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Acute effects of air pollution on ischemic stroke onsets and deaths: A time-series study in Changzhou, China

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ABSTRACT

Objective To investigate the acute effect of air pollutants on ischemic stroke (IS) and IS related death.

Setting 5 urban districts in Changzhou, China between January 9, 2015 and December 31, 2016.

Participants 32,840 IS cases and 4,028 IS deaths were enrolled.

Main outcome measures Time- series design, generalized additive model and multivariable regression model were used to examine the percentage change (95% confidence interval [CI]) in daily IS counts and deaths with an interquartile range (IQR) increase in air pollutants levels for different single or multiple lag days in single-pollutant and two-pollutants models.

Results Daily IS counts increased 0.208% (95% [CI]: 0.036%-0.381%) with an IQR increment in levels of NO_2. The estimated risk of NO_2 was more robust in males and cold season. For daily IS counts, the estimated effects of NO_2 and SO_2 were more significant when adjusted for $PM_{2.5}$ and PM_{10}. An IQR increment in the concentration of PM_{10}, SO_2 and NO_2 significantly increased IS deaths with 6 days cumulative effects (0.268%, 95% CI: 0.007%-1.528%; 0.34%, 0.088%-0.592%; 0.263%, 0.004%-0.522%, respectively). Young individuals (< 65) had higher IS mortality risk for $PM_{2.5}$, PM_{10}, NO_2 and CO. For IS death, the effects estimates of SO_2 in the elderly, female and cold season were more pronounced, statistical significance was also identified for SO_2 when adjusted for CO.

Conclusions This study suggested that short term exposure to ambient NO_2 was associated with increased IS risk. And SO_2 were associated with increased IS onsets and deaths.

Article Summary

Strengths and limitations of this study

- This is the first comprehensive research in China that exploring the short-term effects of air pollutants on the morbidity and mortality of stroke at the same time.
- The statistical power to detect the association was sufficient because of the tremendous numbers of IS cases and IS deaths recorded in our work.
- This time-series study evaluated the association of modifiers between air pollution and daily IS counts or deaths, which may provide clues for the primary prevention of IS and IS related death.
- We could not precisely quantify the air pollutants exposure of individuals using the fixed-site monitoring data.
- This is a single city-based study and the results should be extrapolated with caution.

INTRODUCTION

Air pollution is considered as a primary risk factor for a mounting body of pollution-related diseases and deaths [1]. Moreover, as a modifiable risk factor, air pollution accounts for more than 7 million deaths annually [2]. Studies have extensively explored the association between air pollution and risk of respiratory, reproductive and cardiovascular diseases [3-5]. Epidemiological studies suggested that exposure to air pollutants may increase the risk of cardiovascular diseases in haze days [6-8]. For instance, a study conducted in Stockholm suggested that short-term exposure to $PM_{2.5}$ was associated with out of hospital cardiac arrest. Increments in particulate matters with aerodynamic diameters < 2.5 μm ($PM_{2.5}$), < 10 μm (PM_{10}), nitrogen dioxide (NO_2), sulfur dioxide (SO_2) and carbon monoxide (CO) were associated with higher risk of heart failure hospitalization and death. A meta- analysis indicated that long-terms exposure to $PM_{2.5}$ was related to a dramatic increase in cardiovascular mortality [9].

Stroke is a persistent neurological deficit caused by cerebrovascular damages. It can be classified into ischemic stroke (IS) and hemorrhagic stroke (HS) on the basis of pathophysiologic mechanisms [10-12]. Stroke is one of the most leading causes of death and disability-adjusted life years worldwide [13, 14]. In 2010, the estimated number of individuals suffered from stroke was 50 million and among them, approximately 17million cases were newly identified that year. China bears enormous stroke burden in the world with the highest stroke prevalence, incidence and mortality among other developing world [15]. As the major subtype of stroke in China, IS

accounts for 43% to 79% of all strokes and provokes growing concerns recently [16]. Given the tremendous disease burden of stroke, risk factors identification and prevention are critical and effective public health strategies [17]. However, in terms of stroke, the effect of air pollutants remains a matter of debate, which might be, at least in part, explained by the heterogeneity in different stroke subtypes [18, 19]. Thus, it is a pressing need to synthetically explore the association between air pollutants and specific subtype of stroke related incidence and mortality.

Changzhou stands on the southern bank of the Yangtze River. The city serves as part of the Suzhou-Wuxi-Changzhou metropolitan area and by the year of 2010, there were about 4 million residents in five urban districts (Tianning district, Wujin district, Jintan district, Xinbei district and Liyang city) [20]. Moreover, with the rapid industrialization and economic development in the past years, Changzhou is now experiencing severe air pollution. The concentrations of several air pollutants (such as $PM_{2.5}$, PM_{10}, SO_2 and NO_2) were equal or higher than the national ambient air quality in the recent years [21, 22]. Therefore, Changzhou was chosen as a suitable place to study the effect of air pollution on incidence and mortality of IS. In this study, a comprehensive time-series study was conducted in Changzhou to examine the association between main air pollutants and daily IS counts and deaths. To explore the effect modification, the association of modifiers between air pollution and daily IS counts and deaths was also evaluated. This work will help provide insights into the relationship between stroke and air pollutants and have implication for prevention of IS and IS related deaths.

MATERIALS AND METHODS

Data collection

Air pollution data, including daily 24-hour average levels of $PM_{2.5}$, PM_{10}, NO_2, SO_2 and CO between January 9, 2015 and December 31, 2016 were obtained from ten air quality monitoring stations run by Changzhou Environmental Monitoring Center. The daily mean levels for air pollutants were averaged from all the stations and approximately all the cases recorded in this study resided less than 40 km from the nearest monitoring station. Thus, the monitoring data could be used as an appropriate proxy for personal exposure. Daily meteorological data on temperature (℃) and relative humidity (%) were collected from Changzhou Meteorological Bureau.

Daily IS and IS mortality data from January 9, 2015 to December 31, 2016 were obtained from the database of Changzhou Center for Disease Control and Prevention (CDC). This database belonged to the government -controlled network reporting system for chronic diseases, which was established in Changzhou, 2012. Cardiovascular physicians were responsible for the diagnosis of IS according to the cerebrovascular ICD-10 codes for IS (I63). Demographic data were collected from Changzhou Municipal Bureau of Statistics. Daily IS counts and deaths were also stratified into groups by gender (male and female) and age group (<65 and ≥65 year-old) to explore the effect modification of individual characteristics.

Statistical analysis

Daily IS counts, daily IS death counts, air pollutants levels and weather data were linked by date for the subsequent time-series study, which was applied to analyze the

effects of each air pollutant on IS counts and death. After evaluating the distribution patterns of daily IS counts and deaths, Poisson distributions were identified. Poisson regression was used in generalized additive model (GAM) to calculate the data. Multivariable regression model was used to detect the air pollutants related associations and control the potential confounding factors, such as calendar time, day of week (DOW), daily average relative humidity and temperature. The five degree of freedom (df) was used to adjust for all the confounding factors mentioned above except for DOW (df= 7). Furthermore, to explore the potential modification, data were stratified by sex, age (<65 as young individuals and ≥65 as the elderly) and season (warm season as 1 May to 31 October and cold season as 1 November to 30 April). Associations between air pollutants and IS and IS related death were separately examined according to the following equation:

$$\text{Log}[E(T/D_n)] = \text{intercept} + \beta_1 \text{AirPollution}_{n-i} + \beta_2 \text{DOW} + ps(\text{calendar time, df} = 7) + ps(\text{Temp}_{n-i}, \text{df} = 5) + ps(\text{Relative Humidity}_{n-i}, \text{df} = 5)$$

$E(T/D_n)$ represents the estimated numbers of IS onsets or deaths on day n; $\text{AirPollution}_{n-i}$ represented the average levels of different air pollutants on day n and i is the day lag; β was vector of the coefficients; DOW was the day of week; ps () presented penalized spline function; Temp_{n-i} and $\text{Relative Humidity}_{n-i}$ represented average temperature and relative humidity on day n and i is the day lag.

In addition, for both daily IS counts and deaths, single-pollutant models were fitted with different single lag days (lag0, lag1, lag3 and lag5) to detect the effects of air pollution on IS. As underestimation may occur in single-day models, multiple-day lag models (lag0-1, lag0-3 and lag0-5) were also applied in this study. Smoothing

function was used in the analysis of the exposure-response relationship between the log-relative risk of daily IS counts or deaths and air pollutants concentrations using a 5 df in single-pollutant model.

Statistical software R (version 3.2.3, R Foundation for Statistical Computing, Austria) was used for data analysis and result output. The baseline data was presented as mean ± standard deviation (SD) for continuous variables. All tests were two-sided, and $P<0.05$ was considered as statistically significant.

RESULTS

Descriptive Analysis

During this study period, there were 32,840 IS cases and 4,028 IS deaths recorded, respectively. For IS, on average, 45.4 cases were identified each day (Table S1). Of these, 52.2% were males and 81.3% were the elderly (≥65). And for IS related death, there were 5.6 deaths recorded on average each day (Table S1). Of these, 48.2% were males and 96.4% were the elderly.

Daily average meteorological data were 17.0℃ for temperature and 75% for relative humidity. The daily average levels were 51.8 μg/m^3 for $PM_{2.5}$, 85.3 μg/m^3 for PM_{10}, 22.3 μg/m^3 for SO_2, 38.7 μg/m^3 for NO_2 and 1 mg/ m^3 for CO (Table 2). According to the WHO air quality guidelines (25 μg/m^3 for $PM_{2.5}$, 50 μg/m^3 for PM_{10} and 20 μg/m^3 for SO_2), the corresponding average levels of $PM_{2.5}$, PM_{10} and SO_2 in Changzhou were far beyond the WHO standard.

Estimated Effects of Air Pollutants

Fig. 1 summarizes the percentage change (95% CI) in daily IS counts and deaths with

an interquartile range (IQR) increase in air pollutants levels for different single or multiple lag days in single-pollutant models. For daily IS counts, statistically positive association was observed only in IQR increment of NO_2 levels and this association was the more significant at multi-day exposure than single day exposure. A highest association was observed for lag 0-5 (0.208%, 0.036%-0.381%) and was used in the subsequent study. For daily IS deaths, statistically positive associations were observed in different IQRs increments of PM_{10}, SO_2 and NO_2. All the associations were the most significant at lag5 (0.268%, 0.007%-0.528% for PM_{10}; 0.34%, 0.088%-0.592% for SO_2; 0.263%, 0.004%-0.522% for NO_2) than other days and was thus used in the subsequent analysis.

Concentration-response relationships of air pollutants with daily IS counts or deaths were conducted in Fig. 2. For IS counts, the curve of NO_2 was linear positive and flat at higher concentrations. For daily IS deaths, the concentration-response curves of PM_{10} and SO_2 were similar, flat at low levels and dramatically linear positive at high levels, whereas the curve for NO_2 suggest a linear rise in daily IS deaths.

The effect of NO_2 on the increase of daily IS counts was more significant among males than that of females and the effects of NO_2 and SO_2 on daily IS counts were significant in the cold season, but not in the warm season. No significant association was observed when modifying by age (Table 3). As for daily IS deaths, the estimated effect of PM_{10} was more pronounced among males, whereas the effects of NO_2 and SO_2 were more robust among females. Moreover, $PM_{2.5}$, PM_{10}, NO_2 and CO were

found to have greater deleterious effects among young individuals and SO_2 showed more risk in the elderly. And when modifying be seasons, statistical significance was identified only for SO_2 in the cold season (Table 3).

Table 4 shows the percent increases in daily IS counts and daily IS deaths with each IQR increment in air pollutants levels in two-pollutants models. For daily IS counts, the estimated effects of NO_2 and SO_2 were more significant when adjusted for $PM_{2.5}$ and PM_{10}. For daily IS deaths, statistical significance was identified for SO_2 when adjusted for CO.

DISCUSSION

In this population-based time-series study, a total of 32,840 IS cases and 4,028 IS death were identified in Changzhou from 2015 to 2016. Significant association of NO_2 with daily IS counts was observed even when adjusted by $PM_{2.5}$ and PM_{10}. The estimated IS risk of NO_2 was more robust in the elderly and the cold season. In addition, PM_{10}, SO_2 and NO_2 were positively associated with daily IS deaths. The young individuals had higher IS mortality risk for $PM_{2.5}$, PM_{10}, NO_2 and CO. And IS mortality risk of SO_2 was more robust in the elderly, female and cold season, statistical significance was also identified for SO_2 when adjusted for CO. To the best of our knowledge, in China, this is the first comprehensive study to explore the acute effect of air pollutants on the morbidity and mortality of stroke at the same time.

PM_{10} could cause endothelial dysfunction, inflammatory response and neuro-functional impairment and subsequently induce ischemia-like injuries in the brain [23]. In the current study, an IQR increment in PM_{10} concentrations was

associated with 0.268% increment in daily IS deaths, which was in consistent with the previous investigations [24, 25]. Moreover, the function curve for PM_{10}/ daily IS deaths was flat at <150 μg/m^3 and became dramatically sharp at high levels. Previous multicity time-series study indicated an acute, deleterious and significant effect of PM_{10} on IS mortality and the estimated rate ratios were 1.016 [26]. Long-term exposure to PM_{10} was also demonstrated to positively associate with IS relate death and the estimated relative risk was 1.37 [27]. Moreover, in this study, greater estimated IS mortality risk was observed among males and young individuals. It is plausible that males and young individuals spent more time outdoors and exposed to higher levels of PM_{10} than females and the elderly [28].

NO_2, a representative pollutant of vehicle exhaust, was found to induce excitotoxicity, endothelial and inflammatory responses and damage synaptic plasticity in the brain [29],[30, 31]. In China, a multicity case-crossover study demonstrated that an IQR increase (25.4 μg/m^3) in NO_2 was corresponded to 2.6% increment in IS admissions. Stroke mortality was also positively associated with ambient NO_2 levels [32]. In the present study, we indicated that IQR increments in NO_2 (12 μg/m^3) was associated with 0.208% and 0.263% increases in daily IS counts and IS deaths, respectively. An approximately linear concentration-response relationship was identified for NO_2 and daily IS counts or daily IS deaths, which was consistent with the previous report [33, 34]. The positive association for NO_2 and daily IS counts could be observed even below the National Ambient Air Quality Standards of China (80 μg/m^3). For daily IS counts, the effects of NO_2 were statistically positive and

stronger when adjusted for other pollutants except for SO_2 (no statistical but stronger association was observed), suggesting that the adverse effect of NO_2 on IS onsets was stable an NO_2 contributed most to the increased risk of IS. Moreover, the effects of NO_2 were more significant in cold season and males. For IS deaths, young females were found to be experienced higher risk. The underlying reasons may be that the exposed levels of NO_2 were much higher at these conditions, which was similar to that of PM_{10}. The risk factors for stroke, such as hypertension, atrial fibrillation and diabetes mellitus are more frequent and the estimated effects are stronger among females [35]. Females are considered to be more sensitive to stroke onsets and deaths. Thus, our findings regarding the higher risk of IS deaths observed among females were reasonable.

SO_2 was considered as a strong irritant ambient gaseous pollutant, and could elevate the expression of vasoregulatory pathways and proinflammatory enzymes such as ET-1, iNOS, COX-2, ICAM-1 and TNF-α [36]. And its exposure was demonstrated to contribute to the development and progression of IS [18]. In the present study, SO_2 was significantly associated with daily IS counts when adjusted by $PM_{2.5}$ or PM_{10}. In addition, exposure to SO_2 also had statistical associations with daily IS deaths (the percentage increase was 0.34%), which was in agreement with previous studies [19, 37].The function curve for SO_2/ daily IS deaths was flat at <35 $\mu g/m^3$ and became dramatically sharp at high levels, suggesting that acute and robust increments of SO_2 might result in exacerbating IS death. Furthermore, the associations were more pronounced among females, suggesting that females are more susceptible to IS related

deaths when exposed to gaseous pollutants (NO_2 and SO_2). In addition, SO_2 exhibited higher risk of IS deaths in the elderly [38]. And it can be deduced that the elderly are more likely to bear preexisting respiratory or cardiovascular burdens and the vulnerable condition could influence the effect of SO_2 on stroke [39]. We also found that exposure to SO_2 had more significant effects on both IS onsets and deaths in cold season than in warm season.

In conclusion, our results suggested that short term exposure to ambient NO_2 was associated with increased IS risk. And SO_2 were associated with increased IS counts and deaths. These findings may have significant public health implications for prevention of IS and IS related death. Further studies are recommended to validate our research.

Contributors The study was conceived and designed by Huibin Dong, Yan Lu and Zhan Zhang, and overall study management was by Xingjuan Yao, Shou-Lin Wang and Zhan Zhang. Zhiyong Chen, Guiying Li and Yao Yao contributed to data collection and processing. Shen Yao performed the statistical analysis, Huibin Dong, Yao Yao and Yan Lu helped manuscript preparation and interpret the results. All authors contributed to the revision of the manuscript and reviewed and approved the final version.

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Ethical approval The present study was approved by the Institutional Review Board of Changzhou Center for Disease Control and Prevention, and all procedures were in accordance with prevailing ethical principles.

Competing interests None.

Patient consent Not needed.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement No additional data are available.

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Figure legends

Figure 1 The percentage change in daily ischemic stroke (IS) counts and daily IS deaths with an interquartile range increase in air pollutants levels on different lag days in single-pollutant models in Changzhou, 2015-2016. The data was expressed as mean with 95% confidence interval.

Figure 2 The concentration-response relationships of daily ischemic stroke (IS) counts, daily IS deaths with air pollutants on different lag days (lag 0-5 for daily IS counts and lag 5 for daily IS deaths) in single-pollutant models in Changzhou, 2015-2016.

Table 1 The means, medians and interquartile ranges (IQRs) for daily air pollutants and weather conditions variables in Changzhou, 2015-2016

Variables	Mean± SD	Min	25th	Median	75th	Max	IQR
Weather Condition							
Temperarure (□)	17.1±8.9	-6.6	9.4	18.3	24.1	34.6	5.8
Humidity (%)	75.3±14.2	35.0	66.0	76.0	86.0	100.0	10.0
Air Pollutions							
$PM_{2.5}$ (μg/m^3)	51.8±30.6	6.8	29.4	44.4	64.4	181.1	20.0
PM_{10} (μg/m^3)	85.3±43.3	10.5	51.5	76.6	108.6	289.1	32.0
NO_2 (μg/m^3)	38.7±16	10.9	26.5	35.8	47.8	117.3	12.0
SO_2 (μg/m^3)	22.3±10	6.9	14.7	20.2	27.0	67.8	6.9
CO (mg/m^3)	1±0.3	0.4	0.8	1.0	1.2	2.6	0.2

$PM_{2.5}$, particulate matter <2.5 mm in diameter; PM_{10}, particulate matter <10 mm in diameter; NO_2, nitrogen dioxide; SO_2, sulfur dioxide; CO, carbon monoxide.

Table 2 The percentage change in daily IS counts and deaths with an interquartile range increase in air pollutants levels modifying by age, gender and seasons in Changzhou, 2015-2016

Variable	Total	Age		Sex		Seasons	
		<=65	>65	Female	Male	Warm	Cold
Daily IS counts*							
$PM_{2.5}$	-0.063 (-0.215-0.088)	-0.246 (-0.568-0.076)	-0.042 (-0.21-0.127)	-0.097 (-0.311-0.119)	-0.08 (-0.289-0.128)	-0.149 (-0.525-0.228)	-0.098 (-0.267-0.071)
PM_{10}	-0.053 (-0.22-0.114)	-0.219 (-0.572-0.136)	-0.004 (-0.191-0.184)	-0.086 (-0.324-0.153)	-0.047 (-0.279-0.185)	-0.057 (-0.41-0.297)	-0.088 (-0.282-0.107)
NO_2	0.208 (0.036-0.381)‡	0.258 (-0.075-0.592)	0.166 (-0.025-0.357)	0.168 (-0.055-0.391)	0.238 (0.002-0.475)‡	-0.136 (-0.481-0.21)	0.382 (0.169-0.596)‡
SO_2	0.125 (-0.034-0.284)	-0.044 (-0.364-0.277)	0.113 (-0.062-0.289)	0.15 (-0.068-0.369)	0.056 (-0.162-0.275)	-0.309 (-0.651-0.033)	0.298 (0.099-0.498)‡
CO	-0.078 (-0.22-0.064)	-0.213 (-0.511-0.086)	-0.061 (-0.222-0.1)	-0.151 (-0.355-0.053)	-0.007 (-0.206-0.192)	-0.175 (-0.432-0.083)	-0.053 (-0.237-0.131)
Daily IS deaths†							
$PM_{2.5}$	0.228 (-0.005-0.462)	1.144 (0.329-1.965)‡	0.152 (-0.091-0.395)	0.124 (-0.19-0.439)	0.29 (-0.043-0.625)	-0.199 (-0.77-0.375)	0.184 (-0.088-0.456)
PM_{10}	0.268 (0.007-0.528)‡	1.52 (0.656-2.391)‡	0.152 (-0.12-0.424)	0.093 (-0.261-0.448)	0.404 (0.03-0.78)‡	-0.012 (-0.53-0.509)	0.247 (-0.075-0.57)
NO_2	0.263 (0.004-0.522)‡	0.894 (0.058-1.736)‡	0.195 (-0.073-0.463)	0.37 (0.027-0.715)‡	0.132 (-0.247-0.512)	0.275 (-0.222-0.775)	0.198 (-0.13-0.528)
SO_2	0.34 (0.088-0.592)‡	0.67 (-0.175-1.523)	0.307 (0.045-0.57)‡	0.434 (0.096-0.772)‡	0.215 (-0.15-0.58)	0.017 (-0.493-0.531)	0.388 (0.081-0.696)‡
CO	0.175 (-0.042-0.392)	0.9 (0.166-1.639)‡	0.108 (-0.118-0.335)	0.189 (-0.11-0.49)	0.154 (-0.162-0.471)	0.02 (-0.401-0.443)	0.169 (-0.104-0.443)

*The multivariable regression model fits the pollutants for daily IS counts data at lag 0-5 and adjusts for calendar time (df=5), DOW (df=7), daily average humidity (df=5) and average temperature (df=5).

†The multivariable regression model fits the pollutants for daily IS deaths data at lag 5 and adjusts for calendar time (df=5), DOW (df=7), daily average humidity (df=5) and average temperature (df=5).

‡ Statistically positive association between air pollutants and daily IS counts or deaths

The data was expressed as mean with 95% confidence interval.

Table 3 The percent increases in daily IS counts and deaths with each interquartile range increment in air pollutants levels in two-pollutants models in Changzhou, 2015-2016

Variables	$PM_{2.5}$	PM_{10}	NO_2	SO_2	CO
Daily IS counts					
$PM_{2.5}$	-0.063 (-0.215-0.088)	0.25 (-0.314-0.817)	0.497 (0.259-0.735)‡	0.305 (0.083-0.527) ‡	-0.059 (-0.274-0.157)
PM_{10}	-0.423 (-0.881-0.038)	-0.053 (-0.22-0.114)	0.539 (0.29-0.788)‡	0.262 (0.034-0.491) ‡	-0.065 (-0.293-0.163)
NO_2	-0.346 (-0.558--0.134)	-0.438 (-0.691--0.185)	0.208 (0.036-0.381)‡	-0.007 (-0.267-0.253)	-0.341 (-0.556--0.124)
SO_2	-0.24 (-0.46--0.019)	-0.244 (-0.49-0.003)	0.22 (-0.067-0.507)	0.125 (-0.034-0.284)	-0.166 (-0.356-0.023)
CO	-0.089 (-0.314-0.137)	0.029 (-0.223-0.281)	0.45 (0.215-0.685)‡	0.172 (-0.016-0.361)	-0.078 (-0.22-0.064)
Daily IS deaths					
$PM_{2.5}$	0.228 (-0.005-0.462)	0.436 (-0.36-1.238)	0.22 (-0.127-0.567)	0.28 (-0.071-0.632)	0.065 (-0.314-0.446)
PM_{10}	0.211 (-0.463-0.89)	0.268 (0.007-0.528)‡	0.218 (-0.135-0.573)	0.295 (-0.055-0.646)	0.137 (-0.23-0.504)
NO_2	0.106 (-0.201-0.414)	0.149 (-0.21-0.509)	0.263 (0.004-0.522)‡	0.351 (-0.096-0.8)	0.057 (-0.269-0.384)
SO_2	0.066 (-0.25-0.383)	0.126 (-0.242-0.496)	0.066 (-0.387-0.521)	0.34 (0.088-0.592)‡	0.035 (-0.281-0.351)
CO	0.186 (-0.199-0.571)	0.233 (-0.187-0.656)	0.23 (-0.14-0.601)	0.346 (0.002-0.691)‡	0.175 (-0.042-0.392)

*The multivariable regression model fits the pollutants for daily IS counts data at lag 0-5 and adjusts for the other pollutants, calendar time (df=5), DOW (df=7), daily average humidity (df=5) and average temperature (df=5).

†The multivariable regression model fits the pollutants for daily IS deaths data at lag 5 and adjusts for the other pollutants, calendar time (df=5), DOW (df=7), daily average humidity (df=5) and average temperature (df=5).

‡ Statistically positive association between air pollutants and daily IS counts or deaths

The data was expressed as mean with 95% confidence interval.

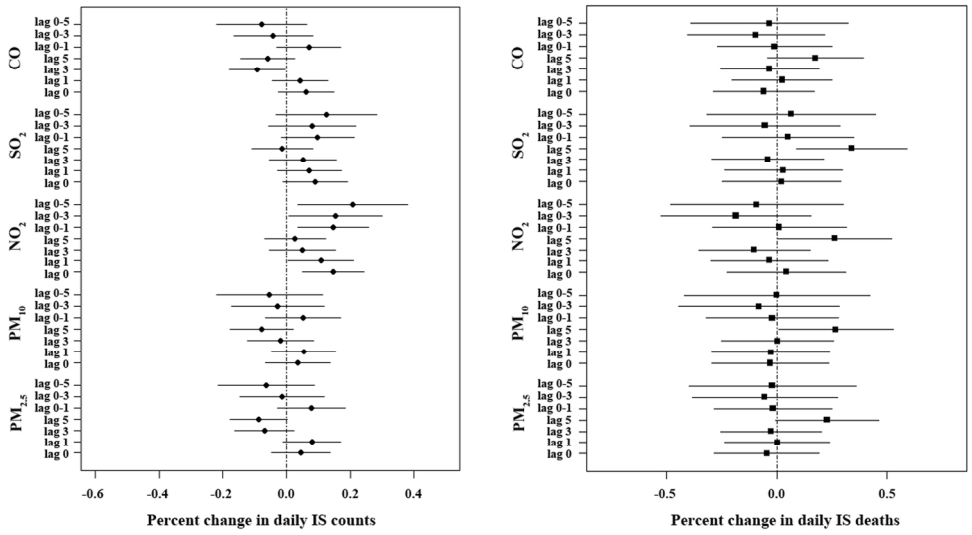

Figure 1 The percentage change in daily ischemic stroke (IS) counts and daily IS deaths with an interquartile range increase in air pollutants levels on different lag days in single-pollutant models in Changzhou, 2015-2016. The data was expressed as mean with 95% confidence interval.

86x48mm (600 x 600 DPI)

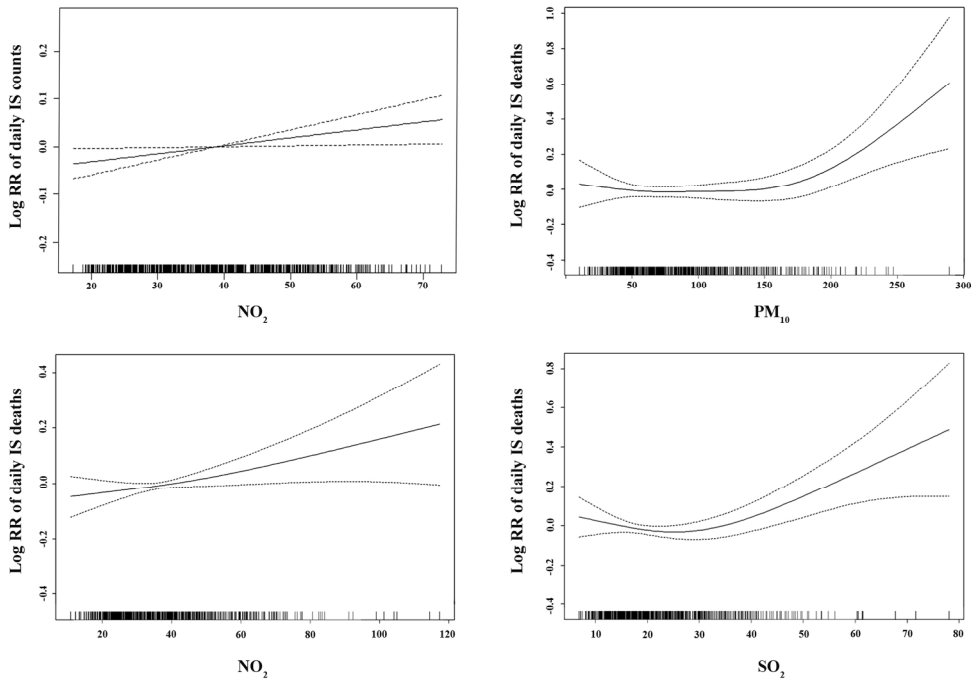

Figure 2 The concentration-response relationships of daily ischemic stroke (IS) counts, daily IS deaths with air pollutants on different lag days (lag 0-5 for daily IS counts and lag 5 for daily IS deaths) in single-pollutant models in Changzhou, 2015-2016.

107x75mm (600 x 600 DPI)

Acute effects of air pollution on ischemic stroke onsets and deaths: A time-series study in Changzhou, China

Huibin Dong[1, a], Yongquan Yu[2, a], Shen Yao[2], Yan Lu[3], Zhiyong Chen[1], Guiying Li[1], Yao Yao[1], Xingjuan Yao[1], Shou-Lin Wang[2], Zhan Zhang[4*]

Supplementary data

Table S1 The descriptive statistics for daily ischemic stroke (IS) counts and deaths in Changzhou, 2015-2016*

Variables	Mean± SD	Min	25th	Median	75th	Max
Daily IS counts	45.4±11.2	14.0	38.0	45.0	53.0	99.0
Male	23.7±7	6.0	19.0	23.0	28.0	60.0
Female	21.8±6.1	6.0	18.0	22.0	26.0	48.0
<65	8.5±3.6	0.0	6.0	8.0	11.0	23.0
≥65	36.9±9.2	11.0	31.0	36.0	43.0	80.0
Daily IS deaths	5.6±2.9	0.0	3.0	5.0	7.0	19.0
Male	2.7±1.8	0.0	1.0	3.0	4.0	10.0
Female	2.8±2	0.0	1.0	3.0	4.0	10.0
<65	0.4±0.7	0.0	0.0	0.0	1.0	3.0
≥65	5.1±2.8	0.0	3.0	5.0	7.0	18.0

*A total of 32,840 IS cases and 4,028 IS deaths recorded were recorded during the study period.

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation
Title and abstract	1-4	Acute effects of air pollution on ischemic stroke onsets and deaths: A time-series study in Changzhou, China
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	5-6	Explain the scientific background and rationale for the investigation being reported
Objectives	6	State specific objectives, including any prespecified hypotheses
Methods		
Study design	7-9	Present key elements of study design early in the paper
Setting	7	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	7	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants
Variables	8	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	7*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	7-8	Describe any efforts to address potential sources of bias
Study size	7	Explain how the study size was arrived at
Quantitative variables	8	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	9	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses
Results		
Participants	9*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	9*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
Outcome data	10-11*	Report numbers of outcome events or summary measures
Main results	10-11	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period

Other analyses	10-11	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	11	Summarise key results with reference to study objectives
Limitations	12-13	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	12-13	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	14	Discuss the generalisability (external validity) of the study results
Other information		
Funding	14	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Acute effects of air pollution on ischemic stroke onsets and deaths: A time-series study in Changzhou, China

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Acute effects of air pollution on ischemic stroke onsets and deaths: A time-series study in Changzhou, China

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ABSTRACT

Objective To investigate the acute effect of air pollutants on ischemic stroke (IS) and IS related death.

Setting 5 urban districts in Changzhou, China between January 9, 2015 and December 31, 2016.

Participants 32,840 IS cases and 4,028 IS deaths were enrolled.

Main outcome measures Time- series design, generalized additive model and multivariable regression model were used to examine the percentage change (95% confidence interval [CI]) in daily IS counts and deaths with an interquartile range (IQR) increase in air pollutants levels for different single or multiple lag days in single-pollutant and two-pollutants models.

Results Daily IS counts increased 0.208% (95% [CI]: 0.036%-0.381%) with an IQR increment in levels of NO_2. The estimated risk of NO_2 was more robust in males and cold season. For daily IS counts, the estimated effects of NO_2 and SO_2 were more significant when adjusted for $PM_{2.5}$ and PM_{10}. An IQR increment in the concentration of PM_{10}, SO_2 and NO_2 significantly increased IS deaths with 6 days cumulative effects (0.268%, 95% CI: 0.007%-1.528%; 0.34%, 0.088%-0.592%; 0.263%, 0.004%-0.522%, respectively). Young individuals (< 65) had higher IS mortality risk for $PM_{2.5}$, PM_{10}, NO_2 and CO. For IS death, the effects estimates of SO_2 in the elderly, female and cold season were more pronounced, statistical significance was also identified for SO_2 when adjusted for CO.

Conclusions This study suggested that short term exposure to ambient NO_2 was associated with increased IS risk. And SO_2 were associated with increased IS onsets and deaths.

Article Summary

Strengths and limitations of this study

- This is the first comprehensive research in China that exploring the short-term effects of air pollutants on the morbidity and mortality of stroke at the same time.
- The statistical power to detect the association was sufficient because of the tremendous numbers of IS cases and IS deaths recorded in our work.
- This time-series study evaluated the association of modifiers between air pollution and daily IS counts or deaths, which may provide clues for the primary prevention of IS and IS related death.
- The exposures measurement of this study was at the community level, and we could not precisely quantify the air pollutants exposure of individuals using the fixed-site monitoring data, this may lead to exposure measurement error.
- This is a single city-based study and the results should be extrapolated with caution.

INTRODUCTION

Air pollution is considered as a primary risk factor for a mounting body of pollution-related diseases and deaths [1]. Moreover, as a modifiable risk factor, air pollution accounts for more than 7 million deaths annually [2]. Studies have extensively explored the association between air pollution and risk of respiratory, reproductive and cardiovascular diseases [3-5]. Epidemiological studies suggested that exposure to air pollutants may increase the risk of cardiovascular diseases in haze days [6-8]. For instance, a study conducted in Stockholm suggested that short-term exposure to $PM_{2.5}$ was associated with out of hospital cardiac arrest. Increments in particulate matters with aerodynamic diameters < 2.5 μm ($PM_{2.5}$), < 10 μm (PM_{10}), nitrogen dioxide (NO_2), sulfur dioxide (SO_2) and carbon monoxide (CO) were associated with higher risk of heart failure hospitalization and death. A meta- analysis indicated that long-terms exposure to $PM_{2.5}$ was related to a dramatic increase in cardiovascular mortality [9].

Stroke is a persistent neurological deficit caused by cerebrovascular damages and includes ischemic stroke (IS) and hemorrhagic stroke (HS) [10-12]. It is one of the most leading causes of death and disability-adjusted life years worldwide [13, 14]. In 2010, the estimated number of individuals suffered from stroke was 50 million and among them, approximately 17million cases were newly identified. China bears enormous stroke burden in the world with the highest stroke prevalence, incidence and mortality among other developing world [15]. As the major subtype of stroke in China, IS accounts for 43% to 79% of all strokes and provokes growing concerns

recently [16]. Given the tremendous disease burden of stroke, risk factors identification and prevention are critical and effective public health strategies [17]. However, the effect of air pollutants remains a matter of debate, which might be, at least in part, explained by the heterogeneity in different stroke subtypes [18, 19]. Thus, it is a pressing need to synthetically explore the association between air pollutants and specific subtype of stroke related incidence and mortality.

Changzhou stands on the southern bank of the Yangtze River. The city serves as part of the Suzhou-Wuxi-Changzhou metropolitan area and by the year of 2010, there were about 4 million residents in five urban districts (Tianning district, Wujin district, Jintan district, Xinbei district and Liyang city) [20]. Moreover, with the rapid industrialization and economic development in the past years, Changzhou is now experiencing severe air pollution. The concentrations of several air pollutants (such as $PM_{2.5}$, PM_{10}, SO_2 and NO_2) were equal or higher than the national ambient air quality in the recent years [21, 22]. Therefore, Changzhou was chosen as a suitable place to study the effect of air pollution on incidence and mortality of IS. In this study, a comprehensive time-series study was conducted in Changzhou to examine the association between main air pollutants and daily IS counts and deaths. To explore the effect modification, the association of modifiers between air pollution and daily IS counts and deaths was also evaluated. This work will help provide insights into the relationship between stroke and air pollutants and have implication for prevention of IS onset and IS related deaths.

MATERIALS AND METHODS

Data collection

Air pollution data, including daily 24-hour average levels of $PM_{2.5}$, PM_{10}, NO_2, SO_2 and CO between January 9, 2015 and December 31, 2016 were obtained from ten air quality monitoring stations run by Changzhou Environmental Monitoring Center. The daily mean levels for air pollutants were averaged from all the stations and approximately all the cases recorded in this study resided less than 40 km from the nearest monitoring station. Thus, the monitoring data could be used as an appropriate proxy for personal exposure [23-25]. Daily meteorological data on temperature (℃) and relative humidity (%) were collected from Changzhou Meteorological Bureau.

Daily IS and IS mortality data from January 9, 2015 to December 31, 2016 were obtained from the database of Changzhou Center for Disease Control and Prevention (CDC). This database belonged to the government- controlled network reporting system for chronic diseases, which was established in Changzhou, 2012. Cardiovascular physicians were responsible for the diagnosis of IS according to the cerebrovascular ICD-10 codes for IS (I63). Demographic data were collected from Changzhou Municipal Bureau of Statistics. Daily IS counts and deaths were also stratified into groups by gender (male and female) and age group (<65 and ≥65 year-old) to explore the effect modification of individual characteristics.

Statistical analysis

Daily IS counts, daily IS death counts, air pollutants levels and weather data were linked by date for the subsequent time-series study, which was applied to analyze the

effects of each air pollutant on IS counts and death. After evaluating the distribution patterns of daily IS counts and deaths, Poisson distributions were identified. Poisson regression was used in generalized additive model (GAM) to calculate the data. Multivariable regression model was used to detect the air pollutants related associations and control the potential confounding factors, such as calendar time, day of week (DOW), daily average relative humidity and temperature. The five degree of freedom (df) was used to adjust for all the confounding factors mentioned above except for DOW (df= 7). Furthermore, to explore the potential modification, data were stratified by sex, age (<65 as young individuals and ≥65 as the elderly) and season (warm season as 1 May to 31 October and cold season as 1 November to 30 April). Associations between air pollutants and IS and IS related death were separately examined according to the following equation:

$$\text{Log}[E(T/D_n)] = \text{intercept} + \beta_1 \text{AirPollution}_{n-i} + \beta_2 \text{DOW} + ps(\text{calendar time, df} = 7) + ps(\text{Temp}_{n-i}, \text{df} = 5) + ps(\text{Relative Humidity}_{n-i}, \text{df} = 5)$$

$E(T/D_n)$ represents the estimated numbers of IS onsets or deaths on day n; $\text{AirPollution}_{n-i}$ represented the average levels of different air pollutants on day n and i is the day lag; β was vector of the coefficients; DOW was the day of week; ps () presented penalized spline function; Temp_{n-i} and $\text{Relative Humidity}_{n-i}$ represented average temperature and relative humidity on day n and i is the day lag.

In addition, for both daily IS counts and deaths, single-pollutant models were fitted with different single lag days (lag0, lag1, lag3 and lag5) to detect the effects of air pollution on IS. As underestimation may occur in single-day models, multiple-day lag models (lag0-1, lag0-3 and lag0-5) were also applied in this study. Smoothing

function was used in the analysis of the exposure-response relationship between the log-relative risk of daily IS counts or deaths and air pollutants concentrations using a 5 df in single-pollutant model.

Statistical software R (version 3.2.3, R Foundation for Statistical Computing, Austria) was used for data analysis and result output. The baseline data was presented as mean ± standard deviation (SD) for continuous variables. All tests were two-sided, and $P<0.05$ was considered as statistically significant.

Public and patient involvement

Public and patient involvement (PPI) was central and integral in the present study. The PPI group consisted of three male and four female representative patients who were recruited and chaired by HB. D. The members of the existing group were involved throughout all stages of this research to make contributions. At the outset, they helped researchers with topics choosing and study designing by providing constructive suggestions. Furthermore, they took part in the conference presentations and group discussions to help filter and interpret data collected from database mentioned above. They also contributed to discussion about the implications of the results by using their experiences. These findings have been disseminated to all the PPI members at the end of the study.

RESULTS

Descriptive Analysis

During this study period, there were 32,840 IS cases and 4,028 IS deaths recorded,

respectively. For IS, on average, 45.4 cases were identified each day (Table S1). Of these, 52.2% were males and 81.3% were the elderly (⩾65). And for IS related death, there were 5.6 deaths recorded on average each day (Table S1). Of these, 48.2% were males and 96.4% were the elderly.

Daily average meteorological data were 17.0℃ for temperature and 75% for relative humidity. The daily average levels were 51.8 μg/m^3 for $PM_{2.5}$, 85.3 μg/m^3 for PM_{10}, 22.3 μg/m^3 for SO_2, 38.7 μg/m^3 for NO_2 and 1 mg/ m^3 for CO (Table 1). According to the WHO air quality guidelines (25 μg/m^3 for $PM_{2.5}$, 50 μg/m^3 for PM_{10} and 20 μg/m^3 for SO_2), the corresponding average levels of $PM_{2.5}$, PM_{10} and SO_2 in Changzhou were far beyond the WHO standard.

Estimated Effects of Air Pollutants

Fig. 1 summarizes the percentage change (95% CI) in daily IS counts and deaths with an interquartile range (IQR) increase in air pollutants levels for different single or multiple lag days in single-pollutant models. For daily IS counts, statistically positive association was observed only in IQR increment of NO_2 levels and this association was the more significant at multi-day exposure than single day exposure. A highest association was observed for lag 0-5 (0.208%, 0.036%-0.381%) and was used in the subsequent study. For daily IS deaths, statistically positive associations were observed in different IQRs increments of PM_{10}, SO_2 and NO_2. All the associations were the most significant at lag5 (0.268%, 0.007%-0.528% for PM_{10}; 0.34%, 0.088%-0.592% for SO_2; 0.263%, 0.004%-0.522% for NO_2) than other days and was thus used in the subsequent analysis.

Concentration-response relationships of air pollutants with daily IS counts or deaths were conducted in Fig. 2. For IS counts, the curve of NO_2 was linear positive and flat at higher concentrations. For daily IS deaths, the concentration-response curves of PM_{10} and SO_2 were similar, flat at low levels and dramatically linear positive at high levels, whereas the curve for NO_2 suggest a linear rise in daily IS deaths.

The effect of NO_2 on the increase of daily IS counts was more pronounced among males than that of females and the effects of NO_2 and SO_2 on daily IS counts were more serious in the cold season, but not in the warm season. No significant association was observed when modifying by age (Table 2). As for daily IS deaths, the estimated effect of PM_{10} was more pronounced among males, whereas the effects of NO_2 and SO_2 were more robust among females. Moreover, $PM_{2.5}$, PM_{10}, NO_2 and CO were found to have greater deleterious effects among young individuals and SO_2 showed more risk in the elderly. And when modifying be seasons, statistical significance was identified only for SO_2 in the cold season (Table 2).

Table 3 shows the percent increases in daily IS counts and daily IS deaths with each IQR increment in air pollutants levels in two-pollutants models. For daily IS counts, the estimated effects of NO_2 and SO_2 were more serious when adjusted for $PM_{2.5}$ and PM_{10}. For daily IS deaths, statistical significance was identified for SO_2 when adjusted for CO.

DISCUSSION

In this population-based time-series study, a total of 32,840 IS cases and 4,028 IS

death were identified in Changzhou from 2015 to 2016. Significant association of NO_2 with daily IS counts was observed even when adjusted by $PM_{2.5}$ and PM_{10}. The estimated IS risk of NO_2 was more robust in the elderly and the cold season. In addition, PM_{10}, SO_2 and NO_2 were positively associated with daily IS deaths. The young individuals had higher IS mortality risk for $PM_{2.5}$, PM_{10}, NO_2 and CO. And IS mortality risk of SO_2 was more robust in the elderly, female and cold season, statistical significance was also identified for SO_2 when adjusted for CO. To the best of our knowledge, in China, this is the first comprehensive study to explore the acute effect of air pollutants on the morbidity and mortality of stroke at the same time.

PM_{10} could cause endothelial dysfunction, inflammatory response and neuro-functional impairment and subsequently induce ischemia-like injuries in the brain [26]. In the current study, an IQR increment in PM_{10} concentrations was associated with 0.268% increment in daily IS deaths, which was in consistent with the previous investigations [27, 28]. Moreover, the function curve for PM_{10}/ daily IS deaths was flat at <150 $\mu g/m^3$ and became dramatically sharp at high levels. Previous multicity time-series study indicated an acute, deleterious and significant effect of PM_{10} on IS mortality and the estimated rate ratios were 1.016 [29]. Long-term exposure to PM_{10} was positively associated with IS relate death and the estimated relative risk was 1.37 [30]. Moreover, in this study, greater estimated IS mortality risk was observed among males and young individuals. It is plausible that males and young individuals spent more time outdoors and exposed to higher levels of PM_{10} than females and the elderly [31].

NO_2, a representative pollutant of vehicle exhaust, was found to induce excitotoxicity, endothelial and inflammatory responses and damage synaptic plasticity in the brain [32-34]. In China, a multicity case-crossover study demonstrated that an IQR increase (25.4 μg/m^3) in NO_2 was corresponded to 2.6% increment in IS admissions. Stroke mortality was also positively associated with ambient NO_2 levels [35]. In the present study, we indicated that IQR increments in NO_2 (12 μg/m^3) was associated with 0.208% and 0.263% increases in daily IS counts and IS deaths, respectively. An approximately linear concentration-response relationship was identified for NO_2 and daily IS counts or daily IS deaths, which was consistent with the previous studies [36, 37]. The positive association for NO_2 and daily IS counts could be observed even below the National Ambient Air Quality Standards of China (80 μg/m^3). For daily IS counts, the effects of NO_2 were statistically positive and stronger when adjusted for other pollutants except for SO_2 (no statistical but stronger association was observed), suggesting that the adverse effect of NO_2 on IS onsets was stable an NO_2 contributed most to the increased risk of IS. Moreover, the effects of NO_2 were more serious in cold season and males. For IS deaths, young females were found to be experienced higher risk. The underlying reasons may be that the exposed levels of NO_2 were much higher at these conditions, which was similar to that of PM_{10}. The risk factors for stroke, such as hypertension, atrial fibrillation and diabetes mellitus are more frequent and the estimated effects are stronger among females [38]. Females are considered to be more sensitive to stroke onsets and deaths. Thus, our findings regarding the higher risk of IS deaths observed among females were

reasonable.

SO_2 was considered as a strong irritant ambient gaseous pollutant, and could elevate the expression of vasoregulatory pathways and proinflammatory enzymes such as ET-1, iNOS, COX-2, ICAM-1 and TNF-α [39]. And its exposure was demonstrated to contribute to the development and progression of IS [18]. In the present study, SO_2 was significantly associated with daily IS counts when adjusted by $PM_{2.5}$ or PM_{10}. In addition, exposure to SO_2 also had statistical associations with daily IS deaths (the percentage increase was 0.34%), which was in agreement with previous studies [19, 40]. The function curve for SO_2 and daily IS deaths was flat at <35 μg/m^3 and became dramatically sharp at high levels, suggesting that acute and robust increments of SO_2 might result in exacerbating IS death. Furthermore, the associations were more pronounced among females, suggesting that females are more susceptible to IS related deaths when exposed to gaseous pollutants (NO_2 and SO_2). In addition, SO_2 exhibited higher risk of IS deaths in the elderly [41]. And it can be deduced that the elderly are more likely to bear preexisting respiratory or cardiovascular burdens and the vulnerable condition could influence the effect of SO_2 on stroke [42]. We also found that exposure to SO_2 had more serious effects on both IS onsets and deaths in cold season than in warm season.

In conclusion, our results suggested that short term exposure to ambient NO_2 was associated with increased IS risk. And SO_2 were associated with increased IS counts and deaths. These findings may have significant public health implications for prevention of IS and IS related death. Further studies are recommended to validate our

research.

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Contributors The study was conceived and designed by HB. D, Y. L and Z. Z, and overall study management was by XJ. Y, SL. W and Z. Z. ZY. C, GY. L and Y. Y contributed to data collection and processing. S. Y performed the statistical analysis, HB. D, Y. Y and Y. L helped manuscript preparation and interpret the results. All authors contributed to the revision of the manuscript and reviewed and approved the final version.

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Ethical approval The present study was approved by the Institutional Review Board of Changzhou Center for Disease Control and Prevention, and all procedures were in accordance with prevailing ethical principles.

Competing interests None declared.

Patient consent Not needed.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement No additional data are available.

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Figure legends

Figure 1 The percentage change in daily ischemic stroke (IS) counts and daily IS deaths with an interquartile range increase in air pollutants levels on different lag days in single-pollutant models in Changzhou, 2015-2016. The data was expressed as mean with 95% confidence interval.

Figure 2 The concentration-response relationships of daily ischemic stroke (IS) counts, daily IS deaths with air pollutants on different lag days (lag 0-5 for daily IS counts and lag 5 for daily IS deaths) in single-pollutant models in Changzhou, 2015-2016.

Table 1 The means, medians and interquartile ranges (IQRs) for daily air pollutants and weather conditions variables in Changzhou, 2015-2016

Variables	Mean± SD	Min	25th	Median	75th	Max	IQR
Weather Condition							
Temperarure (□)	17.1±8.9	-6.6	9.4	18.3	24.1	34.6	5.8
Humidity (%)	75.3±14.2	35.0	66.0	76.0	86.0	100.0	10.0
Air Pollutions							
$PM_{2.5}$ (μg/m^3)	51.8±30.6	6.8	29.4	44.4	64.4	181.1	20.0
PM_{10} (μg/m^3)	85.3±43.3	10.5	51.5	76.6	108.6	289.1	32.0
NO_2 (μg/m^3)	38.7±16	10.9	26.5	35.8	47.8	117.3	12.0
SO_2 (μg/m^3)	22.3±10	6.9	14.7	20.2	27.0	67.8	6.9
CO (mg/m^3)	1±0.3	0.4	0.8	1.0	1.2	2.6	0.2

$PM_{2.5}$, particulate matter <2.5 mm in diameter; PM_{10}, particulate matter <10 mm in diameter; NO_2, nitrogen dioxide; SO_2, sulfur dioxide; CO, carbon monoxide.

Table 2 The percentage change in daily IS counts and deaths with an interquartile range increase in air pollutants levels modifying by age, gender and seasons in Changzhou, 2015-2016

Variable	Total	Age		Sex		Seasons	
		<=65	>65	Female	Male	Warm	Cold
Daily IS counts*							
$PM_{2.5}$	-0.063 (-0.215-0.088)	-0.246 (-0.568-0.076)	-0.042 (-0.21-0.127)	-0.097 (-0.311-0.119)	-0.08 (-0.289-0.128)	-0.149 (-0.525-0.228)	-0.098 (-0.267-0.071)
PM_{10}	-0.053 (-0.22-0.114)	-0.219 (-0.572-0.136)	-0.004 (-0.191-0.184)	-0.086 (-0.324-0.153)	-0.047 (-0.279-0.185)	-0.057 (-0.41-0.297)	-0.088 (-0.282-0.107)
NO_2	0.208 (0.036-0.381)‡	0.258 (-0.075-0.592)	0.166 (-0.025-0.357)	0.168 (-0.055-0.391)	0.238 (0.002-0.475)‡	-0.136 (-0.481-0.21)	0.382 (0.169-0.596)‡
SO_2	0.125 (-0.034-0.284)	-0.044 (-0.364-0.277)	0.113 (-0.062-0.289)	0.15 (-0.068-0.369)	0.056 (-0.162-0.275)	-0.309 (-0.651-0.033)	0.298 (0.099-0.498)‡
CO	-0.078 (-0.22-0.064)	-0.213 (-0.511-0.086)	-0.061 (-0.222-0.1)	-0.151 (-0.355-0.053)	-0.007 (-0.206-0.192)	-0.175 (-0.432-0.083)	-0.053 (-0.237-0.131)
Daily IS deaths†							
$PM_{2.5}$	0.228 (-0.005-0.462)	1.144 (0.329-1.965)‡	0.152 (-0.091-0.395)	0.124 (-0.19-0.439)	0.29 (-0.043-0.625)	-0.199 (-0.77-0.375)	0.184 (-0.088-0.456)
PM_{10}	0.268 (0.007-0.528)‡	1.52 (0.656-2.391)‡	0.152 (-0.12-0.424)	0.093 (-0.261-0.448)	0.404 (0.03-0.78)‡	-0.012 (-0.53-0.509)	0.247 (-0.075-0.57)
NO_2	0.263 (0.004-0.522)‡	0.894 (0.058-1.736)‡	0.195 (-0.073-0.463)	0.37 (0.027-0.715)‡	0.132 (-0.247-0.512)	0.275 (-0.222-0.775)	0.198 (-0.13-0.528)
SO_2	0.34 (0.088-0.592)‡	0.67 (-0.175-1.523)	0.307 (0.045-0.57)‡	0.434 (0.096-0.772)‡	0.215 (-0.15-0.58)	0.017 (-0.493-0.531)	0.388 (0.081-0.696)‡
CO	0.175 (-0.042-0.392)	0.9 (0.166-1.639)‡	0.108 (-0.118-0.335)	0.189 (-0.11-0.49)	0.154 (-0.162-0.471)	0.02 (-0.401-0.443)	0.169 (-0.104-0.443)

*The multivariable regression model fits the pollutants for daily IS counts data at lag 0-5 and adjusts for calendar time (df=5), DOW (df=7), daily average humidity (df=5) and average temperature (df=5).

†The multivariable regression model fits the pollutants for daily IS deaths data at lag 5 and adjusts for calendar time (df=5), DOW (df=7), daily average humidity (df=5) and average temperature (df=5).

‡ Statistically positive association between air pollutants and daily IS counts or deaths

The data was expressed as mean with 95% confidence interval.

Table 3 The percent increases in daily IS counts and deaths with each interquartile range increment in air pollutants levels in two-pollutants models in Changzhou, 2015-2016

Variables	$PM_{2.5}$	PM_{10}	NO_2	SO_2	CO
Daily IS counts					
$PM_{2.5}$	-0.063 (-0.215-0.088)	0.25 (-0.314-0.817)	0.497 (0.259-0.735)‡	0.305 (0.083-0.527) ‡	-0.059 (-0.274-0.157)
PM_{10}	-0.423 (-0.881-0.038)	-0.053 (-0.22-0.114)	0.539 (0.29-0.788)‡	0.262 (0.034-0.491) ‡	-0.065 (-0.293-0.163)
NO_2	-0.346 (-0.558--0.134)	-0.438 (-0.691--0.185)	0.208 (0.036-0.381)‡	-0.007 (-0.267-0.253)	-0.341 (-0.556--0.124)
SO_2	-0.24 (-0.46--0.019)	-0.244 (-0.49-0.003)	0.22 (-0.067-0.507)	0.125 (-0.034-0.284)	-0.166 (-0.356-0.023)
CO	-0.089 (-0.314-0.137)	0.029 (-0.223-0.281)	0.45 (0.215-0.685)‡	0.172 (-0.016-0.361)	-0.078 (-0.22-0.064)
Daily IS deaths					
$PM_{2.5}$	0.228 (-0.005-0.462)	0.436 (-0.36-1.238)	0.22 (-0.127-0.567)	0.28 (-0.071-0.632)	0.065 (-0.314-0.446)
PM_{10}	0.211 (-0.463-0.89)	0.268 (0.007-0.528)‡	0.218 (-0.135-0.573)	0.295 (-0.055-0.646)	0.137 (-0.23-0.504)
NO_2	0.106 (-0.201-0.414)	0.149 (-0.21-0.509)	0.263 (0.004-0.522)‡	0.351 (-0.096-0.8)	0.057 (-0.269-0.384)
SO_2	0.066 (-0.25-0.383)	0.126 (-0.242-0.496)	0.066 (-0.387-0.521)	0.34 (0.088-0.592)‡	0.035 (-0.281-0.351)
CO	0.186 (-0.199-0.571)	0.233 (-0.187-0.656)	0.23 (-0.14-0.601)	0.346 (0.002-0.691)‡	0.175 (-0.042-0.392)

*The multivariable regression model fits the pollutants for daily IS counts data at lag 0-5 and adjusts for the other pollutants, calendar time (df=5), DOW (df=7), daily average humidity (df=5) and average temperature (df=5).

†The multivariable regression model fits the pollutants for daily IS deaths data at lag 5 and adjusts for the other pollutants, calendar time (df=5), DOW (df=7), daily average humidity (df=5) and average temperature (df=5).

‡ Statistically positive association between air pollutants and daily IS counts or deaths

The data was expressed as mean with 95% confidence interval.

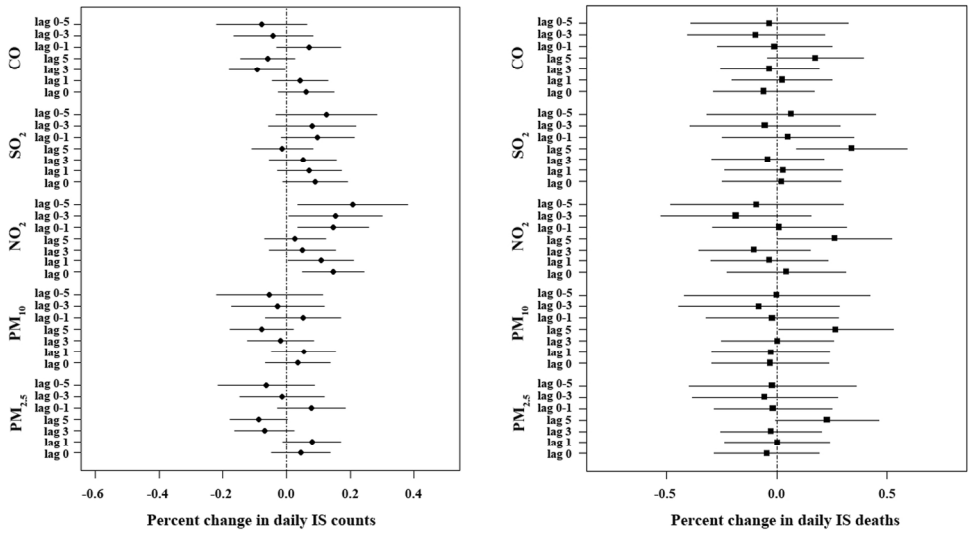

Figure 1 The percentage change in daily ischemic stroke (IS) counts and daily IS deaths with an interquartile range increase in air pollutants levels on different lag days in single-pollutant models in Changzhou, 2015-2016. The data was expressed as mean with 95% confidence interval.

86x48mm (600 x 600 DPI)

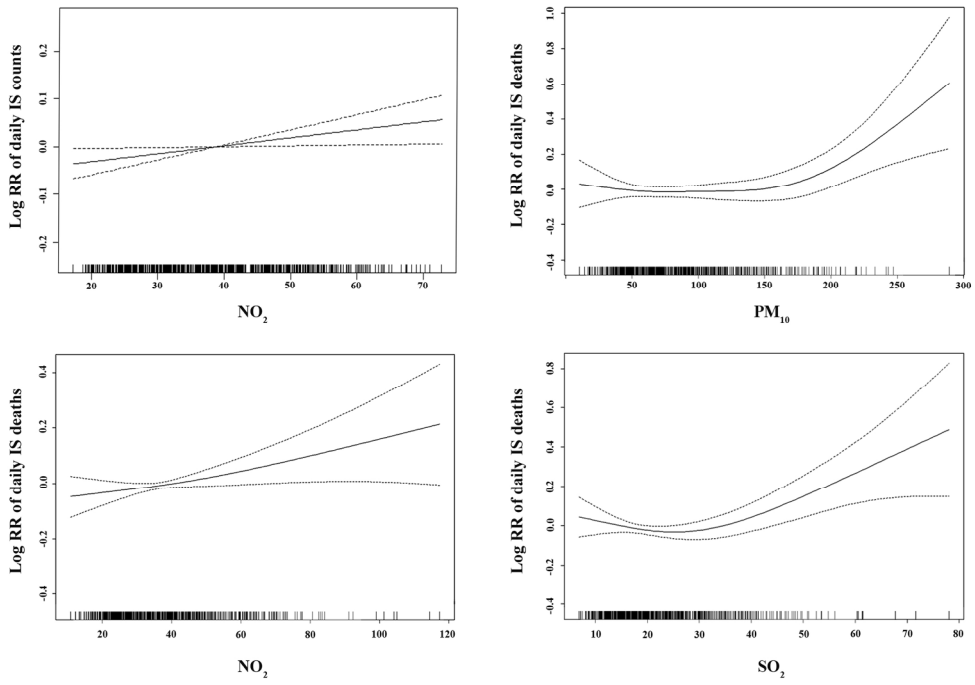

Figure 2 The concentration-response relationships of daily ischemic stroke (IS) counts, daily IS deaths with air pollutants on different lag days (lag 0-5 for daily IS counts and lag 5 for daily IS deaths) in single-pollutant models in Changzhou, 2015-2016.

107x75mm (600 x 600 DPI)

Acute effects of air pollution on ischemic stroke onsets and deaths: A time-series study in Changzhou, China

Huibin Dong[1, a], Yongquan Yu[2, a], Shen Yao[2], Yan Lu[3], Zhiyong Chen[1], Guiying Li[1], Yao Yao[1], Xingjuan Yao[1], Shou-Lin Wang[2], Zhan Zhang[4*]

Supplementary data

Table S1 The descriptive statistics for daily ischemic stroke (IS) counts and deaths in Changzhou, 2015-2016*

Variables	Mean± SD	Min	25th	Median	75th	Max
Daily IS counts	45.4±11.2	14.0	38.0	45.0	53.0	99.0
Male	23.7±7	6.0	19.0	23.0	28.0	60.0
Female	21.8±6.1	6.0	18.0	22.0	26.0	48.0
<65	8.5±3.6	0.0	6.0	8.0	11.0	23.0
≥65	36.9±9.2	11.0	31.0	36.0	43.0	80.0
Daily IS deaths	5.6±2.9	0.0	3.0	5.0	7.0	19.0
Male	2.7±1.8	0.0	1.0	3.0	4.0	10.0
Female	2.8±2	0.0	1.0	3.0	4.0	10.0
<65	0.4±0.7	0.0	0.0	0.0	1.0	3.0
≥65	5.1±2.8	0.0	3.0	5.0	7.0	18.0

*A total of 32,840 IS cases and 4,028 IS deaths recorded were recorded during the study period.

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation
Title and abstract	1-4	Acute effects of air pollution on ischemic stroke onsets and deaths: A time-series study in Changzhou, China
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	5-6	Explain the scientific background and rationale for the investigation being reported
Objectives	6	State specific objectives, including any prespecified hypotheses
Methods		
Study design	7-9	Present key elements of study design early in the paper
Setting	7	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	7	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants
Variables	8	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	7*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	7-8	Describe any efforts to address potential sources of bias
Study size	7	Explain how the study size was arrived at
Quantitative variables	8	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	9	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses
Results		
Participants	9*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	9*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
Outcome data	10-11*	Report numbers of outcome events or summary measures
Main results	10-11	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period

Other analyses	10-11	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	11	Summarise key results with reference to study objectives
Limitations	12-13	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	12-13	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	14	Discuss the generalisability (external validity) of the study results
Other information		
Funding	14	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

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Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Acute effects of air pollution on ischaemic stroke onset and deaths: A time-series study in Changzhou, China

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ABSTRACT

Objective To investigate the acute effect of air pollutants on ischaemic stroke (IS) and IS-related death.

Setting Five urban districts in Changzhou, China, between 9 January 2015 and 31 December 2016.

Participants A total of 32,840 IS cases and 4,028 IS deaths were enrolled.

Main outcome measures A time-series design, generalized additive model and multivariable regression model were used to examine the percentage change (95% confidence interval [CI]) in daily IS counts and deaths with an interquartile range (IQR) increase in air pollutant levels for different single or multiple lag days in single-pollutant and two-pollutant models.

Results Daily IS counts increased 0.208% (95% [CI]: 0.036%-0.381%) with an IQR increment in levels of NO_2. The estimated risk of NO_2 was more robust in males and in the cold season. For daily IS counts, the estimated effects of NO_2 and SO_2 were more significant when adjusted for $PM_{2.5}$ and PM_{10}. An IQR increment in the concentration of PM_{10}, SO_2 and NO_2 significantly increased IS deaths with 6 days of cumulative effects (0.268%, 95% CI: 0.007%-1.528%; 0.34%, 0.088%-0.592%; and 0.263%, 0.004%-0.522%, respectively). Young individuals (< 65 years old) had a higher IS mortality risk for $PM_{2.5}$, PM_{10}, NO_2 and CO. For IS death, the effect estimates of SO_2 in the elderly, females and the cold season were more pronounced; statistical significance was also identified for SO_2 when adjusted for CO.

Conclusions This study suggested that short-term exposure to ambient NO_2 was associated with increased IS risk. In addition, SO_2 was associated with increased IS onset and death.

Article Summary

Strengths and limitations of this study

- This is the first comprehensive research in China that explores the short-term effects of air pollutants on the morbidity and mortality of stroke concurrently.
- The statistical power to detect the association was sufficient because of the substantial numbers of IS cases and IS deaths recorded in our work.
- This time-series study evaluated the association of modifiers between air pollution and daily IS counts or deaths, which may provide clues for the primary prevention of IS and IS-related death.
- The exposure measurement of this study was performed at the community level, and we could not precisely quantify the exposure of individuals to air pollutants using the fixed-site monitoring data; this may lead to exposure measurement error.
- This study considers a single city, and the results should be extrapolated with caution.

INTRODUCTION

Air pollution is considered a primary risk factor for a mounting total of pollution-related diseases and deaths [1]. Moreover, as a modifiable risk factor, air pollution accounts for more than 7 million deaths annually [2]. Studies have extensively explored the association between air pollution and the risk of respiratory, reproductive and cardiovascular diseases [3-5]. Epidemiological studies have suggested that exposure to air pollutants on hazy days may increase the risk of cardiovascular diseases [6-8]. For instance, a study conducted in Stockholm suggested that short-term exposure to particulate matter with aerodynamic diameters < 2.5 μm ($PM_{2.5}$) was associated with out-of-hospital cardiac arrest. Increments in $PM_{2.5}$, PM_{10}, nitrogen dioxide (NO_2), sulfur dioxide (SO_2) and carbon monoxide (CO) were associated with higher risk of heart failure hospitalizations and death. A study conducted in Stockholm suggested that short-term exposure to $PM_{2.5}$ was associated with out-of-hospital cardiac arrest. A meta-analysis indicated that long-term exposure to $PM_{2.5}$ was related to a dramatic increase in cardiovascular mortality [9].

Stroke is a persistent neurological deficit caused by cerebrovascular damage and includes ischaemic stroke (IS) and haemorrhagic stroke (HS) [10-12]. Stroke is one of the leading causes of death and disability-adjusted life years worldwide [13, 14]. In 2010, the estimated number of individuals who suffered from stroke was 50 million, and among them, approximately 17 million cases were newly identified. China bears an enormous stroke burden in the world, having the highest stroke prevalence, incidence and mortality among developing countries worldwide [15]. As the major

subtype of stroke in China, IS accounts for 43% to 79% of all strokes and has recently prompted growing concerns [16]. Given the tremendous disease burden of stroke, the identification and prevention of risk factors are critical and effective public health strategies [17]. However, the effect of air pollutants remains a matter of debate, which might be, at least in part, explained by the heterogeneity of different stroke subtypes [18, 19]. Thus, there is a pressing need to systematically explore the association between air pollutants and specific subtypes of stroke-related incidence and mortality.

Changzhou stands on the southern bank of the Yangtze River. The city is as part of the Suzhou-Wuxi-Changzhou metropolitan area, and by the year of 2010, there were approximately 4 million residents in five urban districts (Tianning district, Wujin district, Jintan district, Xinbei district and Liyang city) [20]. Moreover, because of the rapid industrialization and economic development of the past years, Changzhou is now experiencing severe air pollution. The concentrations of several air pollutants (such as $PM_{2.5}$, PM_{10}, SO_2 and NO_2) were equal or higher than the national ambient air quality in the recent years [21, 22]. Therefore, Changzhou was chosen as a suitable place to study the effect of air pollution on the incidence and mortality of IS. In this research, a comprehensive time-series study was conducted in Changzhou to examine the association between main air pollutants and daily IS counts and deaths. To explore the modification effect, the association of modifiers between air pollution and daily IS counts and deaths was also evaluated. This work will help provide insights into the relationship between stroke and air pollutants and have implications for the prevention of IS onset and IS-related deaths.

MATERIALS AND METHODS

Data collection

Air pollution data, including daily 24-hour average levels of $PM_{2.5}$, PM_{10}, NO_2, SO_2 and CO between January 9, 2015, and December 31, 2016, were obtained from ten air quality monitoring stations run by the Changzhou Environmental Monitoring Center. The daily mean levels for air pollutants were averaged from all the stations, and approximately all the patients whose cases were recorded in this study resided less than 40 km from the nearest monitoring station. Thus, the monitoring data could be used as an appropriate proxy for personal exposure [23-25]. Daily meteorological data on temperature (℃) and relative humidity (%) were collected from the Changzhou Meteorological Bureau.

Daily IS and IS mortality data from 9 January 2015 to 31 December 31 2016 were obtained from the database of the Changzhou Center for Disease Control and Prevention (CDC). This database belonged to the government-controlled network reporting system for chronic diseases, which was established in Changzhou in 2012. Cardiovascular physicians were responsible for the diagnosis of IS according to the cerebrovascular ICD-10 codes for IS (I63). Demographic data were collected from the Changzhou Municipal Bureau of Statistics. Daily IS counts and deaths were also stratified into groups by sex (male and female) and age group (<65 and ≥ 65 years) to explore the effect modification of individual characteristics.

Statistical analysis

Daily IS counts, daily IS death counts, air pollutant levels and weather data were

linked by date for the subsequent time-series study, which was used to analyse the effects of each air pollutant on IS counts and deaths. After evaluating the distribution patterns of daily IS counts and deaths, Poisson distributions were identified. Poisson regression was used in a generalized additive model (GAM) to analyse the data. A multivariable regression model was used to detect the air pollutant-related associations and control for the potential confounding factors, such as calendar time, day of week (DOW), daily average relative humidity and temperature. The five degrees of freedom (df) was used to adjust for all the confounding factors mentioned above except for DOW (df=7). Furthermore, to explore the potential modifications, data were stratified by sex, age (<65 as young individuals and ≥65 as elderly individuals) and season (warm season as 1 May to 31 October and cold season as 1 November to 30 April). Associations between air pollutants and IS and IS-related death were separately examined according to the following equation:

$$\text{Log}[E(T/D_n)]=\text{intercept}+\beta_1\,\text{AirPollution}_{n-i}+\beta_2\,\text{DOW}+\text{ps}\,(\text{calendar time, df=7})+\text{ps}\,(\text{Temp}_{n-i}, \text{df=5})+\text{ps}\,(\text{Relative Humidity}_{n-i}, \text{df=5})$$

$E(T/D_n)$ represents the estimated numbers of IS onset or death on day n; $\text{AirPollution}_{n-i}$ represents the average level of different air pollutants on day n and i is the day lag; β is the vector of the coefficients; DOW is the day of week; ps () represents a penalized spline function; Temp_{n-i} and $\text{Relative Humidity}_{n-i}$ represent average temperature and relative humidity on day n and i is the day lag.

In addition, for both daily IS counts and deaths, single-pollutant models were fitted with different single lag days (lag0, lag1, lag3 and lag5) to detect the effects of air pollution on IS. As underestimation may occur in single-day models, multiple-day

lag models (lag0-1, lag0-3 and lag0-5) were also applied in this study. The smoothing function was used in the analysis of the exposure-response relationship between the log-relative risk of daily IS counts or deaths and air pollutant concentrations using a 5 df in a single-pollutant model.

Statistical software R (version 3.2.3, R Foundation for Statistical Computing, Austria) was used for data analysis and result output. The baseline data was presented as the mean ± standard deviation (SD) for continuous variables. All tests were two-sided, and $P<0.05$ was considered as statistically significant.

Public and patient involvement

Public and patient involvement (PPI) was central and integral in the present study. The PPI group consisted of three male and four female representative patients who were recruited and chaired by HD. The members of the existing group were involved throughout all stages of this research to make contributions. At the outset, they helped researchers with choosing topics and designing the study by providing constructive suggestions. Furthermore, they took part in the conference presentations and group discussions to help filter and interpret data collected from the database mentioned above. They also contributed to discussions about the implications of the results by drawing on their experiences. Findings were disseminated to all PPI members at the end of the study.

RESULTS

Descriptive Analysis

During this study period, there were 32,840 IS cases and 4,028 IS deaths recorded. For IS, on average, 45.4 cases were identified each day (Table S1). Of these, 52.2% were males and 81.3% were the elderly (≥65). In addition, for IS-related death, there were 5.6 deaths recorded on average each day (Table S1). Of these, 48.2% were males and 96.4% were the elderly.

The daily average meteorological data were 17.0°C for temperature and 75% for relative humidity. The daily average levels were 51.8 μg/m^3 for $PM_{2.5}$, 85.3 μg/m^3 for PM_{10}, 22.3 μg/m^3 for SO_2, 38.7 μg/m^3 for NO_2 and 1 mg/ m^3 for CO (Table 1). According to the WHO air quality guidelines (25 μg/m^3 for $PM_{2.5}$, 50 μg/m^3 for PM_{10} and 20 μg/m^3 for SO_2), the corresponding average levels of $PM_{2.5}$, PM_{10} and SO_2 in Changzhou were far beyond the WHO standard.

Estimated Effects of Air Pollutants

Figure 1 summarizes the percentage change (95% CI) in daily IS counts and deaths with an interquartile range (IQR) increase in air pollutant levels for different single or multiple lag days in single-pollutant models. For daily IS counts, a statistically positive association was observed only for an IQR increment of NO_2 levels, and this association was more significant for multi-day exposure than for single day exposure. The highest association was observed for lag 0-5 (0.208%, 0.036%-0.381%) and was used in the subsequent study. For daily IS deaths, statistically positive associations were observed for the different IQRs increments of PM_{10}, SO_2 and NO_2. All associations were the most significant at lag5 (0.268%, 0.007%-0.528% for PM_{10}; 0.34%, 0.088%-0.592% for SO_2; 0.263%, and 0.004%-0.522% for NO_2) than at other

days and thus a 5 day lag model was used in the subsequent analysis.

Concentration-response relationships of air pollutants with daily IS counts or deaths are presented in Figure 2. For IS counts, the curve of NO_2 was linear positive and flat at higher concentrations. For daily IS deaths, the concentration-response curves of PM_{10} and SO_2 were similar, that is, flat at low levels and dramatically linear positive at high levels, whereas the curve for NO_2 suggest a linear rise in daily IS deaths.

The effect of NO_2 on the increase of daily IS counts was more pronounced among males than among females, and the effects of NO_2 and SO_2 on daily IS counts were stronger in the cold season than in the warm season. No significant association was observed when modifying by age (Table 2). For daily IS deaths, the estimated effect of PM_{10} was more pronounced among males, whereas the effects of NO_2 and SO_2 were more robust among females. Moreover, $PM_{2.5}$, PM_{10}, NO_2 and CO were found to have greater deleterious effects among young individuals, and SO_2 showed greater risk in the elderly. In addition, when modifying by seasons, statistical significance was noted only for SO_2 in the cold season (Table 2).

Table 3 shows the percentage increases in daily IS counts and daily IS deaths with each IQR increment in air pollutant levels in the two-pollutant models. For daily IS counts, the estimated effects of NO_2 and SO_2 were more serious when adjusted for $PM_{2.5}$ and PM_{10}. For daily IS deaths, statistical significance was identified for SO_2 when adjusted for CO.

DISCUSSION

In this population-based time-series study, a total of 32,840 IS cases and 4,028 IS death were identified in Changzhou from 2015 to 2016. A significant association of NO_2 with daily IS counts was observed even when adjusted by $PM_{2.5}$ and PM_{10}. The estimated IS risk of NO_2 was more robust in the elderly and in the cold season. In addition, PM_{10}, SO_2 and NO_2 were positively associated with daily IS deaths. Young individuals had a higher IS mortality risk for $PM_{2.5}$, PM_{10}, NO_2 and CO. In addition, the IS mortality risk of SO_2 was more robust in the elderly, females and cold season; statistical significance was also identified for SO_2 when adjusted for CO. To the best of our knowledge, in China, this is the first comprehensive study to explore the acute effect of air pollutants on the morbidity and mortality of stroke concurrently.

PM_{10} can cause endothelial dysfunction, inflammatory response and neuro-functional impairment and, subsequently, induce ischaemia-like injuries in the brain [26]. In the current study, an IQR increment in PM_{10} concentrations was associated with a 0.268% increment in daily IS deaths, which was consistent with previous investigations [27, 28]. Moreover, the function curve for PM_{10}/ daily IS deaths was flat at <150 $\mu g/m^3$ and became dramatically sharp at high levels. A previous multicity time-series study indicated an acute, deleterious and significant effect of PM_{10} on IS mortality, and the estimated rate ratios were 1.016 [29]. Long-term exposure to PM_{10} was positively associated with IS-relate death and the estimated relative risk was 1.37 [30]. Moreover, in this study, a greater estimated IS mortality risk was observed among males and young individuals. It is plausible that males and young people spent more time outdoors and were exposed to higher levels

of PM_{10} than females and the elderly [31].

NO_2, a representative pollutant of vehicle exhaust, has been found to induce excitotoxicity and endothelial and inflammatory responses and to damage synaptic plasticity in the brain [32-34]. In China, a multicity case-crossover study demonstrated that an IQR increase (25.4 μg/m^3) in NO_2 corresponded to a 2.6% increment in IS emissions. Stroke mortality was also positively associated with ambient NO_2 levels [35]. In the present study, we indicated that IQR increments in NO_2 (12 μg/m^3) were associated with 0.208% and 0.263% increases in daily IS counts and IS deaths, respectively. An approximately linear concentration-response relationship was identified for NO_2 and daily IS counts or daily IS deaths, which was consistent with the previous studies [36, 37]. The positive association for NO_2 and daily IS counts could be observed even below the National Ambient Air Quality Standards of China (80 μg/m^3). For daily IS counts, the effects of NO_2 were statistically positive and stronger when adjusted for other pollutants, except for SO_2 (no statistical association was detected, but a stronger association was observed), suggesting that the adverse effect of NO_2 on IS onset was stable and that NO_2 contributed most to the increased risk of IS. Moreover, the effects of NO_2 were more serious in the cold season and in males. For IS deaths, young females were found to experience higher risk. An underlying reason may be that the exposure levels of NO_2 were much higher at these conditions, which was similar to that of PM_{10}. The risk factors for stroke, such as hypertension, atrial fibrillation and diabetes mellitus are more frequent, and the estimated effects are stronger among females [38]. Females are

considered to be more sensitive to stroke onset and deaths. Thus, our findings regarding the higher risk of IS deaths observed among females were reasonable.

SO_2 was considered a strong ambient, gaseous pollutant and irritant that could elevate the expression of vasoregulatory pathways and proinflammatory enzymes such as ET-1, iNOS, COX-2, ICAM-1 and TNF-α [39]. In addition, exposure to it was demonstrated to contribute to the development and progression of IS [18]. In the present study, SO_2 was significantly associated with daily IS counts when adjusted by $PM_{2.5}$ or PM_{10}. In addition, exposure to SO_2 also had statistical associations with daily IS deaths (the percentage increase was 0.34%), which agreed with previous studies [19, 40]. The function curve for SO_2 and daily IS deaths was flat at <35 μg/m^3 and became dramatically sharp at high levels, suggesting that acute and robust increments of SO_2 might exacerbate IS deaths. Furthermore, the associations were more pronounced among females, suggesting that females are more susceptible to IS-related deaths when exposed to gaseous pollutants (NO_2 and SO_2). In addition, SO_2 exhibited a higher risk of IS deaths in the elderly [41]. Thus, it can be deduced that the elderly are more likely to have pre-existing respiratory or cardiovascular burdens, and these vulnerable conditions could influence the effect of SO_2 on stroke [42]. We also found that exposure to SO_2 had more serious effects on both IS onset and deaths in the cold season than in the warm season.

Examining the deleterious effect of air pollutants on the incidence and outcomes of stroke is of significance, and previous studies mainly have focused on one aspect of IS in China. This study is one of the first to comprehensively explore the relationship

between air pollutant levels and morbidity or mortality of IS. As numerous individuals were included to explore the associations, the results of this work strongly support the conclusion and may help provide insights into the relationship between air pollutants and IS. Furthermore, due to the severe air pollution and enormous stroke burden that Changzhou is now experiencing, the findings of this study may provide clues for the primary prevention of the onset or death related to IS, especially for sensitive populations. However, limitations should be noted in this study. Because individuals were identified retrospectively and not all hospitals were included, diagnostic errors and selection bias may exist. We relied on routine measurements from ten fixed-site monitoring stations instead of more accurate measurement based on the individuals' residence and indoor exposure. Thus, we were not able to quantify the exposure of individuals precisely. Moreover, the data regarding IS cases and air pollutant levels were collected from only one city, and it is difficult to extrapolate the results to other areas in China. Further national or multi-cities studies are required.

In conclusion, our results suggested that short-term exposure to ambient NO_2 was associated with increased IS risk. In addition, SO_2 was associated with increased IS counts and deaths. These findings may have significant public health implications for the prevention of IS and IS-related death. Further studies are recommended to validate our research.

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Contributors The study was conceived and designed by HD, YL and ZZ, and overall study management was by XY, S-LW and ZZ, ZC, GL and YYao contributed to data collection and processing. SY performed the statistical analysis, HD, YYu and YL helped in manuscript preparation and interpretation of the results. All authors contributed to the revision of the manuscript and reviewed and approved the final version.

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Ethical approval The present study was approved by the Institutional Review Board of the Changzhou Center for Disease Control and Prevention, and all procedures were in accordance with prevailing ethical principles.

Competing interests None declared.

Patient consent Not needed.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement No additional data are available.

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Figure legends

Figure 1 The percentage change in daily ischaemic stroke (IS) counts and daily IS deaths with an interquartile range increase in air pollutant levels on different lag days in single-pollutant models in Changzhou, 2015-2016. The data are expressed as the mean with a 95% confidence interval.

Figure 2 The concentration-response relationships of daily ischaemic stroke (IS) counts, daily IS deaths with air pollutants on different lag days (lag 0-5 for daily IS counts and lag 5 for daily IS deaths) in single-pollutant models in Changzhou, 2015-2016.

Table 1 The means, medians and interquartile ranges (IQRs) for daily air pollutants and weather conditions variables in Changzhou, 2015-2016

Variables	Mean±SD	Min	25th	Median	75th	Max	IQR
Weather Condition							
Temperature (□)	17.1±8.9	-6.6	9.4	18.3	24.1	34.6	5.8
Humidity (%)	75.3±14.2	35.0	66.0	76.0	86.0	100.0	10.0
Air Pollutions							
$PM_{2.5}$ (μg/m^3)	51.8±30.6	6.8	29.4	44.4	64.4	181.1	20.0
PM_{10} (μg/m^3)	85.3±43.3	10.5	51.5	76.6	108.6	289.1	32.0
NO_2 (μg/m^3)	38.7±16	10.9	26.5	35.8	47.8	117.3	12.0
SO_2 (μg/m^3)	22.3±10	6.9	14.7	20.2	27.0	67.8	6.9
CO (mg/m^3)	1±0.3	0.4	0.8	1.0	1.2	2.6	0.2

$PM_{2.5}$, particulate matter <2.5 mm in diameter; PM_{10}, particulate matter <10 mm in diameter; NO_2, nitrogen dioxide; SO_2, sulfur dioxide; CO, carbon monoxide.

Table 2 The percentage change in daily IS counts and deaths with an interquartile range increase in air pollutants levels modifying by age, sex and seasons in Changzhou, 2015-2016

Variable	Total	Age		Sex		Seasons	
		≤65	>65	Female	Male	Warm	Cold
Daily IS counts*							
$PM_{2.5}$	-0.063 (-0.215-0.088)	-0.246 (-0.568-0.076)	-0.042 (-0.21-0.127)	-0.097 (-0.311-0.119)	-0.08 (-0.289-0.128)	-0.149 (-0.525-0.228)	-0.098 (-0.267-0.071)
PM_{10}	-0.053 (-0.22-0.114)	-0.219 (-0.572-0.136)	-0.004 (-0.191-0.184)	-0.086 (-0.324-0.153)	-0.047 (-0.279-0.185)	-0.057 (-0.41-0.297)	-0.088 (-0.282-0.107)
NO_2	0.208 (0.036-0.381)‡	0.258 (-0.075-0.592)	0.166 (-0.025-0.357)	0.168 (-0.055-0.391)	0.238 (0.002-0.475)‡	-0.136 (-0.481-0.21)	0.382 (0.169-0.596)‡
SO_2	0.125 (-0.034-0.284)	-0.044 (-0.364-0.277)	0.113 (-0.062-0.289)	0.15 (-0.068-0.369)	0.056 (-0.162-0.275)	-0.309 (-0.651-0.033)	0.298 (0.099-0.498)‡
CO	-0.078 (-0.22-0.064)	-0.213 (-0.511-0.086)	-0.061 (-0.222-0.1)	-0.151 (-0.355-0.053)	-0.007 (-0.206-0.192)	-0.175 (-0.432-0.083)	-0.053 (-0.237-0.131)
Daily IS deaths†							
$PM_{2.5}$	0.228 (-0.005-0.462)	1.144 (0.329-1.965)‡	0.152 (-0.091-0.395)	0.124 (-0.19-0.439)	0.29 (-0.043-0.625)	-0.199 (-0.77-0.375)	0.184 (-0.088-0.456)
PM_{10}	0.268 (0.007-0.528)‡	1.52 (0.656-2.391)‡	0.152 (-0.12-0.424)	0.093 (-0.261-0.448)	0.404 (0.03-0.78)‡	-0.012 (-0.53-0.509)	0.247 (-0.075-0.57)
NO_2	0.263 (0.004-0.522)‡	0.894 (0.058-1.736)‡	0.195 (-0.073-0.463)	0.37 (0.027-0.715)‡	0.132 (-0.247-0.512)	0.275 (-0.222-0.775)	0.198 (-0.13-0.528)
SO_2	0.34 (0.088-0.592)‡	0.67 (-0.175-1.523)	0.307 (0.045-0.57)‡	0.434 (0.096-0.772)‡	0.215 (-0.15-0.58)	0.017 (-0.493-0.531)	0.388 (0.081-0.696)‡
CO	0.175 (-0.042-0.392)	0.9 (0.166-1.639)‡	0.108 (-0.118-0.335)	0.189 (-0.11-0.49)	0.154 (-0.162-0.471)	0.02 (-0.401-0.443)	0.169 (-0.104-0.443)

*The multivariable regression model fits the pollutants for daily IS count data at lag 0-5 and adjusts for calendar time (df=5), DOW (df=7), daily average humidity (df=5) and average temperature (df=5).

†The multivariable regression model fits the pollutants for daily IS death data at lag 5 and adjusts for calendar time (df=5), DOW (df=7), daily average humidity (df=5) and average temperature (df=5).

‡ Statistically positive association between air pollutants and daily IS counts or deaths

The data are expressed as the mean with a 95% confidence interval.

Table 3 The percent increases in daily IS counts and deaths with each interquartile range increment in air pollutant levels in two-pollutant models in Changzhou, 2015-2016

Variables	$PM_{2.5}$	PM_{10}	NO_2	SO_2	CO
Daily IS counts					
$PM_{2.5}$	-0.063 (-0.215-0.088)	0.25 (-0.314-0.817)	0.497 (0.259-0.735)‡	0.305 (0.083-0.527) ‡	-0.059 (-0.274-0.157)
PM_{10}	-0.423 (-0.881-0.038)	-0.053 (-0.22-0.114)	0.539 (0.29-0.788)‡	0.262 (0.034-0.491) ‡	-0.065 (-0.293-0.163)
NO_2	-0.346 (-0.558--0.134)	-0.438 (-0.691--0.185)	0.208 (0.036-0.381)‡	-0.007 (-0.267-0.253)	-0.341 (-0.556--0.124)
SO_2	-0.24 (-0.46--0.019)	-0.244 (-0.49-0.003)	0.22 (-0.067-0.507)	0.125 (-0.034-0.284)	-0.166 (-0.356-0.023)
CO	-0.089 (-0.314-0.137)	0.029 (-0.223-0.281)	0.45 (0.215-0.685)‡	0.172 (-0.016-0.361)	-0.078 (-0.22-0.064)
Daily IS deaths					
$PM_{2.5}$	0.228 (-0.005-0.462)	0.436 (-0.36-1.238)	0.22 (-0.127-0.567)	0.28 (-0.071-0.632)	0.065 (-0.314-0.446)
PM_{10}	0.211 (-0.463-0.89)	0.268 (0.007-0.528)‡	0.218 (-0.135-0.573)	0.295 (-0.055-0.646)	0.137 (-0.23-0.504)
NO_2	0.106 (-0.201-0.414)	0.149 (-0.21-0.509)	0.263 (0.004-0.522)‡	0.351 (-0.096-0.8)	0.057 (-0.269-0.384)
SO_2	0.066 (-0.25-0.383)	0.126 (-0.242-0.496)	0.066 (-0.387-0.521)	0.34 (0.088-0.592)‡	0.035 (-0.281-0.351)
CO	0.186 (-0.199-0.571)	0.233 (-0.187-0.656)	0.23 (-0.14-0.601)	0.346 (0.002-0.691)‡	0.175 (-0.042-0.392)

*The multivariable regression model fits the pollutants for daily IS counts data at lag 0-5 and adjusts for the other pollutants, calendar time (df=5), DOW (df=7), daily average humidity (df=5) and average temperature (df=5).

†The multivariable regression model fits the pollutants for daily IS deaths data at lag 5 and adjusts for the other pollutants, calendar time (df=5), DOW (df=7), daily average humidity (df=5) and average temperature (df=5).

‡ Statistically positive association between air pollutants and daily IS counts or deaths

The data was expressed as the mean with 95% confidence interval.

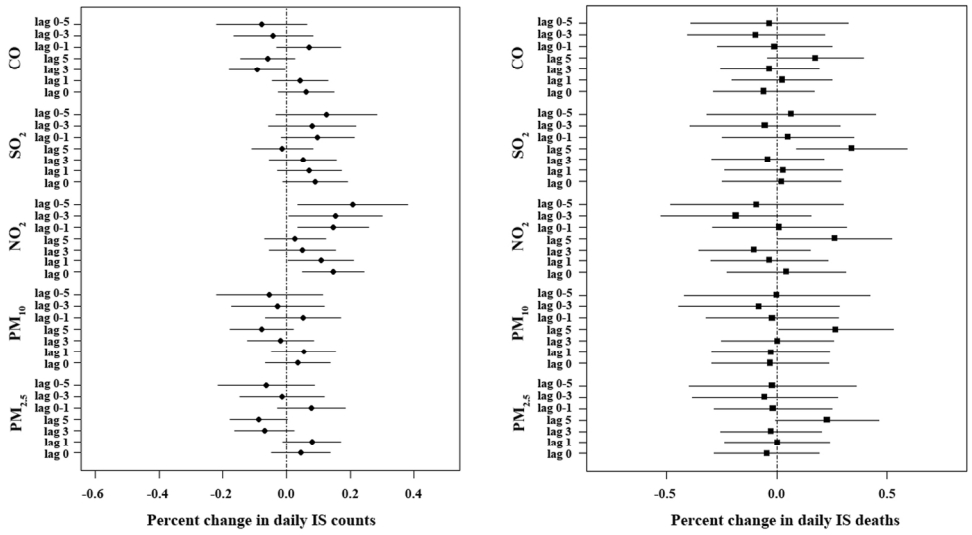

Figure 1 The percentage change in daily ischemic stroke (IS) counts and daily IS deaths with an interquartile range increase in air pollutants levels on different lag days in single-pollutant models in Changzhou, 2015-2016. The data was expressed as mean with 95% confidence interval.

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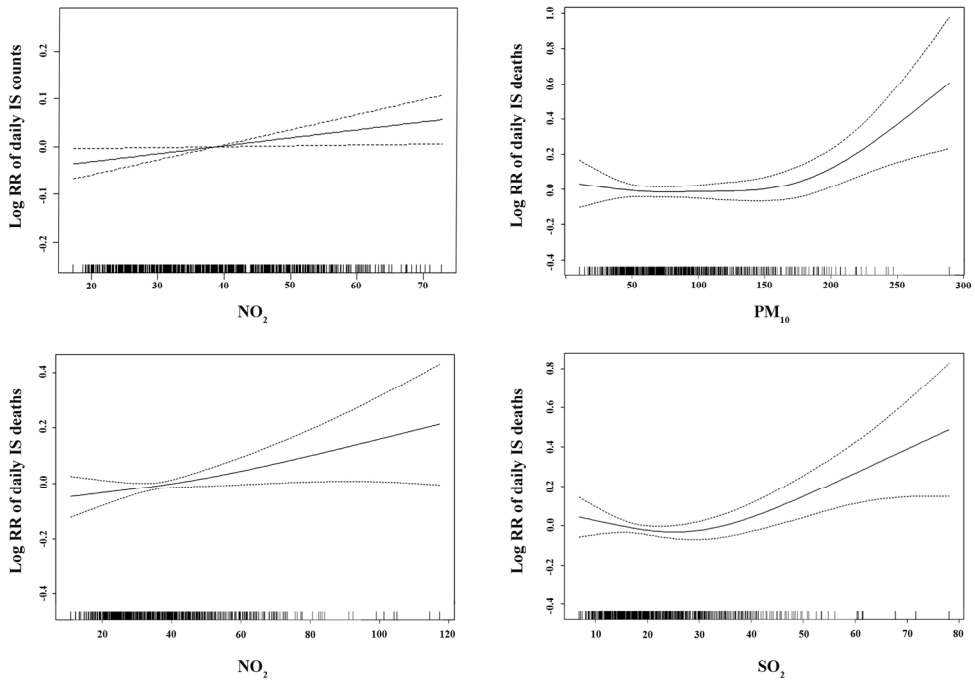

Figure 2 The concentration-response relationships of daily ischemic stroke (IS) counts, daily IS deaths with air pollutants on different lag days (lag 0-5 for daily IS counts and lag 5 for daily IS deaths) in single-pollutant models in Changzhou, 2015-2016.

107x75mm (600 x 600 DPI)

Acute effects of air pollution on ischemic stroke onsets and deaths: A time-series study in Changzhou, China

Huibin Dong[1, a], Yongquan Yu[2, a], Shen Yao[2], Yan Lu[3], Zhiyong Chen[1], Guiying Li[1], Yao Yao[1], Xingjuan Yao[1], Shou-Lin Wang[2], Zhan Zhang[4*]

Supplementary data

Table S1 The descriptive statistics for daily ischemic stroke (IS) counts and deaths in Changzhou, 2015-2016*

Variables	Mean± SD	Min	25th	Median	75th	Max
Daily IS counts	45.4±11.2	14.0	38.0	45.0	53.0	99.0
Male	23.7±7	6.0	19.0	23.0	28.0	60.0
Female	21.8±6.1	6.0	18.0	22.0	26.0	48.0
<65	8.5±3.6	0.0	6.0	8.0	11.0	23.0
≥65	36.9±9.2	11.0	31.0	36.0	43.0	80.0
Daily IS deaths	5.6±2.9	0.0	3.0	5.0	7.0	19.0
Male	2.7±1.8	0.0	1.0	3.0	4.0	10.0
Female	2.8±2	0.0	1.0	3.0	4.0	10.0
<65	0.4±0.7	0.0	0.0	0.0	1.0	3.0
≥65	5.1±2.8	0.0	3.0	5.0	7.0	18.0

*A total of 32,840 IS cases and 4,028 IS deaths recorded were recorded during the study period.

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation
Title and abstract	1-4	Acute effects of air pollution on ischaemic~~ischemic~~ stroke onset~~s~~ and deaths: A time-series study in Changzhou, China
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	5-6	Explain the scientific background and rationale for the investigation being reported
Objectives	6	State specific objectives, including any prespecified hypotheses
Methods		
Study design	7-9	Present key elements of study design early in the paper
Setting	7	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	7	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants
Variables	7-8	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	7*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	7-8	Describe any efforts to address potential sources of bias
Study size	7	Explain how the study size was arrived at
Quantitative variables	8	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	7-9	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses
Results		
Participants	9-10*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	9-10*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
Outcome data	~~10~~9-~~11~~10*	Report numbers of outcome events or summary measures
Main results	10-11	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period

Other analyses	10-11	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	11-12	Summarise key results with reference to study objectives
Limitations	~~12-~~ ~~13~~15	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	12- ~~13~~14	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	14-15	Discuss the generalisability (external validity) of the study results
Other information		
Funding	1~~6~~4	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.