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Title page

Ozone air pollution and ischemic stroke occurrence: identification of a population at risk.

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Abstract

Objectives: Relationship between low level air pollution and stroke are conflicting. This study was conducted to document the relationship between outdoor air pollution and ischemic stroke occurrence.

Methods: We studied the association between daily levels of outdoor pollutants (ozone (O3), nitrogen dioxide (NO2), particulate matter (PM10) and sulphur dioxide (SO2)) and ischemic stroke occurrence (Hospital registry) using a 5 years (2007-2011) time-stratified case-crossover analysis in Nice (France). The short-term effects on stroke occurrence for every 10 µg/m3 increase in each pollutant was calculated using conditional logistic regression model adjusted for temperature, humidity, flu and holidays.

Results: 1729 ischemic stroke patients were enrolled (mean age: 76.1±14.0 years; men: 46.7%). No significant association was found between stroke occurrence and short term effects of all pollutants tested. In stratified analysis, we observed significant associations only between recurrent (n=280) and large artery ischemic stroke (n=578) onset and short term effect of ozone exposure. For an increase of 10 µg/m^3 of ozone level, recurrent stroke risk (mean D-1, D-2, D-3 lag) was increased by 12.1% (95% CI 1.5 to 23.9) and large artery stroke risk (mean D-3, D-4 lag) was increased by 8.0% (95% CI 2.0 to 16.6). Linear dose-response relationship for both subgroups was found.

Conclusions: Our results confirm the relationship between low level ozone exposure and ischemic stroke in high vascular risk subgroup with linear exposure-response relation, independently of other pollutants and meteorological parameters**.**

Strengths and limitations of this study: Relationship between low level air pollution and stroke are conflicting. This paper confirm the relationship between low level ozone exposure and ischemic stroke in high vascular risk subgroup with linear exposure-response relation, independently of other pollutants and meteorological parameters**.** PM2.5 was not studied because not monitored in Nice.

Main Text

INTRODUCTION

Outdoor air pollution is considered as a major environmental health issue, responsible for an excess of death in the world. It is defined as any undesirable modification of air by substances either toxic or likely to have adverse effects on health. Outdoor air pollutants are known to increase morbidity and mortality of respiratory diseases [1]. However, in the 1950's and 1960's, epidemiological studies of acute severe pollution episodes have also shown an increasing cardiovascular and cerebrovascular mortality risk [2]. A link between acute air pollution and stroke mortality has been reported for the first time in the London fog incident study in December 1952 [2]. In the last decades, the consequences of low-level air pollution on cardiovascular mortality and morbidity have been clearly described [3-6]. By analogy, few studies have examined the role of short-term air pollution on ischemic stroke but actually no conclusion could be generalized [3, 5-24]. The purpose of the present study was to document the relationship between the characteristics of outdoor air pollution and the occurence of ischemic stroke.

MATERIALS AND METHODS

Population studied

We performed a 5 years (2007-2011) case-crossover analysis in Nice, France. We retrospectively enrolled consecutive stroke patients admitted at the University Hospital of Nice between January 2007 and December 2011. Querying French DRG based database (PMSI: Programme de Médicalisation des Systèmes d'Information) with I60 to I69 codes from the International Classification of Diseases (10th Revision), we screened all patients

hospitalized for stroke. We filtered the sample to patients living in Nice (geographic area defined by zip codes: 06000, 06100, 06200 and 06300). The diagnosis of ischemic stroke was reviewed and confirmed by a panel of neurologists using clinical and radiological data of medical records. Patients with another diagnosis than stroke were excluded. Demographic data, vascular risk factors, clinical and radiological characteristics of stroke were also collected from medical records.

Outdoor air pollution and meteorological data

Nice is an urban city situated in the south-eastern part of France on the Mediterranean coast. According to the latest census, Nice has a population of 340 735 in 2009. Its climate is temperate and qualified as Mediterranean type. Surrounded by hills and mountains (south Alps), the city of Nice is sheltered from continuous of violent winds. Outdoor air pollution comes mainly from traffic due to high density of roads and international airport (first one in France after Paris airports).

Air pollution data were obtained from the regional agency for air quality monitoring (AirPACA). Exposure measurements during the study period were carried out in 2 of 13 permanent monitoring stations in study area. Measures (µg/m3) were performed in urban station (Cagnes Ladoumègue) for following atmospheric pollutants: particulate matter (PM10) (Tapered Element Oscillating Microbalance), nitrogen dioxide (NO2) (chemiluminescence), sulphur dioxide (SO2) (ultraviolet photometry), and ozone (O3) (ultraviolet photometry). Missing values were replaced by measures performed by the observational monitoring station located at Nice Airport. We computed for each pollutant 24-hours average and specifically for ozone 8-hours daytime periods.

Daily meteorological data were obtained from the National Meteorological Office of Nice including temperature (° Celcius) and humidity (%). Moreover, data on influenza epidemics (weekly count) in region of Provence-Alpes-Cote-d'Azur were obtained from Sentiweb network.

Statistical analysis

Continuous variables were expressed as mean (standard deviation [SD]) or median (interquartile range [IQR]), and categorical variables as percentages. Spearman correlation coefficients (r) between air pollutants and atmospheric parameters were calculated. The time-stratified case-crossover design was used to examine the relationship between short-term effects of outdoor air pollutants and stroke. In this design, each subject enrolled was his own control. Case days were defined as the day of stroke. Control days were defined as the same day of the same stratum as the case day. Study time was stratified by months. Therefore, explicative variables levels at the case day were compared to levels of the same variables at control days. This method has the main advantage to control individual factors, the day of the week, season, time trend [24]. Conditional logistic regression was performed to estimate the association between short-term effects of each air pollutants measured and stroke onset. Odds ratio (OR) and 95% confidence intervals (CI) for a 10 $\mu g/m^3$ increase of pollutant level were adjusted for temperature and humidity with 1-day lag, influenza epidemics and holidays without day lag. The pollutant exposure was tested in models for 1-day lag, 2-day lag, or 3-day lag. Stratified analyses by subgroups were performed according to age, gender, risk vascular factors (tobacco use, diabetes mellitus, hypercholesterolemia, hypertension), and stroke etiological subtypes according the Trial of ORG 10172 in acute stroke treatment (TOAST). We evaluated dose-response relationships across four exposure levels of pollutant

studied and the first quartile was used as the reference group. A p-value less than 0.05 was considered as significant. The data were analyzed using Stata 10.0 SE software.

RESULTS

During the study period (January 2007 to December 2011), there were 2067 patients living Nice and admitted in University Hospital Center for ischemic stroke based on DRG database. After neurologists review of medical records, 1729 ischemic stroke patients were enrolled for final analysis. 620 (35.9%) of these patients were hospitalized in stroke unit. According to the last population census 2009, annual ischemic stroke incidence rates (by 100.000) in studied area were respectively from 2007 to 2011: 100, 100, 98, 96 and 112. Mean age was 76.1±14.0 years, and 46.7% were men (Table 1).

The distribution of air pollutants and meteorological variables is shown in Table 2. Spearman correlation coefficients (r) were ranged from 0.01 to 0.25 between each studied pollutants, except between 0_3 and NO_2 (r=-0.54). Correlation coefficient between minimal temperature and O_3 was r=0.67 (Online only data supplement Table I).

No significant association was found between stroke occurrence and short term effects of all pollutants tested. In addition, we performed stratified subgroup analysis according to gender, age by decade, incident/recurrent stroke status, vascular risk factors, presence of atrial fibrillation, stroke etiological subgroups. We observed significant associations only between recurrent (n=280) and large artery ischemic stroke (n=578) onset and short-term effect of ozone with (Table 3). In recurrent stroke subgroup, for an increase of 10 $\mu g/m^3$ of ozone level (mean D-1, D-2, D-3 lag), stroke risk was significantly increased by 12.1% (95% CI 1.5 to 23.9). Adjusted OR between ozone exposure (mean D-3, D-4) and large artery stroke subgroup was 1.080 (95% CI 1.002 to 1.166). No significant association was observed with

other pollutants than ozone. Adjusted in two-pollutant models, OR were not affected. Using ozone quartiles (1st quartile as the reference group), linear dose-response relationship for both subgroups was observed (Figure 1). Baseline characteristics in recurrent stroke subgroup and large artery stroke subgroup were shown in Figure 2.

DISCUSSION

Our study assessed the short-term effect of ozone exposure on selected population of ischemic stroke in a city specially polluted by ozone. An elevation of 10 µg/m^3 of ozone concentration increases stroke risk with few days lag in recurrent (≈12%) and large artery stroke (≈8%) subgroups only. Linear dose-response relation was observed systematically in both groups. In these groups, the common feature of the patients was that they cumulate vascular risk factors. No significant association was found between all ischemic stroke groups and atmospheric pollutants studied (O_3, NO_2, SO_2 and PM10).

Several studies have investigated the association between outdoor air pollution and stroke [3, 5-24]. Results of these studies are conflicting and hamper generalization of conclusions. Heterogeneous methodological considerations are the main explanation of this conflict. Methodological differences are observed in patient selection, study design, outcomes choice (incidence, hospital admission, mortality), and assessment of individual exposure to selected pollutants [20]. Few published studies investigated specially the association between occurrence of ischemic stroke and ozone exposure using case crossover design [8, 15, 17, 19, 21] or time series analysis method [3, 12, 16, 22]. Consistent with our results, the majority of these studies do not observe relationship between ozone exposure and occurrence of ischemic stroke [3, 8, 17, 19, 21, 22]. Whenever relationship was revealed, association was borderline significant [16], or not confirmed by a second study on the same area of investigation [15, 19]. Despite the fact that the link between ischemic stroke and ozone exposure is not obvious,

results in subgroup analyses seem to identify a population at risk for ozone exposure. In recurrent ischemic stroke subgroup, a significant increase of 12.1% (95% CI 1.5 to 23.9) in stroke risk was observed for each increase of 10 $\mu g/m^3$ of ozone concentration in previous days (mean D-1, D-2, D-3 lag). Consistent with this result, population-based study in Dijon (France), revealed the same association (OR: 1.150; 95% CI 1.027 to 1.209) with 3 days lag [19]. Similarly, a significant association was observed in large artery stroke subgroup (mean D-3, D-4, OR: 1.080; 95% CI 1.002 to 1.166). This link was observed in previous study (Dijon) especially in this stroke etiological subgroup [15]. Associations in other ischemic stroke subgroups are not systematically confirmed (age, gender, vascular risk factors, and season) [3, 15, 19, 21, 22]. Our study confirms the short-term effects of ozone exposure on stroke patients with high vascular risk [15, 19].

Our findings suggest that exposure to ozone, main photochemical pollutant, could increase the risk of ischemic stroke in population subgroups (recurrent stroke, large arteries stroke) particularly exposed to vascular risks factors inducing atherosclerosis. Physiopathological pathways linking ischemic stroke and ozone exposure still remain largely unclear and probably complex. Some studies support a delayed effect (1 to 3 days lag) between acute exposure of ozone pollution and stroke onset [15, 19]. Ozone urban pollution effects on healthy subjects are associated with systemic inflammatory responses, oxidative stress, blood coagulation [25, 26]. These acute phenomena induced by even low levels of ozone could be the trigger of ischemic event consecutively to atherosclerotic plaque instability, alterations in endothelial function, and increased coagulation and thrombosis [27]. As suggested by Henrotin et al., we hypothesized that short term effect of ozone exposure could be involved especially among subjects with high vascular risk [19].

In order to establish a causal relationship between ozone exposure and stroke onset, we studied the exposure-response relation, main criteria identified by Hill [28]. Consistent with previous reports, we show a linear exposure-response relationship between ozone concentration and ischemic stroke in subgroups identified in previous reports [15, 19].

Our study has several limitations. The question of exhaustiveness of stroke patients living Nice in this hospital-based study was considered. In Nice, patients with suspicion of stroke are admitted in priority in University Hospital Center. Likewise, incidence of ischemic stroke was consistent with epidemiological data in France. The question of individual exposure measurement is generally discussed. The main limit is that we used air pollution levels from air monitoring station to represent individuals' exposure. However, we limited our investigations in small geographic area (72 km^2) not considered as a polluted town except for ozone (median 53.3 [32.6-69.2] $\mu g/m^3$). Moreover, in the stroke population studied, elderly patients are mostly retired and have daily activity in study area. Since ozone concentration is correlated with meteorological parameters, temperature and humidity were incorporated into our models. Association between ozone pollution and stroke can be confounded by other pollutants studied, especially particles. Effects of ozone alone are not modified using adjusted models for each of the other pollutants (NO2, SO2 and PM10). PM2.5 was not studied because not monitored in Nice.

SUMMARY

The consequences of ozone pollution on respiratory system and mortality are well documented [1]. Our results confirm the relationship between low level ozone exposure and ischemic stroke in high vascular risk subgroup with linear exposure-response relation, independently of other pollutants and meteorological parameters. Reproducibility of previous results is one of the main Hills criterion to induce causality of ozone exposure. Even if the

individual risk is low, to identify an association between ozone and ischemic stroke incidence is important from a public health point of view, since a large population is concerned. Physiopathological processes underlying this association between ischemic stroke and ozone exposure remain to be investigated.

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Figure Legends

Figure 1: Dose relationship between ozone and ischemic stroke events (1A Recurrent ischemic stroke subgroup, 1B Large artery ischemic stroke subgroup).

Figure 2: Baseline characteristics according recurrent stroke subgroup (A) and large artery stroke subgroup (B) (*: $p<.05$).

Tables

	All patients (n=1729)	Incident n=1449 (83.81%)	Recurrent n=280 (16.19%)	p
Demographic data				
Men	808 (46.73%)	683 (47.14%)	125 (44.64%)	0.0044
Age	76.06±14.04	75.48±14.29	79.01±12.33	<0.0001
< 55 years	141 (8.16%)	132 (9.11%)	9 (3.21%)	0.0011
55 to 64 years	186 (10.76%)	155 (10.70%)	31 (11.07%)	0.8532
65 to 74 years	324 (18.74%)	279 (19.25%)	45 (16.07%)	0.2114
75 to 84 years	524 (30.31%)	438 (30.23%)	86 (30.71%)	0.8712
≥ 85 years	554 (32.04%)	445 (30.71%)	109 (38.93%)	0.0071
Cardiovascular risk factors				
Diabetes mellitus	311 (17.99%)	249 (17.18%)	62 (22.14%)	0.0481
Hypertension	998 (57.72%)	803 (55.42%)	195 (69.64%)	<0.0001
Dyslipidemia	441 (25.51%)	348 (24.02%)	93 (33.21%)	0.0012
Current smoker	410 (23.71%)	357 (24.64%)	53 (18.93%)	0.0398
Overweight	226 (13.07%)	204 (14.08%)	22 (7.86%)	0.0047
Coronary artery desease	263 (15.21%)	209 (14.42%)	54 (19.29%)	0.0381
Atrial fibrillation	527 (30.48%)	433 (29.88%)	94 (33.57%)	0.2198
Classification of stroke etiologic subtypes (TOAST)				
Large artery	578 (33.43%)	479 (33.06%)	99 (35.36%)	0.4552
Cardioembolic	563 (32.56%)	469 (32.37%)	94 (33.57%)	0.6938
Lacunar stroke	153 (8.85%)	129 (8.90%)	24 (8.57%)	0.8582
Other determined etiology	43 (2.49%)	40 (2.76%)	3 (1.07%)	0.0966
Undetermined etiology	392 (22.67%)	332 (22.91%)	60 (21.43%)	0.5872
Hospitalization in stroke unit	620 (35.86%)	546 (37.68%)	74 (26.43%)	0.0003

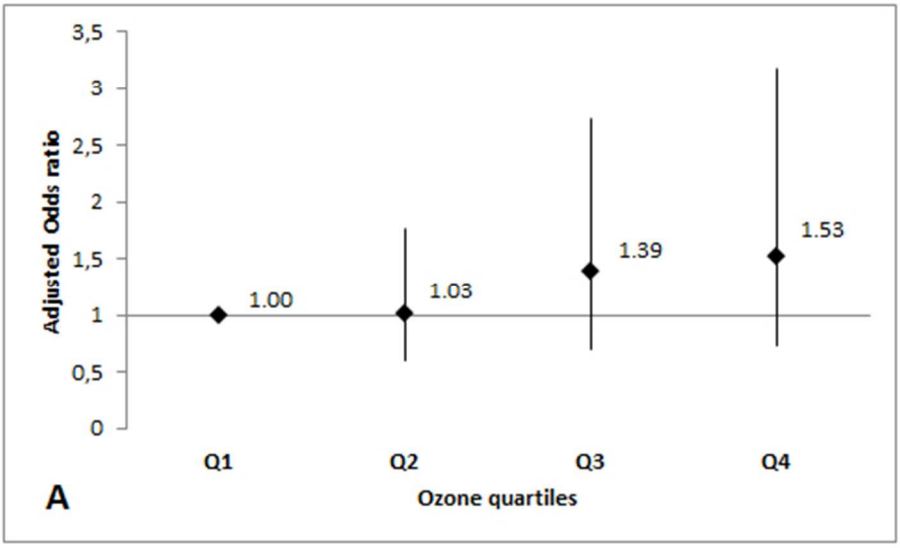
Table 1: Baseline characteristics of patients with ischemic stroke (incident and recurrent) hospitalized in Nice University Hospital from 2007 to 2011.

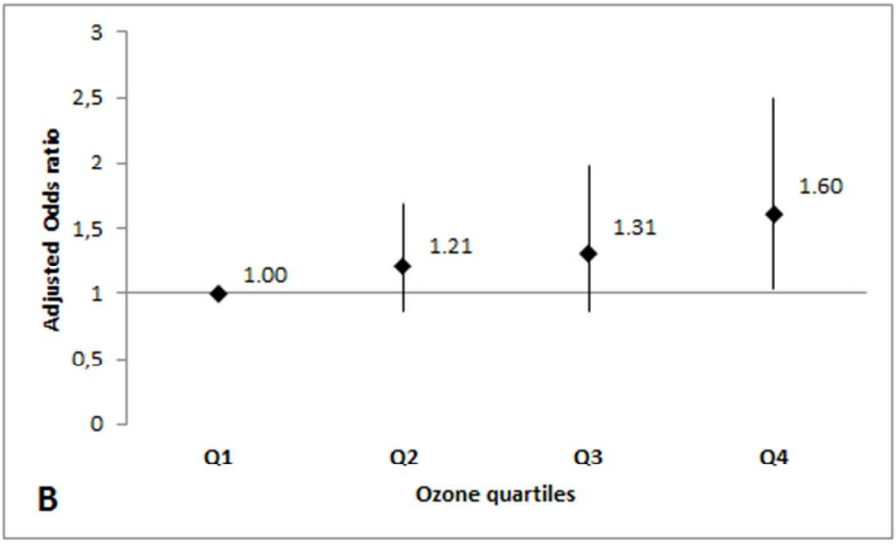
	Mean	SD	Minimum	Quartile 1	Median	Quartile 3	Maximum
Ozone daily 8-hours average (µg/m3)	80.74	31.78	4.63	54.75	84.38	105.38	157.27
Ozone daily 1-hour maximum (µg/m3)	92.37	31.32	7.00	69.00	94.00	115.00	197.00
Ozone 24-hours average (µg/m3)	52.20	22.89	4.00	32.58	53.29	69.02	111.13
PM10 (µg/m3)	28.48	9.81	1.00	22.00	28.00	34.00	74.00
NO2 (µg/m3)	26.22	8.69	3.00	20.00	25.00	32.00	59.00
SO2 (µg/m3)	1.23	1.18	0.00	0.00	1.00	2.00	10.00
Minimum temperature (°C)	13.02	5.98	-1.60	7.80	12.90	18.20	25.90
Maximum humidity (%)	81.40	9.07	40.00	76.00	83.00	88.00	97.00

Table 2: Distribution of air pollution concentrations and meteorological parameters in Nice (France) between 2007 to 2012.

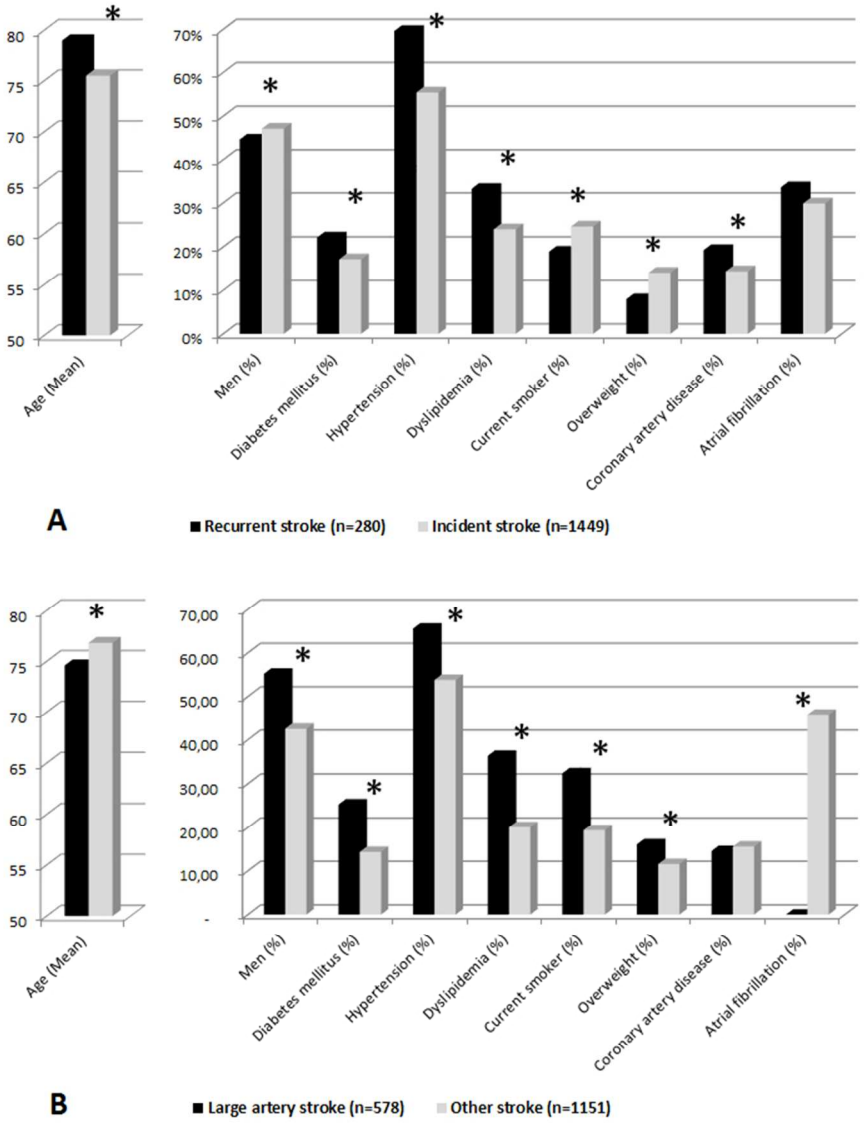
			All ischemic stroke (n=1729)					Recurrent stroke (n=280)					Large artery stroke (n=578)				
			Ora	95% IC			p	Ora	95% IC			p	Ora	95% IC			p
Ozone	D-1	8h average	0.9917	0.9584	to	1.0261	0.633	1.0899	1.0009	to	1.1867	0.047	0.9697	0.9119	to	1.0310	0.326
		1h maximum	0.9957	0.9644	to	1.0281	0.795	1.0641	0.9824	to	1.1527	0.127	0.9881	0.9334	to	1.0460	0.682
		24h average	1.0036	0.9578	to	1.0517	0.877	1.0793	0.9616	to	1.2115	0.195	0.9983	0.9190	to	1.0843	0.968
	D-2	8h average	0.9976	0.9657	to	1.0306	0.888	1.0957	1.0086	to	1.1903	0.030	0.9903	0.9347	to	1.0491	0.741
		1h maximum	1.0040	0.9738	to	1.0351	0.795	1.0955	1.0144	to	1.1831	0.020	0.9979	0.9457	to	1.0529	0.940
		24h average	1.0015	0.9598	to	1.0451	0.942	1.0638	0.9587	to	1.1804	0.244	1.0097	0.9365	to	1.0885	0.801
	D-3	8h average	0.9987	0.9670	to	1.0314	0.939	1.0601	0.9784	to	1.1487	0.154	1.0261	0.9703	to	1.0852	0.366
		1h maximum	0.9968	0.9671	to	1.0273	0.836	1.0380	0.9635	to	1.1184	0.326	1.0254	0.9728	to	1.0808	0.349
		24h average	1.0046	0.9644	to	1.0466	0.822	1.0838	0.9788	to	1.2000	0.122	1.0519	0.9802	to	1.1289	0.160
	D-4	8h average	1.0067	0.9751	to	1.0393	0.681	1.0169	0.9395	to	1.1006	0.678	1.0359	0.9808	to	1.0941	0.205
		1h maximum	0.9978	0.9684	to	1.0280	0.887	1.0038	0.9321	to	1.0811	0.918	1.0290	0.9777	to	1.0829	0.272
		24h average	1.0114	0.9711	to	1.0534	0.583	1.0248	0.9260	to	1.1342	0.635	1.0787	1.0065	to	1.1561	0.032
PM10	D-1		1.0143	0.9518	to	1.0806	0.659	1.0041	0.5586	to	1.7995	0.989	1.0347	0.9282	to	1.1527	0.536
	D-2		0.9861	0.9238	to	1.0523	0.674	0.9518	0.8106	to	1.1167	0.545	0.9350	0.8349	to	1.0464	0.242
	D-3		0.9788	0.9203	to	1.0405	0.493	1.0047	0.8532	to	1.182	0.955	0.9436	0.8475	to	1.0501	0.288
	D-4		0.9780	0.9202	to	1.0391	0.473	0.9911	0.8520	to	1.152	0.908	0.9544	0.8572	to	1.0620	0.392
NO2	D-1		1.0307	0.9367	to	1.1336	0.533	0.8960	0.7689	to	1.0434	0.158	1.0293	0.8699	to	1.2169	0.735
	D-2		0.9931	0.9029	to	1.0918	0.887	0.9427	0.7403	to	1.1991	0.631	1.0494	0.8894	to	1.2372	0.565
	D-3		0.9462	0.8607	to	1.0396	0.250	1.1262	0.8767	to	1.4449	0.349	0.9147	0.7743	to	1.0796	0.292
	D-4		0.9462	0.8607	to	1.0396	0.250	0.8931	0.7047	to	1.1306	0.348	0.9147	0.7743	to	1.0796	0.292
SO2	D-1		1.0069	0.5986	to	1.6893	0.979	0.653	0.164	to	2.5822	0.544	1.0789	0.4507	to	2.5712	0.864
	D-2		0.8763	0.5138	to	1.4905	0.626	0.8916	0.2525	to	3.1284	0.858	1.1784	0.4790	to	2.8858	0.719
	D-3		1.2539	0.7405	to	2.1174	0.397	0.7231	0.1983	to	2.6188	0.622	0.9351	0.3630	to	2.3973	0.889
	D-4		1.4852	0.8956	to	2.4567	0.123	1.3587	0.3735	to	4.9101	0.640	1.6564	0.7140	to	3.8260	0.237

Table 3: Adjusted odds ratios between ischemic stroke and outdoor pollutants exposure for an increase of 10 µg/m3 in Nice (France) between 2007 to 2011.





Dose relationship between ozone and ischemic stroke events (1A Recurrent ischemic stroke subgroup, 1B Large artery ischemic stroke subgroup).
45x54mm (300 x 300 DPI)



Baseline characteristics according recurrent stroke subgroup (A) and large artery stroke subgroup (B) (*: p<.05).
62x81mm (300 x 300 DPI)

	Ozone (8h average)	Ozone (1h maximum)	Ozone (24h average)	PM10	NO2	SO2	Temperature (minimum)	Humidity (Maximum)
Ozone (8h average)	1.000							
Ozone (1h maximum)	0.980	1.000						
Ozone (24h average)	0.954	0.927	1.000					
PM10	0.100	0.140	0.009	1.000				
NO2	-0.472	-0.421	-0.538	0.251	1.000			
SO2	-0.075	-0.068	-0.069	0.109	0.148	1.000		
Temperature (minimum)	0.680	0.672	0.670	0.094	-0.535	-0.096	1.000	
Humidity (maximum)	-0.068	-0.072	-0.099	-0.064	-0.122	-0.010	0.085	1.000

Data supplement Table I: Spearman correlation between air pollutant level and meteorological variables.

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Title page

Ozone air pollution and ischemic stroke occurrence: a case-crossover study in Nice, France.

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Abstract

Objectives: Relationship between low level air pollution and stroke are conflicting. This study was conducted to document the relationship between outdoor air pollution and ischemic stroke occurrence.

Methods: We studied the association between daily levels of outdoor pollutants (ozone (O3), nitrogen dioxide (NO2), particulate matter (PM10) and sulphur dioxide (SO2)) and ischemic stroke occurrence (Hospital registry) using a 5 years (2007-2011) time-stratified case-crossover analysis in Nice (France). The short-term effects on stroke occurrence for every 10 µg/m3 increase in each pollutant was calculated using conditional logistic regression model adjusted for temperature, humidity, flu and holidays.

Results: We enrolled 1729 ischemic stroke patients (mean age: 76.1±14.0 years; men: 46.7%). No significant association was found between stroke occurrence and short term effects of all pollutants tested. In stratified analysis, we observed significant associations only between recurrent (n=280) and large artery ischemic stroke (n=578) onset and short term effect of ozone exposure. For an increase of 10 µg/m^3 of ozone level, recurrent stroke risk (mean D-1, D-2, D-3 lag) was increased by 12.1% (95% CI 1.5 to 23.9) and large artery stroke risk (mean D-3, D-4 lag) was increased by 8.0% (95% CI 2.0 to 16.6). Linear dose-response relationship for both subgroups was found.

Conclusions: Our results confirm the relationship between low level ozone exposure and ischemic stroke in high vascular risk subgroup with linear exposure-response relation, independently of other pollutants and meteorological parameters**.**

Strengths and limitations of this study: Relationship between low level air pollution and stroke are conflicting. This paper confirm the relationship between low level ozone exposure and ischemic stroke in high vascular risk subgroup with linear exposure-response relation, independently of other pollutants and meteorological parameters**.** PM2.5 was not studied because not monitored in Nice.

Main Text

INTRODUCTION

Outdoor air pollution is considered as a major environmental health issue, responsible for an excess of death in the world. It is defined as any undesirable modification of air by substances either toxic or likely to have adverse effects on health. Outdoor air pollutants are known to increase morbidity and mortality of respiratory diseases [1]. However, in the 1950's and 1960's, epidemiological studies of acute severe pollution episodes have also shown an increasing cardiovascular and cerebrovascular mortality risk [2]. A link between acute air pollution and stroke mortality has been reported for the first time in the London fog incident study in December 1952 [2]. In the last decades, the consequences of low-level air pollution on cardiovascular mortality and morbidity have been clearly described [3-6]. By analogy, few studies have examined the role of short-term air pollution on ischemic stroke but actually no conclusion could be generalized [3, 5-24]. The purpose of the present study was to document the relationship between the characteristics of outdoor air pollution and the occurence of ischemic stroke.

MATERIALS AND METHODS

Population studied

We performed a 5 years (2007-2011) case-crossover analysis in Nice, France. We retrospectively enrolled consecutive stroke patients admitted at the University Hospital of Nice between January 2007 and December 2011. Querying French DRG based database (PMSI: Programme de Médicalisation des Systèmes d'Information) with I60 to I69 codes from the International Classification of Diseases (10th Revision), we screened all patients

hospitalized for stroke. We filtered the sample to patients living in Nice (geographic area defined by zip codes: 06000, 06100, 06200 and 06300). The diagnosis of ischemic stroke was reviewed and confirmed by a panel of neurologists using clinical and radiological data of medical records. Patients with another diagnosis than stroke were excluded. Demographic data, vascular risk factors (WHO definitions), clinical and radiological characteristics of stroke were also collected from medical records.

Outdoor air pollution and meteorological data

Nice is an urban city situated in the south-eastern part of France on the Mediterranean coast. According to the latest census, Nice has a population of 340 735 in 2009. Its climate is temperate and qualified as Mediterranean type. Surrounded by hills and mountains (south Alps), the city of Nice is sheltered from continuous of violent winds. Outdoor air pollution comes mainly from traffic due to high density of roads and international airport (first one in France after Paris airports).

Air pollution data were obtained from the regional agency for air quality monitoring (AirPACA). Exposure measurements during the study period were carried out in 2 of 13 permanent monitoring stations in study area. Measures (µg/m3) were performed in urban station (Cagnes Ladoumègue) for following atmospheric pollutants: particulate matter (PM10) (Tapered Element Oscillating Microbalance), nitrogen dioxide (NO2) (chemiluminescence), sulphur dioxide (SO2) (ultraviolet photometry), and ozone (O3) (ultraviolet photometry). Missing values were replaced by measures performed by the observational monitoring station located at Nice Airport. We computed for each pollutant 24-hours average and specifically for ozone 8-hours daytime periods.

Daily meteorological data were obtained from the National Meteorological Office of Nice including temperature (° Celcius) and humidity (%). Moreover, data on influenza epidemics (weekly count) in region of Provence-Alpes-Cote-d'Azur were obtained from Sentiweb network.

Statistical analysis

Continuous variables were expressed as mean (standard deviation [SD]) or median (interquartile range [IQR]), and categorical variables as percentages. Spearman correlation coefficients (r) between air pollutants and atmospheric parameters were calculated. The time-stratified case-crossover design was used to examine the relationship between short-term effects of outdoor air pollutants and stroke. In this design, each subject enrolled was his own control. Case days were defined as the day of stroke. Control days were defined as the same day of the same stratum as the case day. Study time was stratified by months. Therefore, explicative variables levels at the case day were compared to levels of the same variables at control days. This method has the main advantage to control individual factors, the day of the week, season, time trend [24]. Conditional logistic regression was performed to estimate the association between short-term effects of each air pollutants measured and stroke onset. Odds ratio v (OR) and 95% confidence intervals (CI) for a 10 $\mu g/m^3$ increase of pollutant level were adjusted for temperature and humidity with 1-day lag, influenza epidemics and holidays without day lag. The pollutant exposure was tested in models for 1-day lag, 2-day lag, or 3-day lag. Stratified analyses by subgroups were performed according to age, gender, risk vascular factors (tobacco use, diabetes mellitus, hypercholesterolemia, hypertension), and stroke etiological subtypes according the Trial of ORG 10172 in acute stroke treatment (TOAST). We evaluated dose-response relationships across four exposure levels of pollutant

studied and the first quartile was used as the reference group. A p-value less than 0.05 was considered as significant. The data were analyzed using Stata 10.0 SE software.

RESULTS

During the study period (January 2007 to December 2011), there were 2067 patients living Nice and admitted in University Hospital Center for ischemic stroke based on DRG database. After neurologists review of medical records, 1729 ischemic stroke patients were enrolled for final analysis. 620 (35.9%) of these patients were hospitalized in stroke unit. According to the last population census 2009, annual ischemic stroke incidence rates (by 100.000) in studied area were respectively from 2007 to 2011: 100, 100, 98, 96 and 112. Mean age was 76.1±14.0 years, and 46.7% were men (Table 1).

The distribution of air pollutants and meteorological variables is shown in Table 2. Spearman correlation coefficients (r) were ranged from 0.01 to 0.25 between each studied pollutants, except between 0_3 and NO_2 (r=-0.54). Correlation coefficient between minimal temperature and O_3 was r=0.67 (Online only data supplement Table I).

No significant association was found between stroke occurrence and short term effects of all pollutants tested. In addition, we performed stratified subgroup analysis according to gender, age by decade, incident/recurrent stroke status, vascular risk factors, presence of atrial fibrillation, stroke etiological subgroups. We observed significant associations only between recurrent (n=280) and large artery ischemic stroke (n=578) onset and short-term effect of ozone with (Table 3). In recurrent stroke subgroup, for an increase of 10 $\mu g/m^3$ of ozone level (mean D-1, D-2, D-3 lag), stroke risk was significantly increased by 12.1% (95% CI 1.5 to 23.9). Adjusted OR between ozone exposure (mean D-3, D-4) and large artery stroke subgroup was 1.080 (95% CI 1.002 to 1.166). No significant association was observed with

other pollutants than ozone. Adjusted in two-pollutant models, OR were not affected. Using ozone quartiles (1st quartile as the reference group), linear dose-response relationship for both subgroups was observed (Figure 1). Baseline characteristics in recurrent stroke subgroup and large artery stroke subgroup were shown in Figure 2.

DISCUSSION

Our study assessed the short-term effect of ozone exposure on selected population of ischemic stroke in a city specially polluted by ozone. An elevation of 10 μg/m^3 of ozone concentration increases stroke risk with few days lag in recurrent (≈12%) and large artery stroke (≈8%) subgroups only. Linear dose-response relation was observed systematically in both groups. In these groups, the common feature of the patients was that they cumulate vascular risk factors. No significant association was found between all ischemic stroke groups and atmospheric pollutants studied (O_3, NO_2, SO_2 and PM10).

Several studies have investigated the association between outdoor air pollution and stroke [3, 5-24]. Results of these studies are conflicting and hamper generalization of conclusions. Heterogeneous methodological considerations are the main explanation of this conflict. Methodological differences are observed in patient selection, study design, outcomes choice (incidence, hospital admission, mortality), and assessment of individual exposure to selected pollutants [20]. Few published studies investigated specially the association between occurrence of ischemic stroke and ozone exposure using case crossover design [8, 15, 17, 19, 21] or time series analysis method [3, 12, 16, 22]. Consistent with our results, the majority of these studies do not observe relationship between ozone exposure and occurrence of ischemic stroke [3, 8, 17, 19, 21, 22]. Whenever relationship was revealed, association was borderline significant [16], or not confirmed by a second study on the same area of investigation [15, 19]. Despite the fact that the link between ischemic stroke and ozone exposure is not obvious,

results in subgroup analyses seem to identify a population at risk for ozone exposure. In recurrent ischemic stroke subgroup, a significant increase of 12.1% (95% CI 1.5 to 23.9) in stroke risk was observed for each increase of 10 $\mu g/m^3$ of ozone concentration in previous days (mean D-1, D-2, D-3 lag). Consistent with this result, population-based study in Dijon (France), revealed the same association (OR: 1.150; 95% CI 1.027 to 1.209) with 3 days lag [19]. Similarly, a significant association was observed in large artery stroke subgroup (mean D-3, D-4, OR: 1.080; 95% CI 1.002 to 1.166). This link was observed in previous study (Dijon) especially in this stroke etiological subgroup [15]. Associations in other ischemic stroke subgroups are not systematically confirmed (age, gender, vascular risk factors, and season) [3, 15, 19, 21, 22]. Our study confirms the short-term effects of ozone exposure on stroke patients with high vascular risk [15, 19].

Our findings suggest that exposure to ozone, main photochemical pollutant, could increase the risk of ischemic stroke in population subgroups (recurrent stroke, large arteries stroke) particularly exposed to vascular risks factors inducing atherosclerosis. Physiopathological pathways linking ischemic stroke and ozone exposure still remain largely unclear and probably complex. Some studies support a delayed effect (1 to 3 days lag) between acute exposure of ozone pollution and stroke onset [15, 19]. Ozone urban pollution effects on healthy subjects are associated with systemic inflammatory responses, oxidative stress, blood coagulation [25, 26]. These acute phenomena induced by even low levels of ozone could be the trigger of ischemic event consecutively to atherosclerotic plaque instability, alterations in endothelial function, and increased coagulation and thrombosis [27]. As suggested by Henrotin et al., we hypothesized that short term effect of ozone exposure could be involved especially among subjects with high vascular risk [19].

In order to establish a causal relationship between ozone exposure and stroke onset, we studied the exposure-response relation, main criteria identified by Hill [28]. Consistent with previous reports, we show a linear exposure-response relationship between ozone concentration and ischemic stroke in subgroups identified in previous reports [15, 19].

Our study has several limitations. The question of completeness of stroke patients living Nice in this hospital-based study was discussed. In Nice, patients with suspicion of stroke are admitted in priority in University Hospital Center. Likewise, incidence of ischemic stroke was consistent with epidemiological data in France. The question of individual exposure measurement is generally discussed. The main limit is that we used air pollution levels from air monitoring station to represent individuals' exposure. However, we limited our investigations in small geographic area (72 km^2) not considered as a polluted town except for ozone (median 53.3 [32.6-69.2] $\mu g/m^3$). Moreover, in the stroke population studied, elderly patients are mostly retired and have daily activity in study area. Since ozone concentration is correlated with meteorological parameters, temperature and humidity were incorporated into our models. Association between ozone pollution and stroke can be confounded by other pollutants studied, especially particles. Effects of ozone alone are not modified using adjusted models for each of the other pollutants (NO2, SO2 and PM10). PM2.5 was not studied because not monitored in Nice.

SUMMARY

The consequences of ozone pollution on respiratory system and mortality are well documented [1]. Our results confirm the relationship between low level ozone exposure and ischemic stroke in high vascular risk subgroup with linear exposure-response relation, independently of other pollutants and meteorological parameters. Reproducibility of previous results is one of the main Hills criterion to induce causality of ozone exposure. Even if the

individual risk is low, to identify an association between ozone and ischemic stroke incidence is important from a public health point of view, since a large population is concerned. Physiopathological processes underlying this association between ischemic stroke and ozone exposure remain to be investigated.

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Contributorship Statement

All authors have contributed to:

1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data;

2) drafting the article or revising it critically for important intellectual content; and

3) final approval of the version to be published.

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Figure Legends

Figure 1: Dose relationship between ozone and ischemic stroke events (1A Recurrent ischemic stroke subgroup, 1B Large artery ischemic stroke subgroup).

Figure 2: Baseline characteristics according recurrent stroke subgroup (A) and large artery stroke subgroup (B) (*: p<.05).

Tables

	All patients (n=1729)	Incident n=1449 (83.81%)	Recurrent n=280 (16.19%)	p
Demographic data				
Men	808 (46.73%)	683 (47.14%)	125 (44.64%)	0.0044
Age	76.06±14.04	75.48±14.29	79.01±12.33	<0.0001
< 55 years	141 (8.16%)	132 (9.11%)	9 (3.21%)	0.0011
55 to 64 years	186 (10.76%)	155 (10.70%)	31 (11.07%)	0.8532
65 to 74 years	324 (18.74%)	279 (19.25%)	45 (16.07%)	0.2114
75 to 84 years	524 (30.31%)	438 (30.23%)	86 (30.71%)	0.8712
≥ 85 years	554 (32.04%)	445 (30.71%)	109 (38.93%)	0.0071
Cardiovascular risk factors				
Diabetes mellitus	311 (17.99%)	249 (17.18%)	62 (22.14%)	0.0481
Hypertension	998 (57.72%)	803 (55.42%)	195 (69.64%)	<0.0001
Dyslipidemia	441 (25.51%)	348 (24.02%)	93 (33.21%)	0.0012
Current smoker	410 (23.71%)	357 (24.64%)	53 (18.93%)	0.0398
Overweight	226 (13.07%)	204 (14.08%)	22 (7.86%)	0.0047
Coronary artery disease	263 (15.21%)	209 (14.42%)	54 (19.29%)	0.0381
Atrial fibrillation	527 (30.48%)	433 (29.88%)	94 (33.57%)	0.2198
Classification of stroke etiologic subtypes (TOAST)				
Large artery	578 (33.43%)	479 (33.06%)	99 (35.36%)	0.4552
Cardioembolic	563 (32.56%)	469 (32.37%)	94 (33.57%)	0.6938
Lacunar stroke	153 (8.85%)	129 (8.90%)	24 (8.57%)	0.8582
Other determined etiology	43 (2.49%)	40 (2.76%)	3 (1.07%)	0.0966
Undetermined etiology	392 (22.67%)	332 (22.91%)	60 (21.43%)	0.5872
Hospitalization in stroke unit	620 (35.86%)	546 (37.68%)	74 (26.43%)	0.0003

Table 1: Baseline characteristics of patients with ischemic stroke (incident and recurrent) hospitalized in Nice University Hospital from 2007 to 2011.

	Mean	SD	Minimum	Quartile 1	Median	Quartile 3	Maximum
Ozone daily 8-hours average (µg/m3)	80.74	31.78	4.63	54.75	84.38	105.38	157.27
Ozone daily 1-hour maximum (µg/m3)	92.37	31.32	7.00	69.00	94.00	115.00	197.00
Ozone 24-hours average (µg/m3)	52.20	22.89	4.00	32.58	53.29	69.02	111.13
PM10 (µg/m3)	28.48	9.81	1.00	22.00	28.00	34.00	74.00
NO2 (µg/m3)	26.22	8.69	3.00	20.00	25.00	32.00	59.00
SO2 (µg/m3)	1.23	1.18	0.00	0.00	1.00	2.00	10.00
Minimum temperature (°C)	13.02	5.98	-1.60	7.80	12.90	18.20	25.90
Maximum humidity (%)	81.40	9.07	40.00	76.00	83.00	88.00	97.00

Table 2: Distribution of air pollution concentrations and meteorological parameters in Nice (France) between 2007 to 2012.

			All ischemic stroke (n=1729)				Recurrent stroke (n=280)				Large artery stroke (n=578)			
			Ora	95% IC		p	Ora	95% IC		p	Ora	95% IC		p
Ozone	D-1	8h average	0.9917	0.9584	to 1.0261	0.633	1.0899	1.0009	to 1.1867	0.047	0.9697	0.9119	to 1.0310	0.326
		1h maximum	0.9957	0.9644	to 1.0281	0.795	1.0641	0.9824	to 1.1527	0.127	0.9881	0.9334	to 1.0460	0.682
		24h average	1.0036	0.9578	to 1.0517	0.877	1.0793	0.9616	to 1.2115	0.195	0.9983	0.9190	to 1.0843	0.968
	D-2	8h average	0.9976	0.9657	to 1.0306	0.888	1.0957	1.0086	to 1.1903	0.030	0.9903	0.9347	to 1.0491	0.741
		1h maximum	1.0040	0.9738	to 1.0351	0.795	1.0955	1.0144	to 1.1831	0.020	0.9979	0.9457	to 1.0529	0.940
		24h average	1.0015	0.9598	to 1.0451	0.942	1.0638	0.9587	to 1.1804	0.244	1.0097	0.9365	to 1.0885	0.801
	D-3	8h average	0.9987	0.9670	to 1.0314	0.939	1.0601	0.9784	to 1.1487	0.154	1.0261	0.9703	to 1.0852	0.366
		1h maximum	0.9968	0.9671	to 1.0273	0.836	1.0380	0.9635	to 1.1184	0.326	1.0254	0.9728	to 1.0808	0.349
		24h average	1.0046	0.9644	to 1.0466	0.822	1.0838	0.9788	to 1.2000	0.122	1.0519	0.9802	to 1.1289	0.160
	D-4	8h average	1.0067	0.9751	to 1.0393	0.681	1.0169	0.9395	to 1.1006	0.678	1.0359	0.9808	to 1.0941	0.205
		1h maximum	0.9978	0.9684	to 1.0280	0.887	1.0038	0.9321	to 1.0811	0.918	1.0290	0.9777	to 1.0829	0.272
		24h average	1.0114	0.9711	to 1.0534	0.583	1.0248	0.9260	to 1.1342	0.635	1.0787	1.0065	to 1.1561	0.032
PM10	D-1		1.0143	0.9518	to 1.0806	0.659	1.0041	0.5586	to 1.7995	0.989	1.0347	0.9282	to 1.1527	0.536
	D-2		0.9861	0.9238	to 1.0523	0.674	0.9518	0.8106	to 1.1167	0.545	0.9350	0.8349	to 1.0464	0.242
	D-3		0.9788	0.9203	to 1.0405	0.493	1.0047	0.8532	to 1.182	0.955	0.9436	0.8475	to 1.0501	0.288
	D-4		0.9780	0.9202	to 1.0391	0.473	0.9911	0.8520	to 1.152	0.908	0.9544	0.8572	to 1.0620	0.392
NO2	D-1		1.0307	0.9367	to 1.1336	0.533	0.8960	0.7689	to 1.0434	0.158	1.0293	0.8699	to 1.2169	0.735
	D-2		0.9931	0.9029	to 1.0918	0.887	0.9427	0.7403	to 1.1991	0.631	1.0494	0.8894	to 1.2372	0.565
	D-3		0.9462	0.8607	to 1.0396	0.250	1.1262	0.8767	to 1.4449	0.349	0.9147	0.7743	to 1.0796	0.292
	D-4		0.9462	0.8607	to 1.0396	0.250	0.8931	0.7047	to 1.1306	0.348	0.9147	0.7743	to 1.0796	0.292
SO2	D-1		1.0069	0.5986	to 1.6893	0.979	0.653	0.164	to 2.5822	0.544	1.0789	0.4507	to 2.5712	0.864
	D-2		0.8763	0.5138	to 1.4905	0.626	0.8916	0.2525	to 3.1284	0.858	1.1784	0.4790	to 2.8858	0.719
	D-3		1.2539	0.7405	to 2.1174	0.397	0.7231	0.1983	to 2.6188	0.622	0.9351	0.3630	to 2.3973	0.889
	D-4		1.4852	0.8956	to 2.4567	0.123	1.3587	0.3735	to 4.9101	0.640	1.6564	0.7140	to 3.8260	0.237

Table 3: Adjusted odds ratios between ischemic stroke and outdoor pollutants exposure for an increase of 10 µg/m3 in Nice (France) between 2007 to 2011.

Title page

Ozone air pollution and ischemic stroke occurrence: a case-crossover study in Nice, France.

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Abstract

Objectives: Relationship between low level air pollution and stroke are conflicting. This study was conducted to document the relationship between outdoor air pollution and ischemic stroke occurrence.

Methods: We studied the association between daily levels of outdoor pollutants (ozone (O3), nitrogen dioxide (NO2), particulate matter (PM10) and sulphur dioxide (SO2)) and ischemic stroke occurrence (Hospital registry) using a 5 years (2007-2011) time-stratified case-crossover analysis in Nice (France). The short-term effects on stroke occurrence for every 10 µg/m3 increase in each pollutant was calculated using conditional logistic regression model adjusted for temperature, humidity, flu and holidays.

Results: We enrolled 1729 ischemic stroke patients (mean age: 76.1±14.0 years; men: 46.7%). No significant association was found between stroke occurrence and short term effects of all pollutants tested. In stratified analysis, we observed significant associations only between recurrent (n=280) and large artery ischemic stroke (n=578) onset and short term effect of ozone exposure. For an increase of 10 µg/m^3 of ozone level, recurrent stroke risk (mean D-1, D-2, D-3 lag) was increased by 12.1% (95% CI 1.5 to 23.9) and large artery stroke risk (mean D-3, D-4 lag) was increased by 8.0% (95% CI 2.0 to 16.6). Linear dose-response relationship for both subgroups was found.

Conclusions: Our results confirm the relationship between low level ozone exposure and ischemic stroke in high vascular risk subgroup with linear exposure-response relation, independently of other pollutants and meteorological parameters.

Strengths and limitations of this study: Relationship between low level air pollution and stroke are conflicting. This paper confirm the relationship between low level ozone exposure and ischemic stroke in high vascular risk subgroup with linear exposure-response relation, independently of other pollutants and meteorological parameters**.** PM2.5 was not studied because not monitored in Nice.

Main Text

INTRODUCTION

Outdoor air pollution is considered as a major environmental health issue, responsible for an excess of death in the world. It is defined as any undesirable modification of air by substances either toxic or likely to have adverse effects on health. Outdoor air pollutants are known to increase morbidity and mortality of respiratory diseases [1]. However, in the 1950's and 1960's, epidemiological studies of acute severe pollution episodes have also shown an increasing cardiovascular and cerebrovascular mortality risk [2]. A link between acute air pollution and stroke mortality has been reported for the first time in the London fog incident study in December 1952 [2]. In the last decades, the consequences of low-level air pollution on cardiovascular mortality and morbidity have been clearly described [3-6]. By analogy, few studies have examined the role of short-term air pollution on ischemic stroke but actually no conclusion could be generalized [3, 5-24]. The purpose of the present study was to document the relationship between the characteristics of outdoor air pollution and the occurence of ischemic stroke.

MATERIALS AND METHODS

Population studied

We performed a 5 years (2007-2011) case-crossover analysis in Nice, France. We retrospectively enrolled consecutive stroke patients admitted at the University Hospital of Nice between January 2007 and December 2011. Querying French DRG based database (PMSI: Programme de Médicalisation des Systèmes d'Information) with I60 to I69 codes from the International Classification of Diseases (10th Revision), we screened all patients

hospitalized for stroke. We filtered the sample to patients living in Nice (geographic area defined by zip codes: 06000, 06100, 06200 and 06300). The diagnosis of ischemic stroke was reviewed and confirmed by a panel of neurologists using clinical and radiological data of medical records. Patients with another diagnosis than stroke were excluded. Demographic data, vascular risk factors (WHO definitions), clinical and radiological characteristics of stroke were also collected from medical records.

Outdoor air pollution and meteorological data

Nice is an urban city situated in the south-eastern part of France on the Mediterranean coast. According to the latest census, Nice has a population of 340 735 in 2009. Its climate is temperate and qualified as Mediterranean type. Surrounded by hills and mountains (south Alps), the city of Nice is sheltered from continuous of violent winds. Outdoor air pollution comes mainly from traffic due to high density of roads and international airport (first one in France after Paris airports).

Air pollution data were obtained from the regional agency for air quality monitoring (AirPACA). Exposure measurements during the study period were carried out in 2 of 13 permanent monitoring stations in study area. Measures (µg/m3) were performed in urban station (Cagnes Ladoumègue) for following atmospheric pollutants: particulate matter (PM10) (Tapered Element Oscillating Microbalance), nitrogen dioxide (NO2) (chemiluminescence), sulphur dioxide (SO2) (ultraviolet photometry), and ozone (O3) (ultraviolet photometry). Missing values were replaced by measures performed by the observational monitoring station located at Nice Airport. We computed for each pollutant 24-hours average and specifically for ozone 8-hours daytime periods.

Daily meteorological data were obtained from the National Meteorological Office of Nice including temperature (° Celcius) and humidity (%). Moreover, data on influenza epidemics (weekly count) in region of Provence-Alpes-Cote-d'Azur were obtained from Sentiweb network.

Statistical analysis

Continuous variables were expressed as mean (standard deviation [SD]) or median (interquartile range [IQR]), and categorical variables as percentages. Spearman correlation coefficients (r) between air pollutants and atmospheric parameters were calculated. The time-stratified case-crossover design was used to examine the relationship between short-term effects of outdoor air pollutants and stroke. In this design, each subject enrolled was his own control. Case days were defined as the day of stroke. Control days were defined as the same day of the same stratum as the case day. Study time was stratified by months. Therefore, explicative variables levels at the case day were compared to levels of the same variables at control days. This method has the main advantage to control individual factors, the day of the week, season, time trend [24]. Conditional logistic regression was performed to estimate the association between short-term effects of each air pollutants measured and stroke onset. Odds ratio v (OR) and 95% confidence intervals (CI) for a 10 $\mu g/m^3$ increase of pollutant level were adjusted for temperature and humidity with 1-day lag, influenza epidemics and holidays without day lag. The pollutant exposure was tested in models for 1-day lag, 2-day lag, or 3-day lag. Stratified analyses by subgroups were performed according to age, gender, risk vascular factors (tobacco use, diabetes mellitus, hypercholesterolemia, hypertension), and stroke etiological subtypes according the Trial of ORG 10172 in acute stroke treatment (TOAST). We evaluated dose-response relationships across four exposure levels of pollutant

studied and the first quartile was used as the reference group. A p-value less than 0.05 was considered as significant. The data were analyzed using Stata 10.0 SE software.

RESULTS

During the study period (January 2007 to December 2011), there were 2067 patients living Nice and admitted in University Hospital Center for ischemic stroke based on DRG database. After neurologists review of medical records, 1729 ischemic stroke patients were enrolled for final analysis. 620 (35.9%) of these patients were hospitalized in stroke unit. According to the last population census 2009, annual ischemic stroke incidence rates (by 100.000) in studied area were respectively from 2007 to 2011: 100, 100, 98, 96 and 112. Mean age was 76.1±14.0 years, and 46.7% were men (Table 1).

The distribution of air pollutants and meteorological variables is shown in Table 2. Spearman correlation coefficients (r) were ranged from 0.01 to 0.25 between each studied pollutants, except between 0_3 and NO_2 (r=-0.54). Correlation coefficient between minimal temperature and O_3 was r=0.67 (Online only data supplement Table I).

No significant association was found between stroke occurrence and short term effects of all pollutants tested. In addition, we performed stratified subgroup analysis according to gender, age by decade, incident/recurrent stroke status, vascular risk factors, presence of atrial fibrillation, stroke etiological subgroups. We observed significant associations only between recurrent (n=280) and large artery ischemic stroke (n=578) onset and short-term effect of ozone with (Table 3). In recurrent stroke subgroup, for an increase of 10 $\mu g/m^3$ of ozone level (mean D-1, D-2, D-3 lag), stroke risk was significantly increased by 12.1% (95% CI 1.5 to 23.9). Adjusted OR between ozone exposure (mean D-3, D-4) and large artery stroke subgroup was 1.080 (95% CI 1.002 to 1.166). No significant association was observed with

other pollutants than ozone. Adjusted in two-pollutant models, OR were not affected. Using ozone quartiles (1st quartile as the reference group), linear dose-response relationship for both subgroups was observed (Figure 1). Baseline characteristics in recurrent stroke subgroup and large artery stroke subgroup were shown in Figure 2.

DISCUSSION

Our study assessed the short-term effect of ozone exposure on selected population of ischemic stroke in a city specially polluted by ozone. An elevation of 10 µg/m^3 of ozone concentration increases stroke risk with few days lag in recurrent (≈12%) and large artery stroke (≈8%) subgroups only. Linear dose-response relation was observed systematically in both groups. In these groups, the common feature of the patients was that they cumulate vascular risk factors. No significant association was found between all ischemic stroke groups and atmospheric pollutants studied (O_3, NO_2, SO_2 and PM10).

Several studies have investigated the association between outdoor air pollution and stroke [3, 5-24]. Results of these studies are conflicting and hamper generalization of conclusions. Heterogeneous methodological considerations are the main explanation of this conflict. Methodological differences are observed in patient selection, study design, outcomes choice (incidence, hospital admission, mortality), and assessment of individual exposure to selected pollutants [20]. Few published studies investigated specially the association between occurrence of ischemic stroke and ozone exposure using case crossover design [8, 15, 17, 19, 21] or time series analysis method [3, 12, 16, 22]. Consistent with our results, the majority of these studies do not observe relationship between ozone exposure and occurrence of ischemic stroke [3, 8, 17, 19, 21, 22]. Whenever relationship was revealed, association was borderline significant [16], or not confirmed by a second study on the same area of investigation [15, 19]. Despite the fact that the link between ischemic stroke and ozone exposure is not obvious,

results in subgroup analyses seem to identify a population at risk for ozone exposure. In recurrent ischemic stroke subgroup, a significant increase of 12.1% (95% CI 1.5 to 23.9) in stroke risk was observed for each increase of 10 µg/m^3 of ozone concentration in previous days (mean D-1, D-2, D-3 lag). Consistent with this result, population-based study in Dijon (France), revealed the same association (OR: 1.150; 95% CI 1.027 to 1.209) with 3 days lag [19]. Similarly, a significant association was observed in large artery stroke subgroup (mean D-3, D-4, OR: 1.080; 95% CI 1.002 to 1.166). This link was observed in previous study (Dijon) especially in this stroke etiological subgroup [15]. Associations in other ischemic stroke subgroups are not systematically confirmed (age, gender, vascular risk factors, and season) [3, 15, 19, 21, 22]. Our study confirms the short-term effects of ozone exposure on stroke patients with high vascular risk [15, 19].

Our findings suggest that exposure to ozone, main photochemical pollutant, could increase the risk of ischemic stroke in population subgroups (recurrent stroke, large arteries stroke) particularly exposed to vascular risks factors inducing atherosclerosis. Physiopathological pathways linking ischemic stroke and ozone exposure still remain largely unclear and probably complex. Some studies support a delayed effect (1 to 3 days lag) between acute exposure of ozone pollution and stroke onset [15, 19]. Ozone urban pollution effects on healthy subjects are associated with systemic inflammatory responses, oxidative stress, blood coagulation [25, 26]. These acute phenomena induced by even low levels of ozone could be the trigger of ischemic event consecutively to atherosclerotic plaque instability, alterations in endothelial function, and increased coagulation and thrombosis [27]. As suggested by Henrotin et al., we hypothesized that short term effect of ozone exposure could be involved especially among subjects with high vascular risk [19].

In order to establish a causal relationship between ozone exposure and stroke onset, we studied the exposure-response relation, main criteria identified by Hill [28]. Consistent with previous reports, we show a linear exposure-response relationship between ozone concentration and ischemic stroke in subgroups identified in previous reports [15, 19].

Our study has several limitations. The question of completeness of stroke patients living Nice in this hospital-based study was discussed. In Nice, patients with suspicion of stroke are admitted in priority in University Hospital Center. Likewise, incidence of ischemic stroke was consistent with epidemiological data in France. The question of individual exposure measurement is generally discussed. The main limit is that we used air pollution levels from air monitoring station to represent individuals' exposure. However, we limited our investigations in small geographic area (72 km^2) not considered as a polluted town except for ozone (median 53.3 [32.6-69.2] $\mu g/m^3$). Moreover, in the stroke population studied, elderly patients are mostly retired and have daily activity in study area. Since ozone concentration is correlated with meteorological parameters, temperature and humidity were incorporated into our models. Association between ozone pollution and stroke can be confounded by other pollutants studied, especially particles. Effects of ozone alone are not modified using adjusted models for each of the other pollutants (NO2, SO2 and PM10). PM2.5 was not studied because not monitored in Nice.

SUMMARY

The consequences of ozone pollution on respiratory system and mortality are well documented [1]. Our results confirm the relationship between low level ozone exposure and ischemic stroke in high vascular risk subgroup with linear exposure-response relation, independently of other pollutants and meteorological parameters. Reproducibility of previous results is one of the main Hills criterion to induce causality of ozone exposure. Even if the

individual risk is low, to identify an association between ozone and ischemic stroke incidence is important from a public health point of view, since a large population is concerned. Physiopathological processes underlying this association between ischemic stroke and ozone exposure remain to be investigated.

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Figure Legends

Figure 1: Dose relationship between ozone and ischemic stroke events (1A Recurrent ischemic stroke subgroup, 1B Large artery ischemic stroke subgroup).

Figure 2: Baseline characteristics according recurrent stroke subgroup (A) and large artery stroke subgroup (B) (*: $p<.05$).

Tables

	All patients (n=1729)	Incident n=1449 (83.81%)	Recurrent n=280 (16.19%)	p
Demographic data				
Men	808 (46.73%)	683 (47.14%)	125 (44.64%)	0.0044
Age	76.06±14.04	75.48±14.29	79.01±12.33	<0.0001
< 55 years	141 (8.16%)	132 (9.11%)	9 (3.21%)	0.0011
55 to 64 years	186 (10.76%)	155 (10.70%)	31 (11.07%)	0.8532
65 to 74 years	324 (18.74%)	279 (19.25%)	45 (16.07%)	0.2114
75 to 84 years	524 (30.31%)	438 (30.23%)	86 (30.71%)	0.8712
≥ 85 years	554 (32.04%)	445 (30.71%)	109 (38.93%)	0.0071
Cardiovascular risk factors				
Diabetes mellitus	311 (17.99%)	249 (17.18%)	62 (22.14%)	0.0481
Hypertension	998 (57.72%)	803 (55.42%)	195 (69.64%)	<0.0001
Dyslipidemia	441 (25.51%)	348 (24.02%)	93 (33.21%)	0.0012
Current smoker	410 (23.71%)	357 (24.64%)	53 (18.93%)	0.0398
Overweight	226 (13.07%)	204 (14.08%)	22 (7.86%)	0.0047
Coronary artery disease	263 (15.21%)	209 (14.42%)	54 (19.29%)	0.0381
Atrial fibrillation	527 (30.48%)	433 (29.88%)	94 (33.57%)	0.2198
Classification of stroke etiologic subtypes (TOAST)				
Large artery	578 (33.43%)	479 (33.06%)	99 (35.36%)	0.4552
Cardioembolic	563 (32.56%)	469 (32.37%)	94 (33.57%)	0.6938
Lacunar stroke	153 (8.85%)	129 (8.90%)	24 (8.57%)	0.8582
Other determined etiology	43 (2.49%)	40 (2.76%)	3 (1.07%)	0.0966
Undetermined etiology	392 (22.67%)	332 (22.91%)	60 (21.43%)	0.5872
Hospitalization in stroke unit	620 (35.86%)	546 (37.68%)	74 (26.43%)	0.0003

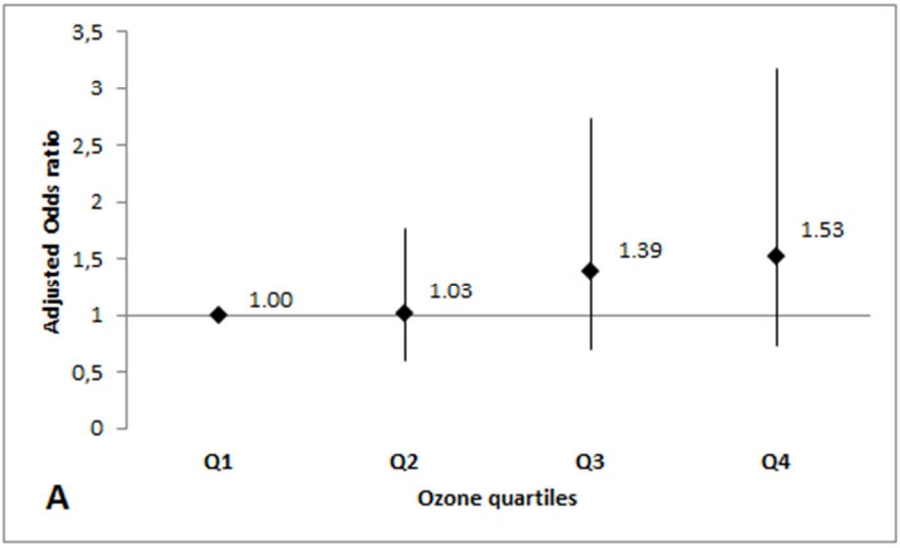
Table 1: Baseline characteristics of patients with ischemic stroke (incident and recurrent) hospitalized in Nice University Hospital from 2007 to 2011.

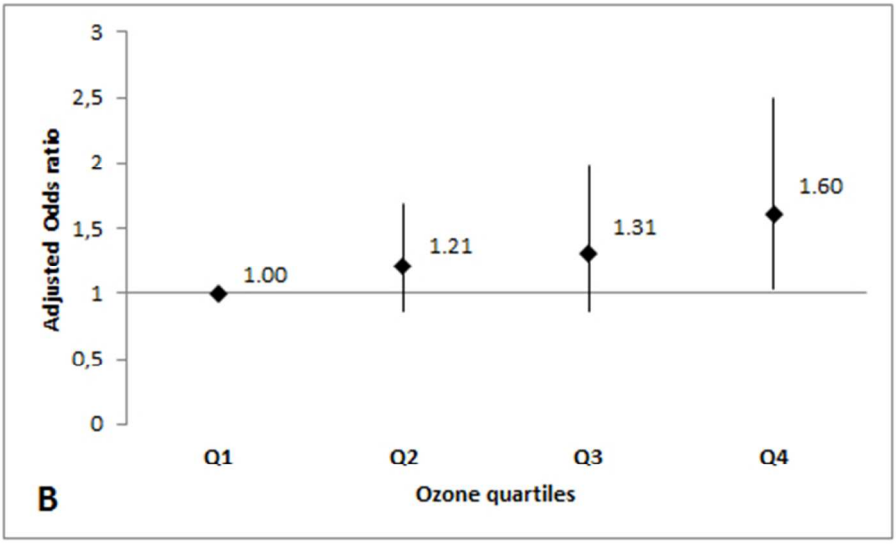
	Mean	SD	Minimum	Quartile 1	Median	Quartile 3	Maximum
Ozone daily 8-hours average (µg/m3)	80.74	31.78	4.63	54.75	84.38	105.38	157.27
Ozone daily 1-hour maximum (µg/m3)	92.37	31.32	7.00	69.00	94.00	115.00	197.00
Ozone 24-hours average (µg/m3)	52.20	22.89	4.00	32.58	53.29	69.02	111.13
PM10 (µg/m3)	28.48	9.81	1.00	22.00	28.00	34.00	74.00
NO2 (µg/m3)	26.22	8.69	3.00	20.00	25.00	32.00	59.00
SO2 (µg/m3)	1.23	1.18	0.00	0.00	1.00	2.00	10.00
Minimum temperature (°C)	13.02	5.98	-1.60	7.80	12.90	18.20	25.90
Maximum humidity (%)	81.40	9.07	40.00	76.00	83.00	88.00	97.00

Table 2: Distribution of air pollution concentrations and meteorological parameters in Nice (France) between 2007 to 2012.

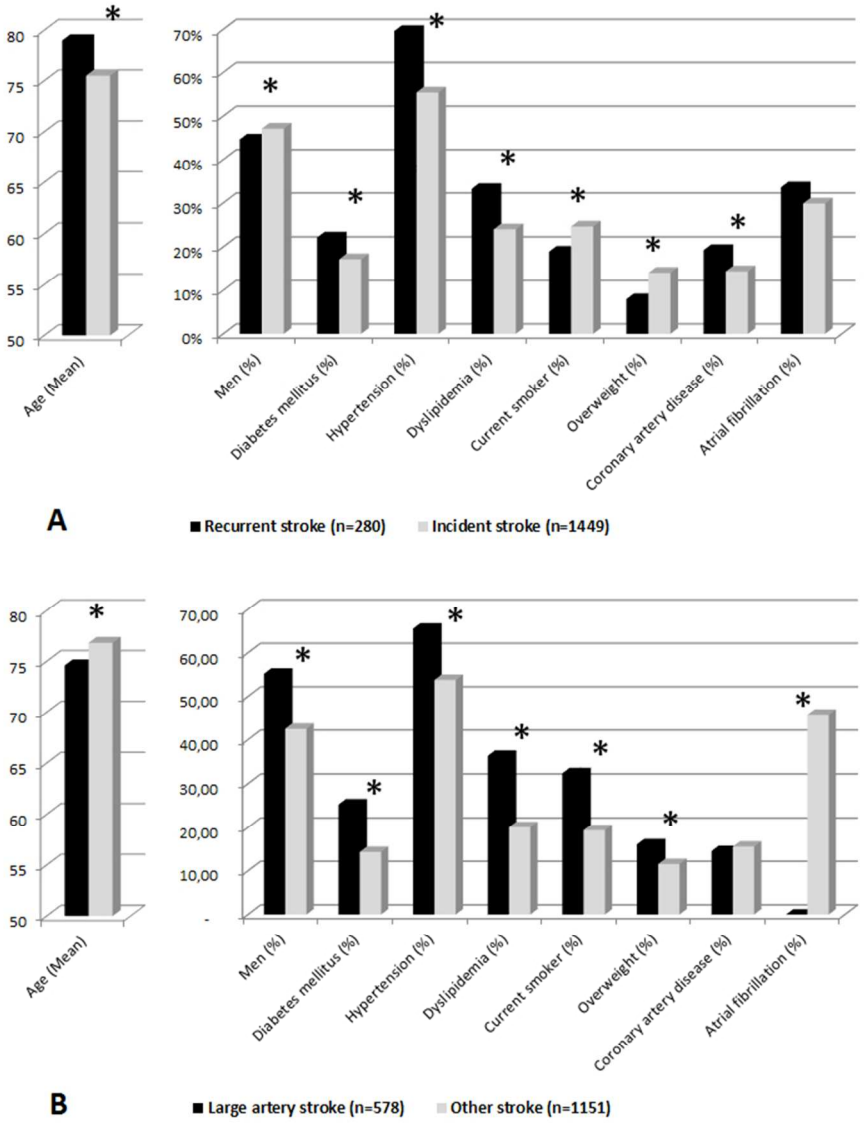
			All ischemic stroke (n=1729)					Recurrent stroke (n=280)					Large artery stroke (n=578)				
			Ora	95% IC			p	Ora	95% IC			p	Ora	95% IC			p
Ozone	D-1	8h average	0.9917	0.9584	to	1.0261	0.633	1.0899	1.0009	to	1.1867	0.047	0.9697	0.9119	to	1.0310	0.326
		1h maximum	0.9957	0.9644	to	1.0281	0.795	1.0641	0.9824	to	1.1527	0.127	0.9881	0.9334	to	1.0460	0.682
		24h average	1.0036	0.9578	to	1.0517	0.877	1.0793	0.9616	to	1.2115	0.195	0.9983	0.9190	to	1.0843	0.968
	D-2	8h average	0.9976	0.9657	to	1.0306	0.888	1.0957	1.0086	to	1.1903	0.030	0.9903	0.9347	to	1.0491	0.741
		1h maximum	1.0040	0.9738	to	1.0351	0.795	1.0955	1.0144	to	1.1831	0.020	0.9979	0.9457	to	1.0529	0.940
		24h average	1.0015	0.9598	to	1.0451	0.942	1.0638	0.9587	to	1.1804	0.244	1.0097	0.9365	to	1.0885	0.801
	D-3	8h average	0.9987	0.9670	to	1.0314	0.939	1.0601	0.9784	to	1.1487	0.154	1.0261	0.9703	to	1.0852	0.366
		1h maximum	0.9968	0.9671	to	1.0273	0.836	1.0380	0.9635	to	1.1184	0.326	1.0254	0.9728	to	1.0808	0.349
		24h average	1.0046	0.9644	to	1.0466	0.822	1.0838	0.9788	to	1.2000	0.122	1.0519	0.9802	to	1.1289	0.160
	D-4	8h average	1.0067	0.9751	to	1.0393	0.681	1.0169	0.9395	to	1.1006	0.678	1.0359	0.9808	to	1.0941	0.205
		1h maximum	0.9978	0.9684	to	1.0280	0.887	1.0038	0.9321	to	1.0811	0.918	1.0290	0.9777	to	1.0829	0.272
		24h average	1.0114	0.9711	to	1.0534	0.583	1.0248	0.9260	to	1.1342	0.635	1.0787	1.0065	to	1.1561	0.032
PM10	D-1		1.0143	0.9518	to	1.0806	0.659	1.0041	0.5586	to	1.7995	0.989	1.0347	0.9282	to	1.1527	0.536
	D-2		0.9861	0.9238	to	1.0523	0.674	0.9518	0.8106	to	1.1167	0.545	0.9350	0.8349	to	1.0464	0.242
	D-3		0.9788	0.9203	to	1.0405	0.493	1.0047	0.8532	to	1.182	0.955	0.9436	0.8475	to	1.0501	0.288
	D-4		0.9780	0.9202	to	1.0391	0.473	0.9911	0.8520	to	1.152	0.908	0.9544	0.8572	to	1.0620	0.392
NO2	D-1		1.0307	0.9367	to	1.1336	0.533	0.8960	0.7689	to	1.0434	0.158	1.0293	0.8699	to	1.2169	0.735
	D-2		0.9931	0.9029	to	1.0918	0.887	0.9427	0.7403	to	1.1991	0.631	1.0494	0.8894	to	1.2372	0.565
	D-3		0.9462	0.8607	to	1.0396	0.250	1.1262	0.8767	to	1.4449	0.349	0.9147	0.7743	to	1.0796	0.292
	D-4		0.9462	0.8607	to	1.0396	0.250	0.8931	0.7047	to	1.1306	0.348	0.9147	0.7743	to	1.0796	0.292
SO2	D-1		1.0069	0.5986	to	1.6893	0.979	0.653	0.164	to	2.5822	0.544	1.0789	0.4507	to	2.5712	0.864
	D-2		0.8763	0.5138	to	1.4905	0.626	0.8916	0.2525	to	3.1284	0.858	1.1784	0.4790	to	2.8858	0.719
	D-3		1.2539	0.7405	to	2.1174	0.397	0.7231	0.1983	to	2.6188	0.622	0.9351	0.3630	to	2.3973	0.889
	D-4		1.4852	0.8956	to	2.4567	0.123	1.3587	0.3735	to	4.9101	0.640	1.6564	0.7140	to	3.8260	0.237

Table 3: Adjusted odds ratios between ischemic stroke and outdoor pollutants exposure for an increase of 10 µg/m3 in Nice (France) between 2007 to 2011.





Dose relationship between ozone and ischemic stroke events (1A Recurrent ischemic stroke subgroup, 1B Large artery ischemic stroke subgroup).
45x54mm (300 x 300 DPI)



Baseline characteristics according recurrent stroke subgroup (A) and large artery stroke subgroup (B) (*: p<.05).
62x81mm (300 x 300 DPI)

	Ozone (8h average)	Ozone (1h maximum)	Ozone (24h average)	PM10	NO2	SO2	Temperature (minimum)	Humidity (Maximum)
Ozone (8h average)	1.000							
Ozone (1h maximum)	0.980	1.000						
Ozone (24h average)	0.954	0.927	1.000					
PM10	0.100	0.140	0.009	1.000				
NO2	-0.472	-0.421	-0.538	0.251	1.000			
SO2	-0.075	-0.068	-0.069	0.109	0.148	1.000		
Temperature (minimum)	0.680	0.672	0.670	0.094	-0.535	-0.096	1.000	
Humidity (maximum)	-0.068	-0.072	-0.099	-0.064	-0.122	-0.010	0.085	1.000

Data supplement Table I: Spearman correlation between air pollutant level and meteorological variables.