

Acute Effect of Air Pollution on Respiratory Complaints, Exhaled NO and Biomarkers in Nasal Lavages of Allergic Children during the Pollen Season

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Key Words

Nitric oxide · Exhaled NO · Eosinophils · Nasal lavage

Abstract

During 2 months of the pollen season, the acute and putative adjuvant effect of traffic-related air pollution on respiratory health was investigated in children sensitised to grass pollen or house dust mite (HDM). Respiratory complaints were objectified via measurement of exhaled NO and inflammatory mediators in nasal lavage (NAL). During the study children, skin prick negative ($n = 31$) or positive to grass pollen ($n = 22$), HDM ($n = 34$) or grass pollen + HDM ($n = 32$), kept a daily diary on respiratory symptoms, and NAL and exhaled air was sampled twice a week. The level of air pollutants and pollen was monitored continuously. Like children sensitised to HDM, those sensitised to pollen reported respiratory complaints (shortness of breath, itchy eyes or blocked nose) more frequently than non-sensitised children during (but not before) the pollen season; the respiratory complaints

of sensitised children were independent of the pollen level. In addition, exposure to increased levels of PM₁₀ induces 'shortness of breath' in pollen- and HDM-sensitised children, whereas ozone induces a blocked nose in HDM-sensitised children. Combined exposure to PM₁₀ + pollen and O₃ + pollen induces a blocked nose in both HDM-sensitised children and children sensitised to pollen + HDM. Significant positive associations were found between eNO and the levels of NO₂, CO, PM_{2.5} and pollen in both sensitised and non-sensitised children. At the start of the pollen season, the NAL concentration of eosinophils and ECP in pollen-sensitised children was increased compared to winter, but their levels were not further affected by increased exposure to pollen or air pollution. In conclusion, during the pollen season, sensitised children continuously report a high prevalence of respiratory complaints which coincides with increased levels of upper and lower airway inflammatory markers. No additional pro-inflammatory effect of air pollution was observed, which indicates that air pollution does not facilitate allergen-induced inflammatory responses.

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Introduction

Air pollution belongs to the factors that are believed to be responsible for the increased prevalence of allergy and exacerbations of asthma. Traffic-generated particulate matter is probably the most important allergy-promoting component here because it can adhere to allergen-carrying pollen grains [1–4].

Exposure to diesel exhaust particles (DEP) and nitrogen oxides (used as indicators for traffic-related air pollution) has been observed to exert an adjuvant effect on allergy in various rodent allergy models. For example, the specific IgE response in rodents sensitised to ovalbumin (OVA) and pollen mixed with DEP was larger than in rodents immunised with OVA or DEP alone [5–11]. Similarly, studies in non-allergic and ragweed-allergic persons showed that DEP increased the release of specific IgE and cytokines in the nose [12–15]. It thus seems that exposure to DEP and noxious gasses elicits the release of cytokines and inflammatory cells in the respiratory tract, which makes the host vulnerable to adverse reactions of allergens [16].

The first piece of epidemiological evidence for an adjuvant effect of traffic-related air pollution on allergy was given by Ishizaki et al. [17], who showed an increased prevalence of sensitisation to *Cryptonia japonica* pollen in areas with high air pollution. These results were later confirmed by others who showed that the frequency of truck traffic was positively associated with reported symptoms of allergic rhinitis and respiratory complaints [18–20], and with the prevalence of pollen sensitisation [21]. Recently, Kramer et al. [22] showed that the prevalence of sensitisation to pollen, HDM, cats, milk or eggs was associated with the mean level of long-term exposure to outdoor NO₂. In other studies, traffic-related air pollution was also found to be associated with respiratory complaints, but not with an increase in the prevalence of allergic symptoms [23–26].

So far, the facilitating effect of traffic-related air pollution on allergic symptoms was investigated in cross-sectional studies that assessed the prevalence of pollen sensitisation in populations living in areas with a high or a low level of air pollution. In addition to the allergy-promoting effect of chronic exposure to air pollution, it is of interest to know whether air pollution acutely aggravates pre-existing allergic airway inflammation and complaints.

Therefore, we studied acute adverse effects of air pollution during two months of the pollen season in four groups of school children (sensitised to pollen, to HDM, to pollen + HDM, and not sensitised) living in an urban environ-

ment. In addition to daily respiratory complaints, we monitored acute inflammatory airway responses twice weekly using inflammatory mediators in nasal lavage and exhaled NO.

Materials and Methods

Study Subjects

The study was performed in children (7–12 years) attending primary schools, and living in the same urban environment. Of the 889 (76%) parents responding to the invitation, 72% gave their child permission to participate and 504 children were included in the study. All participating children were in good health and no specific inclusion or exclusion criteria were applied. The study protocol was approved by the Medical Ethics Committee. In January–February (before the start of the pollen season) children underwent a skin prick test (SPT). In addition, nasal lavage (NAL) was performed, exhaled air was sampled, and the peak expiratory flow (PEF) was measured. Based on the type of sensitisation, 119 children were included in the present study on respiratory health and the effects of air pollution and were further tested on 14 occasions (NAL, exhaled air and PEF) twice a week during the pollen season (from May 1 till June 29) (fig. 1).

Allergy Testing

Allergic status in children was assessed by the SPT using the following five allergen extracts: dog dander, cat dander, *D. pteronyssinus*, grass pollen mixture, and tree pollen mixture (Alk-Abello, Nieuwegein, The Netherlands). Hypersensitivity to HDM and pollen is defined as SPT to *D. pteronyssinus* and grass pollen mixture, respectively. Negative and positive controls were included. Wheal size >2 mm after subtraction of the negative control was considered as a positive allergic reaction.

Questionnaire and Symptom Diary

A modified WHO children questionnaire for chronic respiratory symptoms, extended with questions about smoking habits at home was used and completed in winter. All questionnaires were returned after completion by the 119 participating children assisted by their parents (table 1). At the beginning of the pollen season (May), the children received a booklet in which daily symptoms, i.e. shortness of breath, sore throat, blocked nose, itchy eyes and fever were noted during the pollen season. Twice a week, recording of the daily dairies was controlled by the field workers.

Sampling of Exhaled Air and Assay of Exhaled NO

Exhaled air was sampled using a sampling device made up of a rigid tube connecting mouthpiece and sampling balloon, and equipped with a calibrated narrowing in the exhalation circuit as described before [27]. The sampling device contained an upstream pressure transducer connected to seven different indicator lamps reflecting the exhalation flow rate. While seated, children were asked to take a deep breath and immediately to perform a single exhalation at a constant flow into an inert and impermeable foil bag of 1,000 ml (Mylar balloon, ABC ballonnen, Zeist, The Netherlands). The first 500 ml of exhaled air putatively contaminated with ambient NO was collected at high exhalation flow in a pre-balloon connected via a wide-bore T-piece in the rigid tube of the sampling device and discarded. For subsequent air sampling an exhalation flow rate of

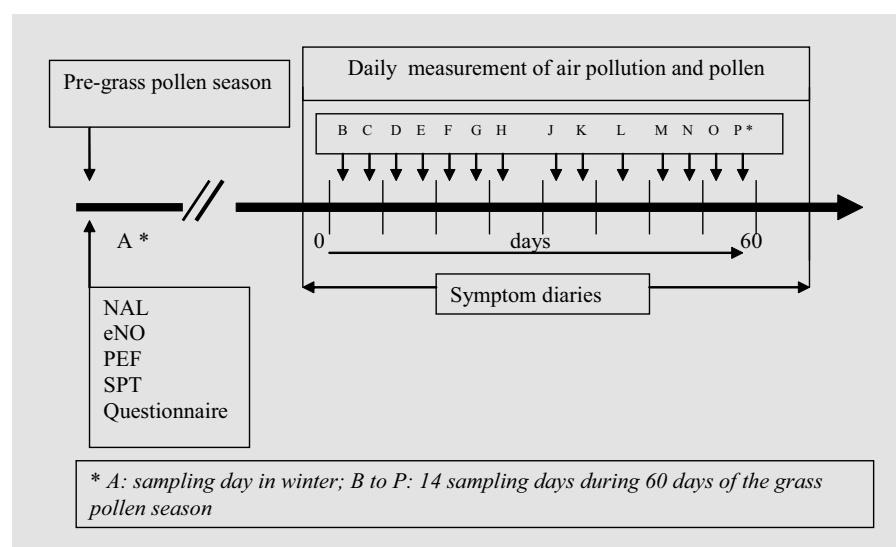


Fig. 1. Study protocol

Table 1. Population characteristics and reported prevalence of respiratory complaints in subgroups of sensitised and non-sensitised children

Variable	Reported prevalence, %			
	not sensitised (n = 29)	HDM-sensitised (n = 34)	pollen-sensitised (n = 23)	HDM + pollen sensitised (n = 29)
Boys, %	50	62	65	36
Wheeze last 12 months	17	44	26	33
Short breath last 12 months	24	42	4	27
Wheezing + short breath last 12 months	14	27	4	24
Blocked nose, no fever	41	71	44	61
Blocked nose last 12 months, but no fever	38	62	26	52
Doctor diagnosed asthma	7	27	4	30
Asthma attacks last 12 months				
1–5 attacks	7	12	0	15
6–25 attacks	0	3	0	0
Asthma medication	4	18	0	27
Bronchitis attacks last 12 months				
1–3 attacks	7	9	4	15
>3 attacks	0	6	0	0
Under medical treatment for respiratory complaints	17	27	13	33
Pets at home				
Cat	21	6	9	6
Dog	21	18	13	15
Other	31	9	17	33

8.3 ml/s was used. Exhalation back pressure was 10–20 cm water, which is sufficient to prevent contamination with nasal NO. Although this method differs from those recommended by the ERS and ATS [28, 29], we have shown previously that it correlates well with on-line measurements made in the laboratory which have followed the ERS and ATS recommendations [30, 31].

The content of exhaled NO (eNO) in the sample balloons was measured within 2 h after sampling by chemiluminescence (Sievers 280B, Boulder, USA) using a response time <200 ms. The analyser sample flow rate was kept constant at 200 ml/min.

Table 2. Average and range of air pollution levels ($\mu\text{g}/\text{m}^3$) during the study period (May–June)

Component	Median	Mean	Minimum	Maximum
Carbon monoxide ($\times 10$)	34.2	34.5	21.9	49.0
Nitrogen oxide	4.9	7.75	1.5	42.3
Nitrogen dioxide	31.5	32.6	16.0	53.2
Ozone	72.0	84.4	40.0	175.0
Sulphur dioxide	2.5	3.36	0.4	14.2
PM ₁₀	26.7	28.7	9.6	70.4
PM _{2.5}	8.9	20.3	7.2	49.1
Pollen	52.6	45.2	0	251.0

Measurement of Peak Expiratory Flow

On each sampling day, peak expiratory flow (PEF) of the children was determined three times under observation using Mini Wright Peak Flow Meters (Clement Clarke, London, UK) and the mean value of 2–3 measurements (difference < 10 ml/min) was used for data analysis. All children performed reproducible peak flow manoeuvres during the study with an averaged PEF value of 357 ± 56 ml/min (range: 180–487).

Nasal Lavage

The NAL was performed as described before [32–35]. Briefly, children bent their necks 45° backwards and elevated the palate to close the nasopharynx. Phosphate-buffered saline (4.0 ml, 37°C) was instilled in one nostril using a pipette, the fluid was held in the nasopharyngeal region for 10 s and then the child flexed its head and let the washing solution flow through a polyamide gauze (100 mesh) and funnel into in a centrifuge tube kept on ice. The procedure was repeated in the other nostril (total recovered volume was approximately 4.5 ml). After centrifugation (10 min, 250 g, 4°C), supernatants were frozen in different aliquots at -80°C until assay of IL-6, IL-8, albumin, uric acid, urea, and eosinophilic cationic protein (ECP). Cells were counted and cell differentiation was performed after Giemsa staining.

IL-6 and IL-8 were measured by a modified ELISA (Biosource, Etten-Leur, The Netherlands) in which the streptavidin poly-HRP activity was measured by chemiluminescence using acridane ester GZ-11 based signal reagent with an intra-day variability of less than 10%. Albumin, uric acid and urea were assayed by a colorimetric test and the Unimate 5 & 7 Urea-kit (both Hoffmann-La Roche, Basel, Switzerland), respectively. ECP was determined by fluoroimmunoassay (FEIA) using the Pharmacia CAP System (range 0.5–200 ng/ml; Pharmacia AB, Uppsala, Sweden).

Levels of Pollen in Ambient Air

Airborne grass pollen was collected on the site with a Burkard pollen sampler and counted. Mean 24-hour level of grass pollen particles on the 4 days preceding the test day in the pollen season was 10–214 pollen grains per cubic metre (mean 79 pollen particles per cubic metre); 95 pollen particles per cubic metre on the day of testing). No grass pollen was observed in January/February (outside the grass pollen season).

Ambient Air Pollution Components

A monitoring station located within 2 km from the schools hourly measured the following air pollutants: CO, O₃, NO, NO₂, SO₂, PM₁₀,

and PM_{2.5}. Ambient levels of air pollutants and pollen were expressed as mean levels from 0.00 to 24.00 (acute exposure), 24-hour mean values of the day before (lag 1), and week before (mean level of last 6 days before sampling). For ozone (O₃), the maximum value between 12.00 and 20.00 was taken as pollution level.

HDM Collection

In houses of children sensitised for HDM, dust was collected on the floor of the living room (m²) and surface of the mattress (m²) in the bedroom. Described by van Stien et al. [36], house dust was collected on a filter by vacuuming for 2 min and Der p 1 and Der f 1 were determined with an enzyme immunoassay.

Statistical Analysis

Associations between inflammatory markers and air pollutants were analysed after log-transformation to normalise residuals by linear and logistic regression models using the SAS statistical analysis software package (table 3, 5). The analyses were adjusted for the following potential confounders: age, gender, gas cooking, unvented water heater, environmental tobacco smoke exposure and having a cold during sampling. Level of significance of the difference between groups was determined using the 95% confidence interval. The longitudinal ordinal data were analysed using MIXOR, mixed model analysis of ordinal data [37] (table 4). Model selection was carried out using AIC [38] in a stepwise fashion. Primary variables added to the model where the sensitivity of the individuals, the mean pollen count of the last 6 days, and interaction with sensitivity. Added were each of the air pollution variables and their interaction with sensitivity. The best model, if any, was tabulated. Significance on threshold values of the logic function of the ordinal variable was assessed using a p value of 0.05. Pearson correlations have been computed per individual and combined with the Fisher Z transformation, as a significant heterogeneity has been found in some cases (table 6).

Results

Descriptive Data on Respiratory Symptoms in Subgroups

Table 1 depicts the prevalence of respiratory complaints of the subgroups reported in winter. The reported prevalence of upper and lower respiratory tract complaints and asthma diagnosed by a doctor was higher in

Table 3. Associations between levels of pollen and air pollution, and reported complaints of sensitised children compared to non-sensitised children

	Sensitised to pollen			Sensitised to HDM			Sensitised to pollen + HDM		
	pollen	air pollution	pollen + air pollution	pollen	air pollution	pollen + air pollution	pollen	air pollution	pollen + air pollution
Shortness of breath	○	PM ₁₀	○	○	PM ₁₀	–	○	○	○
Itchy eyes	○	○	○	+	–	–	○	○	○
Blocked nose	○	○	○	○	O ₃	○	–	○	O ₃
Blocked nose	○	○	○	○	○	PM ₁₀	○	○	○

○ = Complaints not explained by pollen or pollutant; + = increase in complaints explained by pollen;
– = decrease in complaints explained by pollutant.

Table 4. Associations between levels of air pollution versus eNO

	Non-atopic	Pollen sensitised	HDM sensitised	Pollen + HDM sensitised
CO	+	+	○	+
CO _{wk}	–	–	–	–
NO	○	○	○	+
NO _{wk}	○	○	○	○
NO ₂	+	+	+	+
NO _{2, wk}	○	○	○	○
O _{3, lag 1}	+	○	○	○
O _{3, wk}	○	○	+	○
PM ₁₀	○	○	○	○
PM _{10, wk}	○	–	○	–
PM _{2.5}	+	+	+	+
PM _{2.5, wk}	○	–	○	○
Pollen	+	+	+	+
Pollen _{wk}	○	○	○	○

○ = No association; + = positive association (more complaints compared to minimally exposed children); – = negative association (less complaints compared to minimal exposed children). Lag 1 = lag 1 day; wk = mean level of last 6 days before sampling.

the HDM-sensitised children as compared to non-sensitised children and children sensitised to pollen only.

During the pollen season, all children reported respiratory complaints in a diary, such as blocked nose, itchy eye and shortness of breath (fig. 2a–c). Irrespective of the sensitisation type, all sensitised children frequently reported these complaints: 25.0 ± 6.9 , 40.9 ± 8.2 , 28.2 ± 5.5 (mean percentage during pollen season $\pm$ SD); the frequencies in non-sensitised children were low (6.5 ± 3.1 , 15.5 ± 5.8 , 12.8 ± 6.1 , $p < 0.05$).

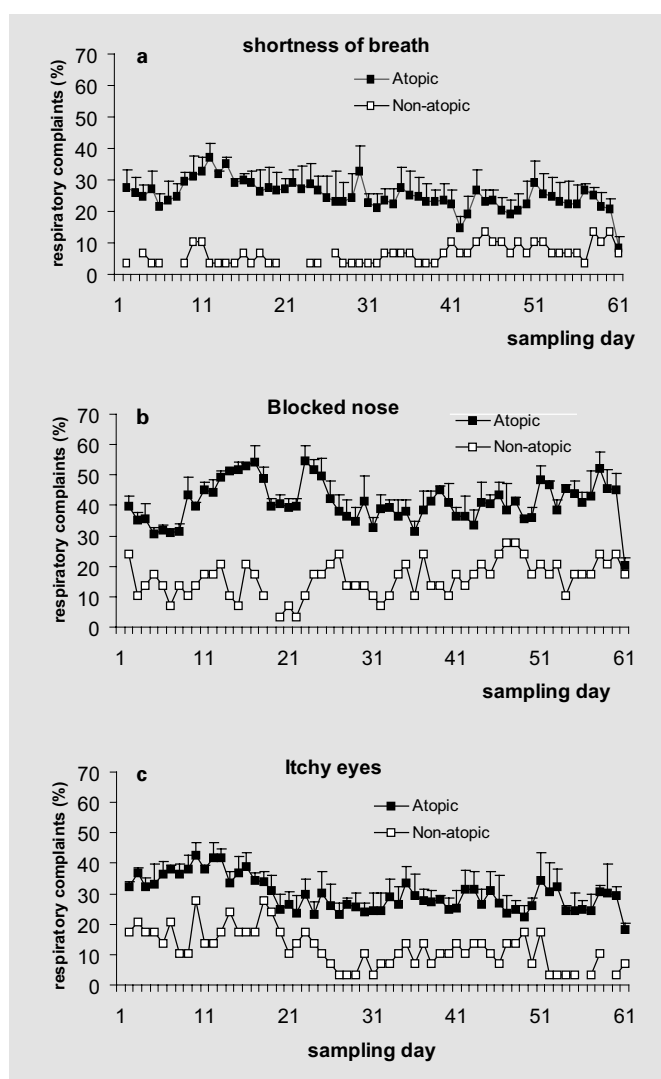


Fig. 2. Prevalence of respiratory complaints during the pollen season in the four subgroups. **a** Shortness of breath. **b** Blocked nose. **c** Itchy eyes.

Table 5. Associations between levels of air pollution and nasal markers

	ECP				Eosinophils				IL-6				IL-8			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
O ₃ , lag 1	○	○	+	○	○	+	○	+	+	○	○	○	○	+	○	○
O ₃ , wk	○	+	○	○	○	○	○	○	○	○	–	○	○	○	○	○
PM ₁₀	+	○	○	○	○	○	○	○	–	–	○	○	○	+	–	○
PM ₁₀ , wk	○	+	○	○	○	○	○	○	○	○	○	○	○	○	○	○
PM _{2.5}	+	○	○	+	+	+	+	○	+	○	○	○	○	○	○	○
PM _{2.5} , wk	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Pollen	○	–	○	○	○	○	○	○	○	○	○	○	+	○	○	○
Pollen _{wk}	○	○	○	○	○	○	○	○	○	○	–	–	–	+	○	○

1 = Non-atopic children; 2 = pollen sensitised; 3 = mite sensitised; 4 = pollen + mite sensitised; ○ = no association; + = positive association (more complaints compared to minimal exposed children); – = negative association (less complaints compared to minimal exposed children). wk = mean level of last 6 days before sampling.

Table 6. Regression coefficients of associations between the various biomarkers

Predicted	IL-6	IL-8	Uric acid	ECP	eNO	PEF	Eos/ml
IL-6	1	0.54*	0.13*	0.31*	0.08*	–0.03	–0.15*
IL-8		1	0.21*	0.52*	0.01	0.06	–0.18*
Uric acid			1	0.18*	0.00	0.04	–0.02
ECP				1	0.05	–0.05	0.03
eNO					1	0.02	0.03
PEF						1	0.02
Eosinophils/ml							1

Values of biomarkers were log-transformed before analysis.

*p < 0.05.

Mean Levels of Air Pollution

The average daily levels of the air pollutants monitored during the study are shown in table 2. During the study period, none of the values exceeded the WHO health guidelines: 10 mg CO/m³ (8-hour average), 70 µg NO₂/m³ (24-hour average), and 150 µg O₃/m³ (maximum value 240 µg O₃/m³) whereas no air quality guidelines are given for NO. Levels of SO₂ were negligible and excluded from further data analysis.

The daily exposure values of pollen, PM₁₀ and ozone are depicted in figure 3. Before the pollen season, the level of grass pollen is negligible but at the beginning of May, the grass pollen level increased to approximately 25 pollen grains/m³ (fig. 3). From the last week of May till the end of the study period (end of June), 5 identifiable grass pollen peaks appeared with levels ranging between 80 and 250 pollen grains/m³.

In May/June, the daily averaged level of PM₁₀ ranged from 10 to 55 µg/m³ (fig. 3) and six identifiable peaks were observed. In this period, the level of PM_{2.5} showed a similar pattern as that of PM₁₀. At the start of the study period, the ozone levels were rather high (120–140 µg/m³; fig. 3), decreased after two weeks to low levels and showed three short-lasting peaks in the second month of the study period. As expected, the levels of NO and NO₂ are traffic related and are therefore low during the weekend, but their averaged daily level remains below safety guidelines (data not shown).

Effect of Pollen on Upper and Lower Respiratory Health Complaints

Environmental Determinants of Respiratory Complaints. Mixed model analysis revealed that an increase in pollen level did not increase the rate of respiratory com-

Fig. 3. Time course of air pollution during 60 days of the pollen season. Left axis: air pollutants; right axis: pollen level. Day 0 is May 1, whereas the first sampling day was May 9 (day B).

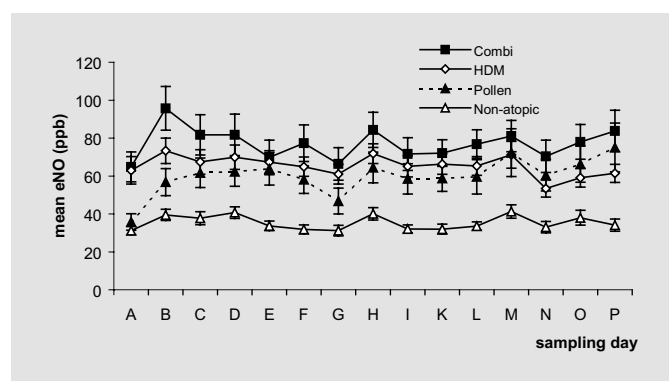
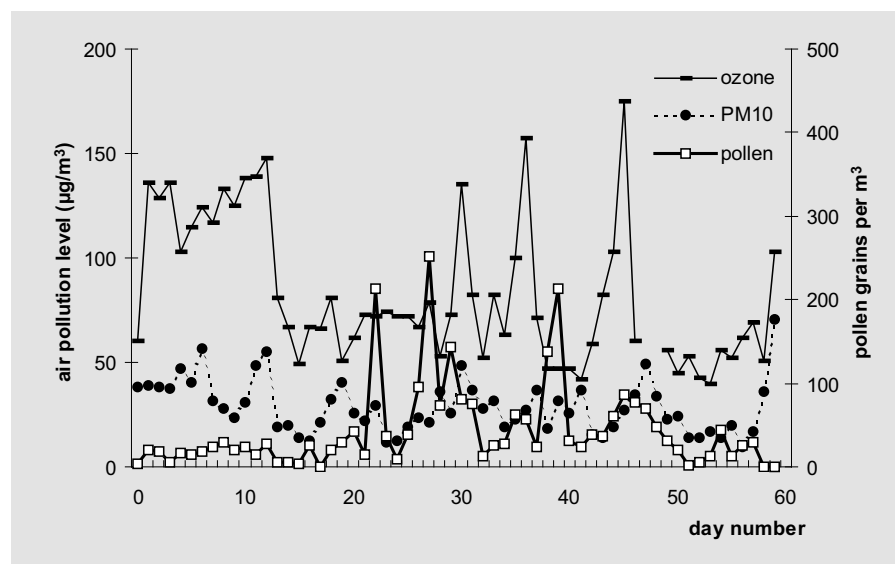


Fig. 4. Mean eNO level of children in the four subgroups before (day A) and during the pollen season (days B-P). Combi: sensitised to pollen + HDM.

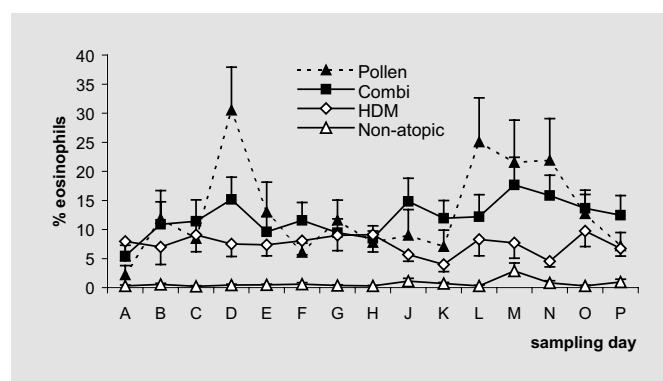


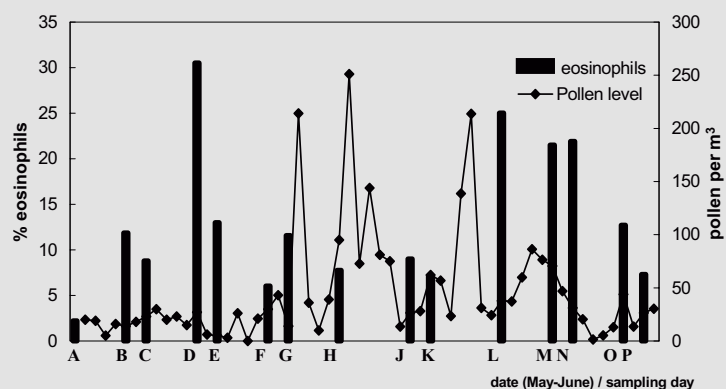
Fig. 5. Mean percentages of eosinophils in NAL of children in the four subgroups before (day A) and during the pollen season (days B-P).

plaints in pollen-sensitive children compared to the non-sensitised children. In children sensitive to mite, pollen increases complaints of itchy eyes. In addition, PM₁₀ exposure induces 'shortness of breath' in pollen and in mite-sensitised children, while ozone induces a blocked nose in mite-sensitised children. Exposure to combinations of PM₁₀ + pollen and O₃ + pollen induces a blocked nose in children sensitised to mite and in those sensitised to pollen + mite, respectively (table 3).

Inflammatory Markers of the Lower Respiratory Tract. Figure 4 shows that mean eNO of sensitised children is consistently higher as compared to non-sensitised children. Before the pollen season, the eNO value in children

sensitive to pollen was comparable to eNO of non-sensitised children ($p < 0.05$) whereas eNO of grass-pollen-sensitive and of non-sensitive children was significantly lower as compared to eNO of HDM-sensitised children. During the pollen season, eNO of non-sensitised children remained low, whereas eNO of children sensitised to pollen was significantly higher at the start of the study period when the first grass pollen had appeared in the air (fig. 3; 15–25 pollen grains/m³). Throughout the grass pollen season, the level of eNO of pollen sensitive children remained elevated compared to the pre-pollen period. In addition, eNO did not steeply increase during or shortly after exposure to 10-fold higher levels of pollen (fig. 3;

Fig. 6. Mean percentages of eosinophils in NAL of children sensitised to pollen only before (day A) and during the pollen season (days B–P; left axis), and local pollen level (days B–P; right axis).



100–250 pollen grains/m³). In HDM-sensitised children, the level of eNO remained consistently high (comparable to pre-pollen value). Regression analysis of the individual eNO values, however, showed a positive correlation ($p < 0.05$) between eNO and acute pollen exposure i.e. pollen level on the day of sampling in all subgroups (table 4). Table 4 also shows similar associations between eNO and the air pollutants CO, NO₂ and PM_{2.5}. Interestingly, in sensitised children, the PEF value was negatively associated with acute exposure to ozone, but no association was found for pollen (data not shown).

Inflammatory Markers in Upper Respiratory Tract. During the pollen season (days B–P; May 9 to June 29), the percentages of eosinophils in NAL of non-sensitised and HDM-sensitised children did not increase above those observed in winter (baseline value on day A; cf. fig. 5). However, the baseline eosinophil level of HDM-sensitive children (8.0%) was significantly higher as compared to non-sensitised and grass pollen-sensitised children. In non-atopic children and children sensitive to grass pollen only, the percentage of eosinophils in NAL was low (0.3 and 2.2%, respectively).

During the pollen season, two clear peaks appeared in the percentage of eosinophils in the NAL of pollen-sensitive children respectively at day D and days L–N which did not coincide with a rise of grass pollen (fig. 6). However, from the very beginning of the pollen season, pollen-sensitive children showed a permanent 5- to 10-fold increase in the NAL concentration of both eosinophils and ECP in comparison with non-atopic children. Indeed, a regression analysis of the data did not show any associations between pollen level and percentage of eosinophils

(or ECP level) in NAL (fig. 6, table 5). Occasionally, positive associations were found for PM_{2.5}, PM₁₀ and ozone, but the results were not consistent. Nor were any associations found for albumin, uric acid or urea (not shown).

Associations between Inflammatory Biomarkers

Analysis of the interrelations between the biomarkers showed that nasal IL-6 level was positively associated ($p < 0.05$) with all other biomarkers (table 6). In addition, IL-8 was positively associated with uric acid, ECP and eosinophils, whereas PEF was not associated with any of the biological markers.

Discussion

The present results show that children sensitised to airborne allergens report a sustained higher prevalence of respiratory complaints during the pollen season, which is objectified by higher concentrations of eosinophils and ECP in the upper airways and eNO as compared to controls. The prevalence of reported respiratory complaints and the levels of the inflammatory markers were not associated with the levels of air pollutants or grass pollen (either alone or combined), which implies that air pollution does not acutely aggravate the allergic response.

During the pollen season, the prevalence of shortness of breath, itchy eyes and blocked nose in grass-pollen-sensitised children was higher than in the pre-grass pollen period. Allergic complaints were reported throughout the 2 months of the pollen season, but were not associated with increased exposure to grass pollen or air pollution.

Similarly, the levels of inflammatory markers in the pollen-sensitised children were permanently increased during this period. These findings suggest that outdoor allergens have elicited an inflammatory response in pollen-sensitised children at the very beginning of the pollen season when pollen levels were still low, but the atopic status of this SPT-positive population did not result in an exacerbation of complaints during peak levels of pollen.

HDM allergens are present continuously over the year, which explains that HDM-sensitised children, in contrast to pollen-sensitised children, reported a high prevalence of respiratory complaints also in winter (table 1). In addition, in winter higher levels of nasal eosinophils and ECP were observed in HDM-sensitised children as compared to pollen-sensitised children. Comparable to the absence of an association between symptoms and pollen levels in pollen-sensitised children, no associations were found between the concentration of HDM in the living room and on the mattress versus wheal size in SPT, and between nasal eosinophils and ECP in HDM-sensitised children (data not shown). In summary, these results show that the pattern of allergic complaints matches that of objective biomarkers in nasal fluid and exhaled air well, although no dose-response effect was observed for pollen and HDM allergens.

Levels of eNO in sensitised children were higher as compared to the non-sensitised children, and the highest eNO was observed in children sensitised to two allergens (HDM and pollen) which is in agreement with previous findings that eNO is positively associated with the number of SPT-positive reactions [39]. During the pollen season, eNO was permanently increased in pollen-sensitive children compared to the baseline value assessed in winter, but unlike the NAL levels of ECP and eosinophils, the pattern of mean eNO showed only minor variation during the pollen season. Regression analysis of the individual eNO values, however, showed significant positive associations in all subgroups ($p < 0.05$) between eNO versus acute exposure to pollen, CO, NO₂ and PM_{2.5} (cf. table 4). These results extend the previous finding that eNO of allergic [40] and asthmatic children was increased in the pollen season, and levelled off again in the post-pollen season [41].

This is the first study that monitored children multiple times during the pollen season to detect a dose-response relationship between levels of pollen and air pollution versus the level of inflammatory markers, including eNO. To our surprise, eNO of pollen-sensitive children was increased on the first sampling day (day B) before the first peaks of grass pollen had appeared, and did not further

increase during or following exposure to high levels of grass pollen. This suggests that minor levels of pollen (<25 pollen/m³) have initiated a prolonged increase in eNO, which is not further affected by subsequent exposure to 10-fold higher levels of pollen.

During the pollen season, the concentration of nasal eosinophils in children sensitised to pollen, to HDM, and to pollen + HDM was, respectively, 8-, 26-, and 19-fold higher as compared to non-sensitised children (not shown). Similarly, eNO of sensitised children is 80–250% higher than in non-sensitised children. Previous reports have shown an association between eNO and the number of blood eosinophils in a random population of children [42] and in children with mild-intermittent allergic asthma [43]. Several studies [44–48] indicate that local inflammatory responses in upper and lower airways generate a mucosal response throughout the respiratory tract. After segmental bronchial provocation of patients with allergic rhinitis, Braunstahl et al. [44] observed an eosinophilic influx, and increased IL-5 and eotaxin staining in nasal epithelium and vice versa. As such, the increase in NAL concentrations of eosinophils and ECP by pollen in children sensitive to pollen suggests that an inflammatory reaction is initiated by low levels of pollen in the lower airways. The simultaneous increase in eNO induced by pollen observed in this group supports such nasobronchial cross-talk and confirms that sensitised children reporting upper airway complaints suffer from an inflammation of the lower airways.

None of the NAL markers we measured were associated with ambient levels of pollen or air pollutants. In children sensitised to grass pollen, the nasal concentration of ECP and eosinophils showed two peaks during the pollen season (fig. 5). Considering the specificity of eosinophilic inflammation, these two peaks are probably due to exposure of this group to grass pollen, since exposure to air pollutants or micro-organisms would also have affected the other two subgroups, including non-sensitised children. On the other hand, these peaks did not appear on days or following days with increased pollen levels suggesting that other environmental factors may be involved. This may be explained by the observations of Knox et al. [3] who emphasised the importance of the starch granules which may be released when grass pollen grains rupture in rainfall. The immunodominant component of rye grass, Lol p 1, is located mainly on the external surface of the cell, but a newly identified allergen, Lol p 9 is located mainly in the starch granules within the pollen grain. A single mature rye grass pollen grain contains about 700 starch granules 0.6–2.5 µm in diameter. These granules

constitute a potential source of micro- and submicro-allergenic particles, which may be responsible for the peaks in the NAL levels of eosinophils and ECP. The aim of our study was to investigate whether acute exposure to traffic-related air pollution aggravates pre-existing allergic airway inflammation and respiratory complaints. So far, various studies have shown that living in areas with high air pollution is associated with an increased prevalence of respiratory and atopic complaints. Kramer et al. [22] recently reported in a random population a significant relation between prolonged exposure to air pollution (using individual yearly mean NO₂ level) and atopic complaints such as high fever, symptoms of allergic rhinitis, wheezing, and sensitisation to pollen and house dust mite. In contrast to Kramer et al. [22], the present study was performed in sensitised children showing a high prevalence of respiratory complaints, i.e. blocked nose, itchy eye, shortness of breath. Our results are more or less in line with those of Hajat et al. [49, 50] who investigated the relation between air pollution and upper respiratory disease using the number of consultations at family practices and showed that PM₁₀, SO₂ and O₃ may be responsible for the worsening of allergic rhinitis symptoms. The observed effect size was, however, rather small.

Based on our data we conclude that aeroallergens induce in sensitised children an ongoing inflammatory response in the upper (increased eosinophils and ECP) and lower airways (increased eNO). However, following subsequent exposure to high levels of air pollution or pollen, there is no further increase in inflammatory reactions in addition to the established inflammatory response that was elicited by low levels of pollen.

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