Environmental exposure to air pollution and allergens and peak flow changes

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ABSTRACT: Laboratory-based studies have shown that ozone and nitrogen dioxide can potentiate the effect of allergen in sensitized asthmatic subjects, but it is not known whether this interaction is important under natural exposure conditions.

Thirty-five subjects with clinical diagnoses of asthma or chronic obstructive pulmonary disease and with a provocative dose causing a 20% fall in forced expiratory volume in one second methacholine <12.25 μmol (using the Yan method) kept peak expiratory flow (PEF) records for a 4-week period during late summer, with concurrent measurement of spore and pollen counts and pollution levels. Multiple regression analysis was then used to determine the effect on PEF of aeroallergen, and of the interaction between aeroallergen and pollutant levels.

A statistically significant interaction was demonstrated between total spore count and ozone, but not nitrogen dioxide. Mean PEF fell in association with increasing spore count (same-day and 24-h lag level) and PEF variability increased with increasing spore count (24-h lag level only); both changes were greater the higher the prior ozone level.

These results suggest that ozone can potentiate the effect of aeroallergens in subjects with bronchial hyperreactivity under natural exposure conditions. However, the effect was small, and the clinical significance of the interaction requires further study.
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Air quality, particularly in inner cities, remains a high profile public health issue and episodes of high air pollution receive prominent media coverage. This public interest is supported by an expanding scientific literature. Various components of urban air including nitrogen dioxide, ozone, sulphur dioxide and acid aerosols have been shown to produce alterations in lung function in the laboratory [1–11] and epidemiological studies have shown that natural exposure to increased pollution can be associated with worsening of asthma [12–17].

Although the scientific evidence broadly indicates that pollution impairs respiratory function it contains some inconsistencies. In particular, significant effects are demonstrated at much lower pollution levels in epidemiological studies than in the laboratory. One possible explanation is that, whilst laboratory experiments generally use single agents, under natural conditions pollutants may interact with one another or with other components of the complex mixture which constitutes urban air. Interactions between combinations of pollutant gases have been tested in the laboratory. Although potentiation has been seen in some studies [18, 19] most combinations have not produced greater changes than the individual agents alone [20–24]. However it has recently been shown that exposure of sensitized asthmatic subjects to ozone in the laboratory, at levels achieved in nature, potentiates the effect of allergen inhalation [25]. Since the particles carried in ambient air include aeroallergens such as pollens and spores, this finding might explain how relatively low levels of pollution produce changes in epidemiological studies above those seen in the artificial conditions of the laboratory.

If this mechanism is important it should be possible to demonstrate the interaction between pollution and allergen in natural conditions. The authors have recently completed a study of the effects of pollution on peak expiratory flow (PEF) measurements in a panel of subjects with asthma or chronic obstructive pulmonary disease (COPD). Local measurements of pollen and spore levels are available and the authors have analysed their data for such an effect.

Methods

The study was performed in Halton Health District in Merseyside (UK). This includes the adjacent towns of Widnes and Runcorn whose centres are only one mile apart. Pollution measurements were made at monitoring sites in both towns, and allergen measurements were made at a site in the centre of Widnes.

Subject

Subjects were selected from two general practices, one in Widnes and one in Runcorn. A 1-in-4 random start systematic sample of patients with diagnoses of asthma or

COPD was taken from the practice disease registers. Entry onto the register is by virtue of a clinical diagnosis of asthma or COPD made by the patient's General Practitioner (GP). The patients selected were each sent a letter countersigned by their GP explaining the nature and purpose of the study and asking them to attend their practice surgery.

Previous analysis in this subject group has shown that the effects of pollution on lung function were only manifest in the subjects who reacted to methacholine [17]. The present analysis was therefore confined to this group.

Protocol

Written informed consent was obtained. Subjects then filled in a respiratory symptom questionnaire (based on that of the International Union against Tuberculosis) and a bronchial challenge test was performed. Subjects were shown how to complete a PEF and symptom record sheet and were asked to keep this daily record for 28 days.

The completed sheets were returned to the practice surgery from where they were collected for analysis. All subjects failing to return record sheets were contacted by telephone or home visit to retrieve the data.

Bronchial challenge tests

Bronchial challenge tests were performed using the method described by YAN et al. [26] but using equivalent doses of methacholine. Methacholine was given in increasing doses with an forced expiratory volume in one second (FEV_1) measurement 1 min after each, starting at a dose of 0.06 mmol and using double increments. The challenge test was stopped when FEV_1 fell to ≤80% of the postsaline value or when a maximum dose of 12.25 µmol methacholine had been given.

Subjects were excluded from challenge testing if they had a baseline FEV_1 <50% of their predicted value.

Serial measurements

Standard PEF technique was taught and subjects were asked to record the highest of three values on each occasion. The PEF record sheets contained spaces for recording of PEF values at 2-h intervals commencing at 02:00 h each day. Subjects were asked to make measurements within 15 min either side of the time shown on the record sheet and to omit measurements at times when they were away from Widnes or Runcorn. They were asked to make measurements for a total of 28 days.

PEF records were accepted for analysis if they included at least 5 days with two or more PEF readings. Most subjects either completed all 28 days, or only returned 1–2 days and were thus excluded.

Symptoms were recorded daily on a 10-cm visual analogue scale, grading each symptom from 0 (worst) to 10 (best). The five symptoms collected were wheeze, dyspnoea, cough, throat irritation and eye irritation.

Pollution and allergen measurement

Pollution levels were measured using the Opsis® system (Opsis, Lund, Sweden) which utilizes differential absorption spectroscopy. A beam of light at visible and ultraviolet frequencies is transmitted to a receiver ~500 m away. Since pollutant gases each absorb light at a characteristic frequency the atmospheric concentration of the selected gases can be determined. The spectrometer is linked to a computerized recording system which stores pollutant levels continuously. The system can therefore be used to determine pollution levels at a particular time point or levels can be averaged over a specified period. In this study 24 h mean levels were used.

Spore and pollen counts were obtained from a standard Burkard volumatic spore trap (Burkard Manufacturing Co. Ltd., Rickmansworth, UK) mounted centrally in the study area. The adhesive strip of the spore trap was changed daily at 16:00 h. Thus the "same-day" spore count used in the main analysis actually ran from 16:00 h the previous day to 16:00 on the index day.

Data transformation

The provocative dose causing a 20% fall in FEV_1 (PD_{20}) methacholine was calculated from the challenge test results using linear interpolation between points on the dose-response curve. Subjects were considered to be reactors if they had a PD_{20} <12.25 µmol. From the PEF records the daily mean PEF value was obtained and the daily PEF variability was calculated at the amplitude percentage mean [27]:

$$\frac{\text{Highest} - \text{lowest PEF} \times 100}{\text{mean}}$$

For further analysis log_e-transformed values of amplitude percentage mean and allergen counts were used in order to normalize the data.

Symptom data could not be satisfactorily normalized because of the high number of zero scores. The daily symptom score was therefore simply treated as negative (zero score) or positive (any other score), and further analysis conducted using logistic regression.

Regression analysis

Previous analysis in this subject group has shown that the effects of pollution on lung function were only manifest in the subjects who reacted to methacholine [17]. The present analysis was therefore confined to this group.

In order to look for pollution/aeroallergen interactions the measurements of ozone and nitrogen dioxide were divided into thirds representing low, medium and high pollution days, using the 33rd and 67th centiles of their respective distributions as cut-off points. After allowance for between-subject differences in PEF the variation in mean PEF across these three levels of pollution was calculated, and the effect on mean PEF of total spore count was examined by simple regression with spore count as a continuous variable.

To test for interactions same-day allergen levels and pollution levels from the previous day (24-h lag-level) were used. This was felt to be the closest possible approximation to laboratory experiments in which allergen exposure followed inhalation of a pollutant. The spore/pollution interaction term was added to a multiple regression model, which already included an adjustment for the differences in between-subject PEF, pollution level from the previous day, and same-day spore level.

Table 1. – Clinical characteristics of the 35 subjects included in the study

Subjects	
Male	14 (40)
Female	21 (60)
Smoking	
Current	9 (26)
Ex	7 (20)
Never	19 (54)
GP diagnosis	
Asthma	31 (91)
COPD	3 (9)
Inhaled steroids	23 (66)
Atopy[#]	22 (63)
Age yrs	46 (20–71)
Baseline FEV1	2.44 (1.40–4.05)
L % pred	88 (52–120)
PD20 µmol	2.18 (0.13–10.44)

Data are absolute number with percentages in parentheses, except for age, forced expiratory volume in one second (FEV1) and provocative dose causing a 20% fall in FEV1 (PD20) which are means with ranges in parentheses. GP: General practitioner; COPD: chronic obstructive pulmonary disease. [#]: atopy = skin weal >2 mm above control to any one of dust mite, grass or cat dander.

The analysis was performed separately for ozone and nitrogen dioxide; repeated for each pollutant using amplitude percentage mean as the dependent variable in place of mean PEF; and repeated using the 24-h lag spore count plus the 48-h lag pollution level to look for a delayed interaction effect.

Symptom data was treated identically, except that multiple logistic regression was used. All analyses were performing using the statistical program Generalised Linear Interactive Modelling (the Royal Statistical Society, London, UK).

Results

Subjects

Thirty-six subjects produced adequate PEF records and reacted to methacholine. However, due to technical problems with the Burkard trap, allergen measurements were not available on 4 days and as a result one subject fell below the predetermined criterion of supplying five complete days of spore, pollution and PEF data. The 35 remaining subjects had a mean age of 46 with a range of 20–71 yrs. Fourteen were male. Only nine were current smokers, but an additional seven were exsmokers. Additional clinical data is shown in table 1.

Pollution and aeroallergen levels

The study was performed from August to mid-September 1991. The pollution levels have been reported previously [17]. Briefly, the maximum 24-h and 8-h ozone levels were 55 $\mu g \cdot m^{-3}$ and 71 $\mu g \cdot m^{-3}$, below the 8-h World Health Organization (WHO) guide-level of 100–120 $\mu g \cdot m^{-3}$. For nitrogen dioxide the maximum 24-h level was 84 $\mu g \cdot m^{-3}$ compared to the WHO guide-level of 150 $\mu g \cdot m^{-3}$.

Daily total spore count varied from 655·m^{-3} to 40,685·m^{-3} with a median value of 6,385·m^{-3}. Pollen counts were much lower, perhaps not surprisingly given the time of year at which the study was conducted, and since spore levels predominated to such a degree pollens were not considered further.

Relationship of mean peak expiratory flow to pollution and aeroallergen

Mean PEF fell across ozone thirds (table 2). The effect was small but highly statistically significant (p<0.005). There was a similar trend for nitrogen dioxide but this result was not significant.

In univariate analysis mean PEF fell as spore count increased but this association was not significant (table 3). When the ozone/spore interaction term was added to the model there was a significant reduction in the variance of the model (p<0.05). There was a greater fall in PEF with spore count following medium-ozone days than following low-ozone days, and a still greater fall following high-ozone days (table 4). There was no evidence of an interaction between spores and nitrogen dioxide.

The pattern of results, and of statistical significance, was virtually identical when 24-h lag spore levels and 48-h lag pollution levels were analysed (tables 2–4).

Relationship of amplitude percentage mean to pollution and aeroallergen

The pattern of results for PEF variability was similar to that with mean PEF although statistical significance differed. Amplitude percentage mean increased with increasing levels of ozone, but this was of marginal statistical significance. In contrast the associations between increasing spore count and amplitude percentage mean were strongly significant for both same-day and 24-h lag-levels (table 3).

The increase in amplitude percentage mean with spore count was more marked after high-ozone days than after medium-ozone days, which in turn was more marked than after low-ozone day. However this interaction was only

Table 2. – Differences in mean peak expiratory flow (PEF) and PEF variability between low, medium and high pollution days

	Mean PEF[#]	Amplitude percentage mean[#]	Mean PEF[##]	Amplitude percentage mean[##]
Medium ozone	-2.90 (2.65)	-0.04 (0.06)	0.47 (2.75)	0.04 (0.06)
High ozone	-9.08 (2.45)***	0.10 (0.05)*	-7.10 (2.51)**	0.06 (0.05)
Medium nitrogen dioxide	-3.26 (2.36)	0.07 (0.05)	-4.34 (2.43)	-0.00 (0.05)
High nitrogen dioxide	-4.69 (2.50)	0.03 (0.05)	-4.34 (2.51)	-0.06 (0.05)

The level of the PEF variables on the low pollution day is taken as the baseline. [#]: versus 24-h lag pollutant; [##]: versus 48-h lag pollutant. The term "24-h lag pollutant" refers to the level 2 days previously. Amplitude percentage mean values are loge-transformed. *: p<0.05; **:p<0.005; ***: p<0.001 versus medium ozone days. Significance values are for the trend across pollution levels.

Table 3. – Univariate regression analysis of the association of mean peak expiratory flow (PEF) and amplitude percentage mean with spore count

	Mean PEF	Amplitude percentage mean
Same day spore count	-0.84 (1.13)	0.08 (0.02)***
24-h lag spore count	-0.62 (1.01)	0.05 (0.02)*

Data are regression coefficients with standard errors of the regression coefficient in parentheses. Amplitude percentage mean and spore count values are $\log_e$-transformed. *: $p<0.05$; ***: $p<0.001$.

significant with the 24-h lag spore/48-h lag ozone combination (table 4).

Relationship of symptoms to pollution and aeroallergen

The symptom data was less well kept than the PEF data by a few subjects. Thirty subjects produced useable records. Among the symptom results only wheeze showed any consistent association with pollution and aeroallergen levels, and the results for other symptoms will not be considered further.

Wheeze was significantly associated with spore, ozone and nitrogen dioxide levels in univariate analysis. In addition there was a significant interaction between spores and ozone, showing a greater chance of wheeze with increasing spore count following high ozone days than following medium ozone days, and the lowest coefficient following low ozone days. The same pattern was seen for the 24-h lagged spore/48-h lagged ozone interaction (table 5).

There was unconvincing evidence of an interaction between spores and nitrogen dioxide. A result of borderline significance was obtained but the pattern was not as expected, with wheeze being less likely as spore count increased after medium ozone days than after low ozone days (table 5). Furthermore, this result was only obtained with the lagged interaction.

Magnitude of change

Although statistically significant the changes from low spore/low ozone days to high spore/high ozone days were not marked. The difference in mean PEF was ~15 L·min^{-1}, and in amplitude percentage mean ~1.3%.

Discussion

The authors have studied the effect of spore count on PEF levels in subjects who are hyperreactive to methacholine, and shown that both the fall in mean PEF and the increase in PEF variability with increasing spore count were greater the higher the prior ozone level. These effects were statistically significant but the absolute change was fairly small. Wheezing also showed an association with spores and ozone, compatible with the PEF results. Similar associations were not seen with nitrogen dioxide.

The possibility that pollution exposure might enhance the effects of aeroallergen has been explored in several recent studies. Interest was aroused particularly by the study of MOLFINO et al. [25] in which seven subjects allergic to ragweed were exposed to 240 µg·m^{-3} ozone or air for 60 min, followed by allergen challenge. The provocative concentration causing a 20% fall in FEV1 allergen was significantly reduced by prior ozone exposure. This work has been extended in a study including larger subject numbers, and again ozone appeared to potentiate the response to allergen challenge [28]. Although this second study was of superior design in some respects, the ozone concentration used was 500 µg·m^{-3}, well above that which would commonly be encountered in the UK. A further study in which the nasal response to allergen was enhanced by ozone also employed unnaturally high ozone levels [29]. However, other laboratory studies have failed to show an interaction [30].

The effect of nitrogen dioxide on allergen challenge has also been studied. TUNNICLIFFE et al. [31] demonstrated a modest increase in bronchial response to house dust mite after exposure to 750 µg·m^{-3} nitrogen dioxide, but not after 190 µg·m^{-3}. The higher level is above that commonly detected by outdoor monitors although it might be achieved indoors. DEVALIA et al. [32] used both nitrogen dioxide and sulphur dioxide; the effect of nitrogen dioxide alone on subsequent mite challenge just failed to reach statistical significance, but in combination with sulphur dioxide a significant increase in mite reactivity was seen. A small study in children failed to show any effect of nitrogen dioxide on allergen sensitivity although the duration of exposure was short [33].

The effect of fungal spores on airway disease has been less widely reported than that of pollution, but there is evidence of an association. In Chicago (IL, USA) spore levels, but not tree, grass or ragweed, were associated with asthma deaths [34]. Symptoms, inhaler use and PEF recordings all varied with summertime spore level in Southern California (USA) [35]. This latter group have also reported a detailed analysis of 12 asthmatic children; both allergen and fungal spores (but not fine particles or pollen) had measurable effects on indices of asthma control, but there was no interaction between them [36].

In analysing the data the authors were guided by the laboratory experiments discussed above. Only ozone and nitrogen dioxide were considered because these pollutants

Table 4. – Change in mean peak expiratory flow (PEF) and amplitude percentage mean with increasing spore count after medium and high ozone days, relative to the change after low ozone days

	Mean PEF[#]	Amplitude percentage mean[#]	Mean PEF[##]	Amplitude percentage mean[##]
Medium ozone	-5.90 (2.51)	0.02 (0.05)	-4.96 (2.14)	0.11 (0.05)
High ozone	-7.02 (2.97)[+]	-0.04 (0.06)	-7.26 (3.22)[+]	0.15 (0.07)[+]

Data are multiple regression coefficients with standard errors of the regression coefficients in parentheses. The main effects of ozone and spore count are omitted from the table. [#]: *versus* same day spore/24-h lag ozone; [##]: *versus* 24-h lag spore/48-h lag ozone. Amplitude percentage mean and spore count values are $\log_e$-transformed. [+]: $p<0.02$ *versus* medium ozone days. Significance values are for the difference in the effect of spores between ozone levels.

Table 5. – Logistic regression coefficients with standard errors of the regression coefficients in parentheses indicating $\log_e$(odds) of change in symptom score from no symptoms to positive symptoms with change in spore count on medium and high pollution days relative to the change on low pollution days

	Wheeze[#]	Wheeze[##]	Wheeze[+]	Wheeze[++]
Medium ozone/nitrogen dioxide	1.57 (0.47)	0.18 (0.41)	0.99 (0.40)	-0.60 (0.40)
High ozone/nitrogen dioxide	1.72 (0.51)***	0.63 (0.48)	1.42 (0.54)**	0.69 (0.40)*

The main effects of pollutant and spore count are omitted from the table. Changes are shown for $\log_e$ (spore count·m^{-3}) and for ozone/ nitrogen dioxide per 10 μg·mm^{-3}. [#]: *versus* same-day spore/24-h lag ozone; [##]: *versus* same-day spore/24-h lag nitrogen dioxide; [+]: *versus* 24-h lag spore/48-h lag ozone; [++]; *versus* 24-h lag spore/48-h lag nitrogen dioxide. *: p<0.05; **:p<0.01; ***: <0.001 *versus* medium ozone/nitrogen dioxide days.

have produced significant potentiation in allergen challenge in human intervention studies, whereas there is less evidence for other agents. These studies involved controlled exposure to the relevant pollutant followed by challenge to a single allergen. It is obviously impossible to reproduce this situation precisely outside the laboratory, but the authors chose to look at the interaction between pollution exposure on one day and aeroallergen level on the following day which seemed a simple way of approximating the same sequential effect. The lag effect of the same sequence (*i.e.* allergen level on the previous day plus pollution level 2 days ago) was also examined since epidemiological studies often show delayed effects of exposure [13, 16, 17, 37–40].

The analysis of spore counts was sufficiently detailed to potentially allow the separate study of various species; there were days during the study when counts of *Cladosporium*, *Alternaria*, *Didymella* and *Sporobolomyces* were very high. However, each individual spore species tended to show fairly low, largely unvarying levels for most of the study period apart from the occasional high days, and analysis of any one alone would have been markedly influenced by these disproportionately high readings. Thus, despite the potential interest in an assessment of the relative importance of individual spore types, it was not felt that the data would permit adequate analysis of anything other than the combined total spore count.

When atopic and nonatopic subjects were considered separately there were no significant differences in the changes seen in the two groups (data not shown). This may reflect the small numbers once subjects are subdivided in this way. In addition, atopy was measured to common allergens only. Subjects were not tested for allergy to an array of fungi, and thus the division into atopic and nonatopic is unlikely to be the most appropriate for the aeroallergens encountered during the study period.

One important aspect of the current study is the implication that an interaction with allergens might amplify the effects of pollution in the epidemiological setting. Such a confounding effect would be more important if pollution and aeroallergen levels also tended to be high at the same times. In the period the authors studied ozone and spore levels were not closely correlated, but data elsewhere suggests that they tend to covary [41].

Although this study has demonstrated a statistically significant interaction between ozone level and spore counts the clinical significance is questionable. The PEF difference between low pollution/low spore days and high pollution/high spore days was of the order of 15 L·min^{-1}. This is similar to the change shown in a previous report from the same subject group when pollution effects were considered alone [17]. However, although the mean changes in PEF were modest there may well be individuals within the population who were more sensitive to pollutants and/or aeroallergens in whom the effects were clinically important. To demonstrate that such individuals exist it would be necessary to have sufficient data to show a consistent repeated change in PEF with varying pollution and allergen levels. The study duration was too short to allow such an assessment. Furthermore the measurements of spores and pollution may not reflect personal exposure. Effects may be more important in individuals, for example, at ground floor level in polluted areas.

In summary this study has shown an interaction between the effects of ozone and spore levels in a group of subjects with clinical diagnosis of airways disease who were also hyperreactive to methacholine. This effect was modest in magnitude but arose at pollution and aeroallergen levels of an average UK summer, and it is possible that the effects would be clinically important in some individuals in more extreme conditions.

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