ORIGINAL ARTICLE

Effect of nitrogen dioxide and ozone on the risk of dying in patients with severe asthma

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Background: A study was performed to assess the acute association between air pollution, pollen and spores, and mortality in a population based cohort of subjects with asthma recruited from emergency room admissions for an asthma exacerbation using a case crossover design.
Methods: Patients in Barcelona aged over 14 years who died during the period 1985–95 who had visited the emergency department of one of the four largest hospitals in the city for asthma during 1985–9 were included in the study (a total of 467 men and 611 women). Deaths were identified by record linkage of the cohort individuals with the Catalonia mortality registry. Causes of death were based on the underlying cause on the death certificate. Air pollution, pollen and spore levels were measured at the city monitoring stations which provide an average for the entire city.
Results: Nitrogen dioxide was associated with mortality for all causes of death (adjusted odds ratio (OR) for an increase of the interquartile range = 1.50, 95% confidence interval (CI) 1.09 to 2.64) in asthmatic patients with more than one emergency room admission for asthma. The association was particularly strong for respiratory causes (OR 1.63, 95% CI 0.93 to 2.86). Ozone also increased the risk of death in asthmatic patients (OR 1.90, 95% CI 1.09 to 3.30) during spring and summer. The association with particles, pollen, and spores was not significant, and no interactions between air pollutants and pollen and spores were found.
Conclusion: Nitrogen dioxide and ozone may exacerbate severe asthma and even cause death among asthmatic subjects.

The role of air pollution in asthma exacerbations is less consistent than that found for chronic obstructive pulmonary disease (COPD). In a comprehensive review of all studies on hospital admissions for asthma and acute effects of daily levels of air pollution, Anderson *et al* found inconclusive results both in adults and children.[1] Similarly, the largest panel study ever carried out (the PEACE study)—which involved 28 panels in nine countries and included 2010 children with respiratory symptoms—did not find any association with symptoms, function or medication,[2] which contradicts the findings of previous panel studies in asthmatic patients in relation to particles and ozone (O_3).[3 4]

One of the explanations for this inconsistency is a lack of statistical power of a rare phenomenon such as daily asthma events. The European multicentre studies Air Pollution Health Effects Approach (APHEA I)[5] which included several cities and the larger new study (APHEA II)[6] have overcome this limitation and reported an association between particle and nitrogen dioxide (NO_2) levels and daily admissions for asthma in adults, and particles, NO_2, and sulfur dioxide (SO_2) levels in children, and an association with O_3 in some areas.

A different explanation for the inconsistent results concerns the differences between cities in the pollution mixtures, particularly the confounding or synergistic role of pollen and spores. Some studies have addressed the potential for a confounding role by pollen and spores and have consistently found a lack of confounding effect on the association between air pollution and asthma admissions[1 7] or symptoms.[8–12] Pollen has rarely been associated with asthma symptoms,[13] while spore counts have consistently been found to affect the symptoms of asthma.[9–12 14] Experimentally, there is firm support for the hypothesis that O_3, NO_2, SO_2, and particles modulate allergic responses in asthmatic subjects,[15] which has been observed for O_3[16–18] and NO_2 in human volunteers.[18–22] However, only a few studies in real air have analysed the synergism between air pollutants and pollen[1] and fungal spores[9–12] in exacerbations of asthma.

A final possible explanation for the inconsistent results arises from the role of susceptibility factors (such as severity[23]) that may enhance the effect of pollutants on asthma. Most of the experimental and epidemiological studies in asthmatics have included subjects with moderate asthma, whereas severe asthmatics appear to be much more susceptible to air pollution effects.

We have analysed the interrelations between urban air pollutants, pollen, and spores in a cohort of asthmatic subjects using a case crossover design The fact that the unit of observation was the individual allowed us to explore which characteristics conferred a major susceptibility to air pollution. Death was the outcome variable. It is not a sensitive outcome of the environmental effects in asthma exacerbations, but it is a very specific indicator in a case crossover approach since cases serve as their own controls which eliminates the confounding by fixed characteristics such as treatment and medical control. Our objective was to assess the acute association between air pollution, pollen and spores, and mortality in a population based cohort of subjects recruited from those admitted to the emergency department for an exacerbation of asthma.

METHODS

Persons living in Barcelona aged over 14 years who attended the emergency department for asthma between 1985 and 1989 were included in the study. Data were collected from the clinical records of the four largest urban hospitals which covered about 80% of all Barcelona asthma emergency department visits.[24] Visits recorded at other hospitals in the city did not differ in terms of social or demographic characteristics. Physicians reviewed the clinical records of all daily visits and selected those with labels in the diagnostics section of the clinical record which matched a list drawn up by a panel of chest physicians. Using this information and, if necessary,

Table 1 Characteristics of subjects (n (%)) admitted for asthma to the emergency department in 1985–9 who died during 1985–95, according to number of admissions and diagnosis

	Only one admission		More than one admission			
	Asthma		Asthma		Asthma/COPD	
	Male	Female	Male	Female	Male	Female
Subjects admitted to emergency department for asthma (1985–9)	1274	2221	399	741	537	438
Asthmatics who died (1985–95) among those admitted to emergency department in 1985–9	183 (14)	327 (15)	31 (8)	92 (12)	253 (47)	192 (44)
Age						
<45	29 (16)	16 (5)	10 (32)	5 (5)	9 (3)	1 (1)
45–64	46 (25)	53 (16)	12 (39)	28 (31)	63 (25)	20 (10)
65–79	73 (40)	112 (34)	7 (23)	39 (42)	128 (51)	92 (48)
≥80	35 (19)	146 (45)	2 (6)	20 (22)	53 (21)	79 (41)
Severity of emergency department visits						
Treated and released within 24 h	105 (59)	155 (49)	19 (61)	51 (55)	124 (49)	76 (40)
Any admission to hospital	70 (39)	155 (49)	12 (39)	41 (45)	127 (51)	111 (59)
Any admission to ICU	3 (2)	7 (2)	0 (0)	0 (0)	0 (0)	2 (1)
Cause of death (ICD-9)						
Cardiovascular (390-459)	36 (20)	123 (38)	8 (26)	26 (28)	48 (19)	61 (32)
Myocardial infarction (410-414)	14 (8)	28 (9)	4 (13)	4 (4)	15 (6)	11 (6)
Respiratory (460-519)	50 (27)	81 (25)	9 (29)	40 (43)	114 (45)	82 (43)
COPD (490-492, 494-496)	25 (14)	34 (10)	1 (3)	8 (9)	84 (33)	41 (21)
Asthma (493)	13 (7)	34 (10)	7 (23)	29 (32)	17 (7)	29 (15)
Number of emergency department visits/1000 days						
≥2.26 (median)	53 (29)	100 (31)	15 (48)	31 (34)	168 (66)	114 (59)
<2.26	130 (71)	227 (69)	16 (52)	61 (66)	85 (34)	78 (41)
Season of death						
April–September (summer)	88 (48)	151 (46)	17 (55)	48 (52)	117 (46)	84 (44)
October–March (winter)	95 (52)	176 (54)	14 (45)	44 (48)	136 (54)	108 (56)

other data in the clinical record, they classified the emergency visit as asthma, chronic obstructive pulmonary disease (COPD), or other respiratory causes as explained elsewhere.[24] Subjects with bronchospasm or an attack of shortness of breath and a concomitant diagnosis of COPD were classified as COPD. Their name and both surnames, age, sex, address, and details of referral or discharge were recorded. Data on treatment were not collected. Entry to the cohort was episode based—that is, a visit to the emergency department for asthma.

Deaths were identified by record linkage of the cohort individuals with the Catalonia mortality registry for the years 1985–95.[24] Fields used for linkage were full name, sex, and year of birth. Those not found in the mortality registry were considered alive at the end of the study period (31 December 1995). Causes of death were based on the underlying cause in the death register and classified according to the ICD-9. A validity study of the goodness of the linkage was carried out in 326 asthmatic subjects contacted by telephone during 1996. Linkage classified death correctly in 98% of cases. The linkage was able to classify as deaths 93% (39 of 42) of those who had died and 99% (282 of 284) of those who were alive.

Air pollution exposure was measured at the city monitoring stations which provide a mean for the entire city. Details of the monitoring sites (the mean of three stations), the measurement methods, and the indicators can be found elsewhere.[25] The 24 hour mean levels of particulate matter (PM_{10}) were measured using the beta-radiation method. Daily and hourly levels of NO_2 and O_3 were measured using a chemiluminescence method and carbon monoxide (CO) by infrared absorption. The 24 hour mean levels of black smoke were assessed by the reflectometry method in a network of 15 samplers. Pollen and spores were measured by the Cour method[26] which provides mean weekly particle concentrations. The measurements are highly accurate for pollen grains, but some fungal spores become damaged during the treatment so fungal spore counts are underestimates. The pollen and spore data were obtained at a single site in the north east of the city. To establish the week number, the international convention that the first week of the year is the one that contains the first Thursday has been followed.

Analysis of data

The analysis followed a case crossover procedure[24] and was limited to those who had a diagnosis of asthma during the period 1985–9 (n=5610). The study included those who had died during the period 1985–95 (1080 of 5610). The analysis was stratified according to the number of admissions and diagnosis since a high proportion of subjects who were admitted more than once received diagnoses of both asthma and COPD at different visits because of the difficulty in diagnosing asthma in elderly subjects and the diagnostic limitations of the emergency department.

The lag structure of the air pollutants was the cumulative lag over the two previous days—that is, the mean of the same and the previous two days. Mean weekly pollen and spore measurements had a non-normal distribution skewed to the right and were logarithmically transformed.

Adjusted odds ratios were obtained by comparing air pollution, pollen and spore levels on the dates of deaths with the levels during the control period using multivariate conditional logistic regression in a model which also including daily values of the confounding variables (temperature, humidity, hot days, influenza epidemics, and soybean asthma epidemics). The selection of the control period dates followed a recent proposal by Levy *et al*[27] which showed that this strategy produces unbiased estimation. It consists of dividing time into strata defined a priori and using the remainder of eligible days in each stratum as referents. In our case the stratification was done on day of the week and calendar month—for example, a case on Thursday 20 July was compared with all other Thursdays in July. This design controls for day of the week by design and avoids short term autocorrelation because the periods are separated by 7 days. To analyse the interaction between air

Table 2 Distribution of air pollutants, pollen and spores

	Years	Min	p25	p50	p75	Max
Daily values (μg/m³)						
PM_{10}	1991–5	17.3	47.6	61.2	80.3	240.7
Black smoke	1985–95	10.6	29.2	40.0	54.8	258.0
NO_2 (1 h)	1986–95	8.0	71.9	89.7	112.4	339.2
NO_2 (24 h)	1986–95	5.2	41.1	52.3	64.0	141.8
O_3 (1 h)	1986–95	6.6	46.0	69.3	94.0	283.4
O_3 (8 h)	1986–95	3.9	31.8	54.4	79.0	244.5
SO_2	1985–95	2.2	11.7	18.8	39.2	160.0
CO (ng/m³)	1991–5	0.6	4.9	7.7	12.1	66.0
Weekly values (counts/m³)						
Total pollen	1985, 1988–94	0.27	11.28	40.09	120.06	3740.1
Gramineas	1985, 1988–94	0	0.27	1.11	4.10	92.70
Spores	1990–4	0.66	7.20	19.74	38.23	518.4
Cladosporium	1990–4	0	0	0.14	1.82	120.0
Alternaria	1990–4	0	0	0	0	6.15
Epicoccum	1990–4	0	0	0	0	2.57
Helminthosporium	1990–4	0	0	0	0	0

pollutant and pollen and spores, weekly values of pollen and spores were used, assuming that the value at the day of death was the value of the week of death. Interaction was assessed by including the multiplicative term in the multivariate model. Analyses were carried out using Stata software, release 6.0 (StataCorp, College Station, TX, USA).

RESULTS

Most of the asthmatic patients were admitted only once to an emergency department (table 1). A smaller proportion of subjects admitted only once to an emergency department (15%) or admitted more than once but always with a diagnosis of asthma (11%) had died during the follow up period than those admitted more than once and those with diagnoses of both asthma and COPD (46%). A total of 467 men and 611 women died and were included in the analysis (table 1). Among those who died, women were older than men and had a higher proportion of admissions to hospital, suggesting a more severe asthma crisis. Subjects admitted more than once and always with a diagnosis of asthma were younger and with less severe admissions. The main causes of death were cardiovascular in women and respiratory in men admitted only once, and respiratory in men and women admitted more than once. Deaths from asthma were more frequent among subjects admitted to the emergency department more than once and always for asthma. The number of admissions to the emergency department in a given time during the years 1985–9 was higher for those admitted more than once for asthma. Finally, those admitted only once or with a concomitant diagnosis of COPD tended to die in winter while those with repeated admissions for asthma were more likely to die in summer.

Air pollution levels, pollen and spore counts are shown in table 2. Both measures of NO_2 (maximum hourly daily mean and daily average) had a strong correlation (r=0.88), as did both measures of O_3 (maximum hourly and average of 8 hours daily mean) (r=0.99). Pollen levels had a very strong seasonal pattern; total pollen peaked in February and March (and Gramineas in May) and became almost zero from August to January, while levels of spores were more constant with maximum levels in March and lowest levels in July.

Table 3 Association† (odds ratio and 95% confidence interval) between mortality and air pollutant, pollen and spores in patients with asthma, stratified according to number of emergency department admissions. Each odds ratio comes from a different model

	Interquartile range (μg/m³)	Only 1 admission	More than 1 admission	
		Asthma	Asthma	Asthma/COPD
Daily values (μg/m³)‡				
PM_{10}	32.7	0.884 (0.672 to 1.162)	1.084 (0.661 to 1.778)	1.011 (0.746 to 1.368)
Black smoke	25.6	1.109 (0.902 to 1.365)	1.184 (0.783 to 1.790)	1.126 (0.910 to 1.394)
NO_2 (1 h)	40.5	1.039 (0.859 to 1.257)	1.483 (1.031 to 2.134)*	1.055 (0.872 to 1.278)
NO_2 (24 h)	22.9	1.056 (0.872 to 1.279)	1.579 (1.063 to 2.344)*	1.024 (0.839 to 1.245)
O_3 (1 h)	48.0	1.096 (0.820 to 1.466)	1.688 (0.978 to 2.643	0.946 (0.695 to 1.288)
O_3 (8 h)	47.2	1.080 (0.787 to 1.483)	1.555 (0.892 to 2.712)	0.946 (0.674 to 1.326)
SO_2	27.5	1.142 (0.809 to 1.611)	1.480 (0.528 to 4.152)	1.193 (0.832 to 1.712)
CO	7.2	1.127 (0.895 to 1.418)	1.125 (0.773 to 1.638)	0.815 (0.614 to 1.082)
Weekly values (counts/m³)				
Total pollen¶		0.756 (0.565 to 1.011)	1.388 (0.723 to 2.663)	1.207 (0.893 to 1.631)
Gramineas¶		0.833 (0.616 to 1.126)	1.064 (0.572 to 1.979)	1.190 (0.880 to 1.609)
Total spores¶		0.875 (0.609 to 1.258)	1.024 (0.462 to 2.272)	1.079 (0.703 to 1.654)
Cladosporium¶		0.998 (0.817 to 1.219)	0.951 (0.649 to 1.394)	0.904 (0.737 to 1.111)
Alternaria (>0)		0.866 (0.594 to 1.264)	1.185 (0.601 to 2.336)	1.323 (0.880 to 1.989)
Epicoccum (>0)		0.860 (0.423 to 1.747)	0.954 (0.247 to 3.752)	1.392 (0.710 to 2.727)

*p<0.05.
†Adjusted for asthma epidemics, temperature, humidity, hot days, and influenza epidemics.
‡Odds ratio for the interquartile difference.
¶Odds ratio for a 10-fold increase.

Table 4 Association (odds ratio (95% CI)† between daily hourly maximum levels of nitrogen dioxide and ozone with mortality by subject's characteristics, season and cause of death

	Nitrogen dioxide			Ozone		
	Only 1 admission	More than 1 admission		Only 1 admission	More than 1 admission	
	Asthma	Asthma	Asthma/COPD	Asthma	Asthma	Asthma/COPD
Mortality for all causes						
Male	1.037 (0.731 to 1.472)	1.172 (0.581 to 2.363)	0.876 (0.668 to 1.147)	1.234 (0.799 to 2.980)	1.115 (0.417 to 2.980)	0.858 (0.563 to 1.162)
Female	1.042 (0.831 to 1.308)	1.617 (1.055 to 2.479)*	1.292 (0.983 to 1.699)	1.033 (0.723 to 1.476)	1.823 (1.019 to 3.2.74)*	1.062 (0.605 to 1.678)
p for interaction	0.90	0.45	0.05	0.59	0.38	0.50
Age						
⩽65 years	0.903 (0.637 to 1.279)	1.783 (0.992 to 3.207)	1.669 (1.063 to 2.623)*	0.625 (0.354 to 1.104)	2.378 (1.075 to 5.265)*	1.442 (0.700 to 2.973)
>65 years	1.120 (0.880 to 1.413)	1.329 (0.827 to 2.134)	0.948 (0.763 to 1.170)	1.360 (0.970 to1.909)	1.235 (0.649 to 2.388)	0.856 (0.603 to 1.205)
p for interaction	0.29	0.44	0.03	0.02	0.21	0.20
Severity of emergency department visits						
Released within 24 h	1.067 (0.828 to 1.382)	1.417 (0.856 to 2.346)	1.267 (0.957 to 1.679)	1.101 (0.742 to 1.625)	1.636 (0.867 to 3.242)	0.694 (0.439 to 1.097)
Hospital referral	1.041 (0.781 to 1.386)	1.570 (0.919 to 2.682)	0.899 (0.684 to 1.125)	1.043 (0.667 to 1.632)	1.525 (0.711 to 3.268)	1.201 (0.789 to 1.825)
p for interaction	0.89	0.81	0.10	0.88	0.78	0.08
Number of emergency department visits/1000 days						
⩾2.26 (median)	1.028 (0.767 to 1.376)	1.853 (1.118 to 3.070)*	1.133 (0.908 to 1.414)	1.075 (0.616 to 1.873)	2.427 (1.081 to 5.450)*	0.960 (0.660 to 1.398)
<2.26	1.018 (0.798 to 1.298)	1.115 (0.648 to 1.920)	0.852 (0.087 to 1.236)	1.067 (0.760 to 1.497)	1.186 (0.629 to 2.238)	0.901 (0.525 to 1.547)
p for interaction	0.96	0.18	0.20	0.98	0.17	0.85
Season of death						
April-September	1.156 (0.897 to 1.489)	1.746 (1.075 to 2.838)*	1.049 (0.812 to 1.356)	1.018 (0.726 to 1.426)	1.900 (1.093 to 3.302)*	1.048 (0.732 to 1.501)
October-March	0.903 (0.679 to 1.202)	1.190 (0.674 to 2.099)	1.049 (0.791 to 1.329)	1.359 (0.761 to 2.427)	0.702 (0.200 to 2.465)	0.697 (0.381 to 1.277)
p for interaction	0.20	0.33	0.88	0.42	0.17	0.25
Cause of death						
Respiratory	0.879 (0.617 to 1.251)	1.630 (0.929 to 2.861)	0.974 (0.712 to 1.331)	0.695 (0.389 to 1.240)	1.792 (0.848 to 3.783)	0.836 (0.509 to 1.278)
Cardiovascular	1.374 (0.978 to 1.930)	1.293 (0.636 to 2.672)	1.337 (0.963 to 1.875)	1.397 (0.854 to 2.285)	1.331 (0.529 to 3.349)	0.985 (0.521 to 1.861)

*p<0.05.
†Adjusted for asthma epidemics.

Table 5 Association (odds ratio (95% CI) for interquartile change) between nitrogen dioxide and ozone and mortality, controlling for pollen and spores

	More than one admission: always asthma*	
	Nitrogen dioxide	Ozone
Not adjusted	1.688 (1.074 to 2.652)	1.755 (0.984 to 3.133)
Adjusted for:		
Total pollen	1.655 (1.057 to 2.621)	1.736 (0.971 to 3.102)
Gramineas	1.689 (1.074 to 2.655)	1.758 (0.984 to 3.142)
Total spores	1.599 (0.911 to 2.809)	1.931 (0.975 to 3.825)
Cladosporium	1.598 (0.904 to 2.826)	1.964 (0.990 to 3.896)
Alternaria	1.610 (0.916 to 2.827)	1.949 (0.968 to 3.928)

*Adjusted for epidemic days. Conducted in years 1985 and 1988–94 for the non-adjusted model and after including pollen; and for the years 1990–4 after including spores.

The association between air pollutants and mortality for all causes was stronger in subjects admitted more than once to the emergency department for asthma than for asthmatic subjects admitted only once or for those admitted for both asthma and COPD (table 3). The association was statistically significant for NO_2 ($p<0.05$) and at the limit of significance for hourly O_3 levels ($p=0.06$). In subjects admitted only once or admitted for asthma and COPD, the associations with air pollutants were lower and non-significant. No interactions between pollutants (such as SO_2 and O_3, or SO_2 and NO_2) were observed ($p>0.4$). Pollen and spores were unrelated to mortality. Among the confounding variables, only asthma epidemics (OR 3.95, 95% CI 0.63 to 25) had a p value of <0.2. The other variables (temperature, hot days, holidays, and influenza epidemics) were unrelated to mortality in these subjects, although these variables were kept in the regression models.

Table 4 shows the association between NO_2 and O_3 and mortality according to the subject's characteristics, season, and cause of death. Few statistically significant interactions were observed. In subjects with more than one admission and with diagnoses of both asthma and COPD, women and young subjects had a higher risk of dying in relation to NO_2 than men and older people. Among those admitted only once for asthma, old people were at higher risk of dying in relation to O_3. In general, women and subjects with more severe asthma—that is, more admissions per 1000 days—were at higher risk, although not all interactions were statistically significant. The association with NO_2 and O_3 was stronger in the warm season (from April to September) in subjects with more than one admission for asthma. Thus, the association with O_3 in summer is much stronger during the warm season when O_3 levels are higher, although the interaction was not statistically significant. In subjects admitted more than once for asthma only the association between respiratory causes of death and NO_2 and O_3 was strongest, although there was also an increase with cardiovascular causes, while in the other groups the association was stronger for cardiovascular than for respiratory causes. When the cause of death was asthma, the associations were not statistically significant. The results obtained with the other measures of NO_2 (such as NO_2-24 h) and O_3 (such as O_3-8 h) were very similar.

The associations between NO_2 and O_3 with mortality were not confounded by pollen and spores (table 5). After adjusting for spores, the OR was only slightly reduced (by 5%). The increase in the confidence intervals was due to the smaller number of observations after adjusting for spores. Adjustment for the other pollutants such as particles did not confound the association with either NO_2 or O_3 (for example, OR for NO_2 after adjustment for black smoke = 1.53, 95% CI 1.00 to 2.37). Neither pollen nor spores had a synergistic effect with NO_2 or O_3 over the whole year or after stratifying by season ($p>0.5$).

DISCUSSION

Patients with severe asthma—that is, those with more than one admission to the emergency department for an asthma exacerbation—had a higher risk of dying on days with higher levels of NO_2, regardless of the season, and O_3 in the warm season. These associations were not confounded by the weekly levels of pollen and fungal spores. No interactions between NO_2 and O_3 with pollen and spores were found. Both NO_2 and O_3 had previously been reported to be associated with admission for an asthma attack in this population[28] and in other populations,[3 6] but never with mortality in asthmatics. Some recent studies have found an acute association between O_3[29–31] (and, to a lesser extent, NO_2[32 33]) and the daily death count in the whole population. The present study suggests that asthmatics with repeated admissions to the emergency department are particularly susceptible to oxidants. Nevertheless, given the low prevalence of severe asthma, it is unlikely that this frail population accounts for the whole effect of oxidants on mortality observed in studies of the general population.

Most panel studies in asthmatic children have shown a significant effect of oxidants on lung function.[3 4] The problem, however, is that experimental and panel studies in asthmatic subjects have not been consistent.[2 3 34] Asthmatics have been found to be more susceptible to the bronchial response to NO_2 than healthy subjects, but not all subjects challenged with NO_2 (30%) fall at 0.2 ppm at rest,[35] a concentration found in many outdoor air locations.[36] Ozone produced similar decrements in lung function during exercise in asthmatic and in non-asthmatic subjects, with a high interindividual variation.[34] However, chamber studies and panel studies have only included patients with moderate asthma. The present study was based on subjects attending the emergency department for an asthma attack (one of the criteria of asthma severity), and in this population the effect of oxidants was found in those with repeated admissions due to repeated attacks. The fact that asthmatic patients with repeated admissions to the emergency department were more vulnerable to the acute effects of NO_2 and O_3 suggests that patients with severe asthma constitute a population who are particularly susceptible to air pollution.

Inconsistencies in the effects of air pollutants in patients with asthma have been attributed to the failure to include pollen and spores in the studies.[1] Pollen and fungal spores were unrelated to mortality in our study subjects. Most studies have not found an independent role for pollen in asthma exacerbations[1 8 9] except during thunderstorms (when pollen degranulates and allergenic proteins became smaller and respirable),[37] but a recent study from the Netherlands reported that the daily variation in pollen levels was related to the daily death count.[38] The fact that environmental pollen levels had a very strong seasonality with levels around zero for most days of the year and extremely high values at the end of winter and

beginning of spring poses methodological difficulties for the statistical analysis. A strategy to overcome this problem is to limit the analysis to the pollen season. Nevertheless, in our study we did not find any effect of pollen during this season. The source of the spore data used in our study (the Cour method) may explain the lack of an independent or synergistic effect of fungal spores, given the previous positive results in panel studies conducted in the USA[9–12] and a recent study in the UK.[14] The use of different sampling methods may also explain why levels of fungal spores were much lower in Barcelona than those observed in Alpine (San Diego),[18 19] in State College, Pennsylvania,[20] and in Los Angeles.[21]

Correlation between NO_2 and O_3 was rather poor in Barcelona and neither confounded the effect of the other. In studies in the general population it is generally accepted that the major air pollutants related to health effects are fine particles,[33] and that NO_2 is a surrogate for fine particles produced by motor vehicles.[36] However, the asthmatic subjects in our study were not affected by particles. In patients with asthma there is a mechanistic basis for the effect of NO_2 and O_3.[3 34] Oxidants are not highly soluble and most inhaled gas is retained in the small airways of the lungs, particularly in the case of NO_2. At high concentrations damage to epithelial cells by oxidant injury in animals and humans reduces the clearance of infecting organisms, stimulates inflammatory cell activity, and releases proinflammatory mediators.

Using the diagnosis of asthma on the death certificate, no association was found between air pollution and death from asthma. However, because the diagnosis of asthma on the death certificate is unreliable,[39] we could not discard the possibility that the mechanism of action of oxidants in causing death was through an asthma attack, since an association was found with death for all respiratory causes. If so, exposure of subjects with severe asthma to high levels of oxidants may appear as a cause of fatal asthma. A different mechanism could be through cardiovascular effects[40] which would agree with the relative risk observed for cardiovascular causes. Nevertheless, the study of the relationship between causes of death and air pollution was limited by the small number of deaths in the present study.

The case crossover design has advantages compared with the aggregated design since the observation of individuals provides a clinically meaningful reasoning, and the design potentially reduces the confounding role of variables such as medical control and treatment. However, the case crossover design is vulnerable to problems of selection of the control period. We have chosen the less biased strategy.[27] In addition, we carried out a sensitivity analysis using the other approaches proposed for selection of control days as different symmetrical ambi-directional periods[41] and found that the maximum bias produced when changing the control period accounted for 7% of the effect observed, and that the selection used is more conservative in terms of the magnitude of the standard error. Hence, we are confident that selection of controls did not bias our findings.

The diagnosis of asthma in the emergency department might be misclassified. However, the group with more than one visit which was always classified as asthma must be considered to be asthmatics. The group with a concomitant diagnosis of COPD had a stronger association with particles than with oxidants, which agrees with the findings in a cohort of patients with COPD[24] and may indicate that, in general, patients with a concomitant diagnosis of COPD really did have COPD. However, women and young subjects with a concomitant diagnosis of COPD had a higher risk of dying with increasing levels of NO_2. These subjects had a lower probability of misclassification as COPD and are probably true asthmatics.[42] If so, the stronger association of these individuals with NO_2 and O_3 would agree with the association observed in subjects with repeated admissions always diagnosed as an asthma exacerbation. This fact would reinforce the validity of a role for NO_2 and O_3 in patients with severe asthma. In addition, the stratified analysis showed a stronger association in women, patients aged less than 65 years, and those with more admissions to the emergency department always diagnosed as asthma. Again, these groups are probably true asthmatics. This strengthens the epidemiological evidence for an acute adverse effect of outdoor levels of NO_2 and O_3 on mortality in this asthmatic population.

Another limitation of this study is the use of weekly values for the biological pollutants, given the timing of their measurement. However, this is unlikely to have biased the results in a conservative way since, for air pollutants for which we had both daily and weekly values, the estimates were higher for the weekly than for the daily values (for example, OR = 1.834 and 1.950, respectively, for NO_2 and O_3 on a weekly basis). It is therefore unlikely that the lack of an association with pollen and spores was due to the timing of the measurements.

Epidemiological findings suggest that, when COPD is the cause of death, most of the mortality is displaced by only a few months except for death from cardiovascular causes.[43] There are no previous data on asthma. The fact that the relative risk was larger in young subjects suggests that the potential years of life lost due to air pollution in patients with asthma is more than a few months and is therefore a relevant public health problem.

This study provides for the first time an analysis of the acute effects of air pollution on mortality in subjects with severe asthma. The results suggest that severe asthmatics are susceptible to the adverse effects of urban air pollutants, particularly oxidants. The finding of a stronger association in a subpopulation with a specific disease that predisposes them to the effects of air pollution strengthens the evidence for a causal association. The results also suggest that oxidants may cause fatal asthma. This study was conducted at a single centre which precluded the use of a large sample and more sophisticated analysis by age, sex, and cause of death. A multicentre study using the same approach is required.

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