 Mortality burden of ambient fine particulate air pollution in six Chinese cities: results from the Pearl River Delta study *Environ. Int.* 96 91–97

Chuang K J, Chan C C, Su T C, Lee C T and Tang C S 2007 The effect of urban air pollution on inflammation, oxidative stress, coagulation, and autonomic dysfunction in young adults *Am. J. Respir. Crit. Care Med.* 176 370–6