

PERSONAL EXPOSURE TO AMBIENT PARTICULATE MATTER (PM_{2.5}) IS ASSOCIATED WITH NASAL INFLAMMATION IN ASTHMATIC CHILDREN LIVING IN PARIS

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Allergic respiratory diseases have recently become a major public and political concern in most industrialized countries over the world. The increased incidence in allergic asthma and allergic rhinitis has paralleled the motor vehicle traffic booming, suggesting a potential role of air pollutants in this major rise. Epidemiological studies have revealed a consistent association between outdoor ambient air pollution (to which ozone, nitrogen dioxide and particulate matter (PM) are major contributors) and various health respiratory outcomes [Lebowitz, 1996]. They have shown an association between small short-term increases in PM levels and increases in daily mortality and morbidity due to cardiovascular and respiratory diseases [Katsouyanni *et al.*, 1990; Dockery and Pope, 1994; Schwartz, 1994]. The role of diesel exhaust particles (DEP) is questioned because DEP is one of the major components of particulate matter pollution in urban environments, accounting for up to 90% of the ultra fine particulate mass [United Nations Environment Program, 1994].

The existence of plausible and reliable underlying mechanisms supports these epidemiological observations. Many studies have thus focused on the biological events that occur after diesel exhaust particles (DEP) exposure. Paralleling observations in the lung, in the nose, short-term DEP exposure results in common qualitative alterations characterized by immediate nasal hyper responsiveness, antioxidant response, marked epithelial inflammation, and a specific humoral response. In addition, studies on combined DEP/allergen challenge report that, beside their intrinsic deleterious properties, DEP have an adjuvant-like effect on the immediate and late-phase response to allergen challenge, since they are able to mimic effects occurring after allergen challenge (for a review, see [Nikasinovic *et al.*, 2003]). The nose is an easily assessable mucosal surface, compared to lung. It is an interesting area for assessing upper airway inflammatory status, as it is the first region of the respiratory tract in contact with airborne pollutants. The nasal lavage (NAL) fluid (NALF) is rich in inflammatory biomarkers, as Naclerio and co-workers demonstrated in 1986 in allergic subjects [Naclerio *et al.*, 1986]. NAL is a useful method for epidemiological studies since it fulfils conditions for large-scale use: it is simple to perform, non-invasive, and atraumatic. However, the rare epidemiological studies dealing with air pollution and nasal inflammation failed to demonstrate a significant effect of PM on NALF inflammatory biomarkers.

In that context, the aim of this study was to determine the impact of personal exposure to fine particles (PM < 2.5 µm diameter, PM_{2.5}) and to another major indicator of traffic air pollutants, NO_x, on nasal inflammation in children with allergic asthma and in healthy children living in Paris area.

METHODS

This prospective study consisted in a 48h personal and indoor measurement of a few major outdoor and indoor air pollutants, followed by a medical examination at the Asthma center (Trousseau hospital, Paris) where a nasal lavage and skin prick tests were performed. The Ethic committee of the Grenoble University Hospital approved the study. Written consent was obtained from all parents and children.

Subjects

A total of 91 children aged 7 to 14 years participated in the study from October 1999 to June 2002. The study population included 46 allergic / asthmatic and 45 healthy children, both living in Paris or its close suburb, since birth. Asthmatic patients were children with a doctor-diagnosis of asthma, recruited among attendees of the pediatric Asthma center of Trousseau Hospital (Paris, France) and by a network of private willing pediatricians or general practitioners. The diagnosis of asthma was attested by history of recurrent wheezing and dyspnea attacks, with proven β_2 agonist reversible airways obstruction characterized by an increase of at least 15% in the FEV₁. All asthmatic children had mild to moderate asthma and received conventional treatment. Healthy participants, recruited by the same network of private pediatricians or general practitioners, had no history of asthma, rhinitis, personal or familial atopy evidenced by the interview and a normal physical examination. Skin prick tests were performed with the following allergens: cat, *Dermatophagoides pteronyssinus*, *Alternaria tenuis*, timothy grass, *Blatta germanica*, olive tree, birch tree, pellitory and ragweed, all provided by Allerbio (Paris, France). In addition, an upper respiratory tract examination was performed routinely by anterior rhinoscopy, and no child presented nasal polyps or septal deviation or previous surgery.

Personal exposure measurements

In order to evaluate short-term effect of air pollutants, personal exposures measurements were realized during the 48h preceding the NAL procedure. A particular effort was made to measure personal exposure to nitrogen oxides (NO_x) and fine particles. Measurements were performed during school days. Before the sampling period, samplers were brought by an interviewer at home where a validated environmental questionnaire based on the Expolis study [Jurvelin *et al.*, 2001] was filled.

Concerning NO_x measurements, all children continuously carried on their clothes near to the breathing zone and for 48h a passive sampler measuring nitrogen monoxide (NO) and nitrogen dioxide (NO₂) (Ogawa & Company, Inc, Pompano Beach, Florida, USA). At night, the sampler was put on the bedside table. These small badges consist of a cylindrical polymer body and a plastic pin-clip holder with two filters. The first is impregnated with triethanolamine that traps NO₂ while the second incorporates an oxidant to convert NO to NO₂, which allows measuring the total NO_x (NO+NO₂) content. The resulting pink color was measured by spectrophotometry at a 545 nm wavelength (Beckmam Model 25 photometer). Blanks and replicates were measured in 10% of the population. Results are expressed in $\mu\text{g} / \text{m}^3$.

Fine particles were collected on a Teflon filter (Pall Gelman, diameter 37 mm, porosity 2 μm) placed in an impactor connected to a pump (SKC, Arelco, Fontenay/Bois, France) with an air flow set at 4 l/min. Particles were selected by a GK 2.05 cyclone KTL (BGI Instruments, model Gil-Air 5, Sensidyne, Clearwater, Florida, USA). Air flow was calibrated before and after each sampling, with a mini-Buck Calibrator™ (Arelco, Fontenay/Bois, France). The level of pump noise was reduced by placing the pump in a shell equipped with cork and rubber. During the day, the shell was carried in a rucksack, facilitating movement during transport. During the night the personal sampler was located in the living room. In all cases, the samplers were placed on a table or a desk in the home or in the office. Children were asked to carry the rucksack during all their moving. Special guidelines were given to professors.

Each filter was weighted before and after sampling with a microbalance (Sartorius, M5P). Filters were stored at 4°C after sampling. Before analysis, they were placed during 24h in a chamber continuously monitored and controlled for temperature ($20 \pm 1^\circ\text{C}$) and humidity ($50 \pm 5\%$). After deionisation (Haug, AMG), filters were weighed two successive times. If the difference between these two measurements was higher than 4 µg, measurements were repeated until the difference was equal or lower to 4 µg. Personal exposure to PM_{2.5} (in µg/m³) was estimated as the difference between the two filter masses before and after sampling, divided by the total air volume. The reproducibility of these methods had been assessed and validated [Mosqueron *et al.*, 2002].

Assessment of confounders or effect modifiers

Standard questionnaires on home environment written in the framework of the European Expolis Study [Jurvelin *et al.*, 2001] were filled during the home visit to collect data on home equipment with sources of pollutants emissions (air and water heating systems, energy used for cooking, ventilation, wood-pressed products, refurbishment...).

The morning after the 48h-measurements, each child was asked to provide a sample of first urine for the cotinine dosage. Urine samples were stored at -20°C until analysis. Cotinine was measured on a Beckman CX7 automated analyzer by an enzyme immunoassay (EIA) adapted by our team [Roche *et al.*, 2001] to assess urinary cotinine in nonsmokers exposed to tobacco smoke and which detection limit is 1 µg/L. This measurement reflects environmental tobacco smoke (ETS) exposure to which the child was exposed during the 48h-measurements.

Indoor concentrations of formaldehyde and acetaldehyde were also measured by placing the sampler in the living room, 48h before nasal lavage procedure. A similar SKC pump with an airflow set at 200 ml/min was connected to a SupelcoTM aldehydes cartridge (SupelcoTM, Bellefonte, Pennsylvania, USA) impregnated with 2,4-dinitrophenylhydrazine. Duplicate samples were taken for 10% of the population chosen randomly, to test the reproducibility of measurements. Sample analyses were performed on a Perkin-Elmer HPLC chromatographic chain (Norwalk, USA) using UV detector operated at a wavelength of 365 nm. Hydrazone separation was carried out on a Kromasil C18 column (250 µm, 4.6 mm id, 3.5 µm) associated with a 5C18 precolumn (Restek, Bellefonte, Pennsylvania, USA). The mobile phase was made of acetonitrile, water and tetrahydrofuran at a flow rate of 1 ml/min. An elution gradient was used to separate the different compounds. Formaldehyde and acetaldehyde are expressed in µg/m³.

Current exposure to house-dust mites was assessed by collecting dust from the mattress and the floor of the bedroom and from the living room with a vacuum cleaner [Dillon *et al.*, 1999]. The Acarex tests® kits (Karapharm, Marseilles, France) allow a semi-quantitative assessment of the house-dust mites concentration with a four-step color scale (negative, mild, moderate, strong).

The pollen data were supplied by the National Survey Network for Aerobiology (RNSA) and are expressed as the total number of grain pollens / m³, counted during the 48h before NAL.

Meteorological data, ambient 8-hour mean and maximal ozone values and 24-hour PM₁₀ mean concentrations were selected for the two days preceding the hospital visit. Ambient ozone and PM₁₀ levels were taken from the Paris air quality-monitoring network (AIRPARIF) and meteorological data were provided by Météo France. All the children lived within the area covered by monitors of AIRPARIF network.

Nasal inflammation assessment

Nasal inflammatory status was evaluated by a NAL method adapted from the Hilding procedure, previously developed by our team for children [Nikasinovic-Fournier *et al.*, 2002]. Briefly, during sampling performed in a sitting position, the subject was instructed to breathe by mouth, and to tilt his/her head forwards at a 60° angle with the vertical. NAL consisted of irrigation of the nasal cavity (three instillation / aspiration cycles for each nostril) with saline solution (NaCl 9‰) *via* a transparent inflated pediatric Foley catheter (Porgès S.A., Le Plessis Robinson, France), gauge 8 or 10, according to nose dimensions, in order to assure a comfortable sampling for the volunteer. Using a needleless syringe, 37° C sterile saline was instilled (3 to 5 mL) and then after 10 seconds aspirated, corresponding to one instillation/aspiration cycle. The NAL fluids (NALF) recovered from each nostril were pooled. The recovered fluid was centrifuged (500 x g) for 15 min at +4°C. The supernatant was aliquoted and stored at - 70°C for later biochemical analysis. The pellet was treated with an equivalent volume of 2,3-dihydroxy 1,4- dithiolbutane (Digest-EUR®, Eurobio, Les Ullis, France) in RPMI media (Sigma Aldrich, Saint Quentin Fallavier, France) and kept at room temperature for 45 min. Total cell count including leukocytes and epithelial cells was determined on a hemocytometer. Differential cell count was performed on cytopins (Shandon cytospin; Shandon, Eragny/Oise, France) and two slides were stained with May-Grünwald Giemsa (Sigma Aldrich, Saint Quentin Fallavier, France). A minimum of 200 nucleated cells was counted in a blind manner and absolute number for each cell type (neutrophils, eosinophils, lymphocytes and epithelial cells) was calculated.

NAL biomarkers assessment

Two permeability markers, albumin and urea, and one antioxidant, uric acid, were measured. Their concentration measurements were conducted on a Monarch IL analyzer (Monarch Instrumental Laboratory, Paris, France) using immunonephelometric method for albumin and enzymatic method for urea and uric acid. The lower detection limits were 2 mg/L, 30 µmol/L and 2 µmol/L, respectively. Inflammation status was evidenced by the pro-inflammatory cytokin IL-6, GM-CSF, and the chemoattractant IL-8, and the two Th2 cytokines, IL-4 and IL-5, using an ELISA kit (R&D systems Europe, Lille, France). The lower detection limits were 3 ng/L, 3 ng/L, 10 ng/L, 10 ng/L, and 3 ng/L, respectively. In addition, ECP, a marker of eosinophil activation was assessed using an ELISA kit (Pharmacia&UpJohn, Saint-Quentin en Yvelines, France) with a lower detection limit of 0,5 µg/L. Protease-anti-protease balance was assessed by measuring total neutrophil elastase, as a marker of neutrophil activity (ELISA kit from VWR International, Fontenay/Bois, France) and its major inhibitor, α 1-antitrypsin using the ELISA method. The lower detection limits were 3 µg/L and 1 µg/L, respectively.

Statistics

Data were processed using BMDP software package (University of California, Los Angeles, USA). The normality of biomarkers distribution was first tested by the Shapiro Wilk's test. Because most of these distributions were skewed, we used log transformation or, if necessary, the Box Cox transformation $f(X) = (X^\lambda - 1)/\lambda$ where X is the biological variable to transform and λ a coefficient (generally, 0.1, 0.2 or 0.3).

For each group of children (asthmatic and healthy), a bivariate and a multivariate analyses were performed. A Pearson correlation test was applied to evaluate correlations

between biomarker concentrations. To evaluate the association between air pollutants and nasal inflammatory biomarkers, a linear regression model was built for each biomarker including traditional confounders such as age, gender, exposure to ETS as assessed by urinary cotinine levels, and all variables associated in the bivariate analysis with a p value less than 0.20. For asthmatic children, nasal corticosteroid treatment, dust-mites allergen exposure and pollen exposure were added in the models. Results of the association are expressed by the standardized regression coefficient and the coefficient of determination (R^2). A p value of less than 0.05 was considered statistically significant.

RESULTS

Description of study populations

A total of 91 children participated in this study. There were 23 boys and 23 girls in the 46 asthmatic children group and 22 boys and 23 girls in the 45 healthy children group. The mean age was 11 ± 2 years for asthmatic children and 10 ± 2 years for controls. The majority of asthmatic children had regular bronchial corticosteroid treatment and most of them reported nasal symptoms the year before interview but only 7% used nasal corticosteroids the week before NAL. Children were mostly sensitized to *Dermatophagoides pteronyssinus*, cat and timothy grass.

The number of children living in Paris or in its close suburb was similar in both groups and about 80% lived in a flat. There was no difference between groups concerning potential sources of indoor pollution, except for ETS exposure. In the asthmatic group, regular paternal smoking was more frequent than in the healthy group. In contrast, no difference was observed in the reported number of cigarettes smoked during the 48h-period preceding the NAL.

Air pollutants and allergen levels

No difference was observed between the two groups, considering the 48h preceding NAL mean ozone levels ($28 \pm 20 \mu\text{g}/\text{m}^3$ for controls and 31 ± 21 for asthmatics) and pollen grains numbering (67 ± 119 for controls and 74 ± 109 for asthmatics).

The personal exposure levels were similar for both groups (Table 1). $\text{PM}_{2.5}$ measurements were available for 82 of 91 samples since nine pumps had technical problems during exposure assessment. $\text{PM}_{2.5}$ mean level was moderately elevated ($36.6 \pm 46.5 \mu\text{g}/\text{m}^3$) although they seemed higher in controls than in asthmatics ($p=0.27$). The mean PM_{10} concentrations for the whole population (22.7 ± 8.0) obtained by the AIRPARIF network were not correlated with personal $\text{PM}_{2.5}$ levels ($r=0.08$; $p=0.47$).

NO_x badges were successfully analyzed for 90 of the 91 participants. NO_x and NO_2 were moderately correlated ($r=0.44$, $p<10^{-3}$). Formaldehyde and acetaldehyde levels did not differ between the two groups but were significantly correlated ($r=0.77$, $p<10^{-3}$). Dust mites allergens classes were mostly negative or mild in both groups. As for cotinine levels, they were similar in both groups and were significantly correlated with parental smoking reports ($r=0.35$, $p<10^{-3}$).

Nasal biomarkers

A total of 91 NAL were performed during the study period, and mostly between September and June. In the healthy children, the cellular profile was marked, for a half, by epithelial cells and by few neutrophils (8%) and eosinophils (less than 4% of total cells). In

asthmatic children, the cellular profile consisted in about 40% of epithelial cells, followed by neutrophils (25%) and eosinophils (11%). The eosinophil percentage was significantly higher in the asthmatics ($p=0.02$).

IL-4, IL-5, ECP and GM-CSF were difficult to detect in all samples. For the other mediators, their concentrations did not differ significantly between the two groups.

Nasal biomarkers, indoor and outdoor air pollution and allergen exposures

In healthy children, urinary cotinine was significantly related to epithelial cell counts that also tended to correlate with NO_x ($p=0.07$). The regression model explained 35% of epithelial cells variability. Neutrophils and eosinophils were not related with any of the determinants tested.

In asthmatics, a consistent effect of PM_{2.5} was evidenced for most of the biomarkers. PM_{2.5} concentrations resulted in significant increases in neutrophils and eosinophils counts, the determination coefficients being 52% and 33% respectively. Exsudation biomarkers were also significantly correlated with PM_{2.5} concentrations, particularly urea levels, since the model explained up to 60% of the variations. Our regression models failed to show any significant relation between PM_{2.5} and interleukins concentrations, but for IL-6, results are close to statistical significance ($p=0.08$). In contrast, no correlation was found between NO_x and any of the biomarkers.

DISCUSSION

The present study shows, for the first time, in children with mild to moderate allergic asthma, an association between NALF inflammatory biomarkers and personal PM_{2.5} exposure. Exposure to PM_{2.5} results in a significant increase in neutrophils and eosinophils numbers, associated with increased levels of exsudation biomarkers, albumin, urea and α 1-antitrypsin. These associations with PM_{2.5} levels were not observed in healthy children.

To the best of our knowledge, this is the first epidemiological study dealing with nasal inflammation that uses personal measurements of the exposure to two major outdoor air pollutants in urban areas, PM_{2.5} and NO_x. In all previous works, the assessment of exposure to air pollution was based on fixed site measurements provided by the local air quality monitoring network [Frischer *et al.*, 1993; Hiltermann *et al.*, 1997; Kopp *et al.*, 1999; Steerenberg *et al.*, 2001]. These studies mainly investigated ozone and PM₁₀ effects on nasal inflammation. PM₁₀ are of particular importance since they are inhalable. Most of them are smaller than 2.5 μ m and are therefore called fine particles [Ormstad *et al.*, 1998]. We chose to measure PM_{2.5} concentrations that are more relevant PM in the context of respiratory health. Moreover, experimental studies on deposition and clearance of inhaled particles in the human nose indicate that, when the subject inhales and exhales through the nose, about 60 to 100% of PM_{2.5} are deposited in the nose [Lippmann, 1970]. Another key point in our study is that all the main potential confounding exposures, except pollen counts, were assessed by personal or indoor measurements, thus reducing misclassification.

With regard to nasal inflammation assessment, a non-invasive well-tolerated and reproducible method was used to estimate mucosal inflammation. Compared to healthy children, a shift in the cellular profile toward a decrease in epithelial cells and an increase in leucocytes and particularly in eosinophils, was observed in the asthmatic group, suggesting that infiltration of these cells in nasal mucosa of allergic children was more important than in

healthy subjects, as previously described [Frischer *et al.*, 1993; Beppu *et al.*, 1994; Benson *et al.*, 1999]. These cellular profiles are in accordance with those obtained in other epidemiological studies. In this study, we also measured several soluble mediators. NALF albumin, urea and uric acid mean concentrations for the total population were in close agreement with previously published data for children [Steerenberg *et al.*, 2001].

All detectable markers but eosinophils did not differ between asthmatic and healthy children. One possible explanation is that all children living in urban areas might present a “physiological inflammation” resulting in elevated NALF biomarkers, whatever their health status. This hypothesis is supported by Frischer *et al.* [1993] who demonstrated that urban children had significantly higher mean levels of IL-8 (32%), urea (39%), uric acid (26%), albumin (15%) and nitric oxide metabolites (21%) in NALF than did suburban children. The similarity in biomarkers levels between asthmatic and healthy children may also be related to the lack of specific allergic mediators such as IL-4 and IL-5 in the comparison. This result thus deserves other investigations.

The most consistent result of the present study is the highly significant impact of PM_{2.5} exposure on leukocytes counts in asthmatic children. The regression model was able to explain more than 50% of the variations for neutrophils and 33% of the variations for eosinophils. To our knowledge, this is the first epidemiological study to show the effect of PM_{2.5} exposure on leukocyte infiltration in nasal mucosa. Previous works had found that NAL inflammatory biomarkers were rather related to ozone levels but none demonstrated leukocytes increase in relation to PM₁₀ exposures [Frischer *et al.*, 1993]. Other authors examined nasal inflammatory response associated with several air pollutants such as nitrogen oxides, carbon monoxide, black smoke, PM₁₀, sulfur dioxide and ozone [Hiltermann *et al.*, 1997; Steerenberg *et al.*, 2001]. In an epidemiological study with repeated measures conducted in Leiden (The Netherlands), effects of photochemical air pollution on nasal inflammation were evidenced in asthmatic adults [Hiltermann *et al.*, 1997]. Ozone was associated with both neutrophilic and eosinophilic inflammation, with the strongest effect on cellular counts for ozone levels recorded the previous days and the strongest effect on soluble mediators for ozone levels of 3 days before. In contrast, no association between nasal inflammation and PM₁₀ concentrations was detectable, except for epithelial cells counts which increased with the mean weekly PM₁₀ levels. Authors indicate that this negative result was likely to be related to low PM₁₀ levels and to low ambient PM₁₀ fractions generated by high diesel traffic emissions. However, their mean PM₁₀ level assessed in an ecological way was 40 µg/m³, with a 95% interval confidence of 21 to 76 µg/m³. Our mean PM₁₀ levels for the 48h preceding the hospital visit (22.7 ± 0.8 µg/m³) are 2-fold lower than those measured in Leiden. We also failed to find a significant association between these mean PM₁₀ and nasal biomarkers. In another study, Steerenberg *et al.* [2001] compared mean levels of nasal inflammatory biomarkers on urban and suburban children. They did not evidence any effect of PM₁₀ exposures on total cell counts (leukocytes counts were not available).

Taken together, these results indicate that the ecological measurements of PM₁₀ might be irrelevant for the study of respiratory inflammation. Indeed, personal PM levels are generally higher than levels obtained by collective measurements from air quality networks; this phenomenon has been described as “personal cloud” [Mosqueron, 2002].

In addition, our results show that albumin, urea and α 1-antitrypsin concentrations were significantly influenced by PM_{2.5} concentrations, suggesting that these particles were

able to increase membrane permeability of nasal epithelium and subsequent inflammatory reaction marked by inflammatory cells influx. Interestingly, these associations were observed at levels of PM_{2.5} considered to be safe according to WHO guidelines [Cotton, 1993].

The association found between PM_{2.5} and NALF inflammatory mediators from asthmatic children is in line with experimental studies conducted recently on DEP. Short-term DEP exposure resulted in marked epithelial inflammation and demonstrated that DEP exposure led to intrinsic effects as well as adjuvant-like effects when DEP was co-administered with allergens [Diaz-Sanchez *et al.*, 1999a]. This latter design suited better to environmental atmosphere where particulate and allergens often co-exist. Moreover, DEP and soot particles have been found to adsorb proteins *in vivo* [Muranaka *et al.*, 1986; Takafuji *et al.*, 1989], suggesting that particles are carriers of allergens. In addition, Ormstad *et al.* [1998] reported that PM_{2.5} fraction of suspended particulate matter have important allergens attached to their surface which could be regarded as a depot of allergens carried into the airways, by concentrating many allergen proteins.

CONCLUSION

In conclusion, this study shows for the first time that personal PM_{2.5} exposure, at levels commonly encountered in urban areas, is related to nasal inflammation in children with allergic asthma but not in healthy children. The strongest association is observed with eosinophil counts, which supports recent speculations on DEP involvement in allergic phenotype over expression.

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Table 1. Personal and indoor exposure levels in Paris study (n=91)

Air pollutants	Healthy children n= 45		Asthmatic children n=46	
	Mean \pm SD	Median [range]	Mean \pm SD	Median [range]
PM2.5 ($\mu\text{g}/\text{m}^3$)	42.0 \pm 59.5	26.1 [18.8-43.8]	30.6 \pm 25.0	22.1 [13.7-35.4]
NOx ($\mu\text{g}/\text{m}^3$ equivalent NO ₂)	49.8 \pm 23.9	46.0 [34-64]	57.2 \pm 35.5	48.0 [39.5-66.8]
NO ₂ ($\mu\text{g}/\text{m}^3$)	34.3 \pm 10.9	33.0 [27.0-44.0]	35.7 \pm 10.6	36.5 [30.0-44.0]
Formaldehyde ($\mu\text{g}/\text{m}^3$)	40.5 \pm 18.7	33.7 [26.8-51.2]	39.1 \pm 22.5	37.7 [24.0-49.7]
Acetaldehyde ($\mu\text{g}/\text{m}^3$)	10.7 \pm 10.4	9.5 [0-16.9]	9.1 \pm 10.0	8.5 [0.0-14.4]
Urinary cotinine ($\mu\text{g}/\text{L}$)	11.9 \pm 15.7	5.0 [0-72]	10.1 \pm 16.2	6.1 [0-60]
Dust mites allergens (%)				
<i>Negative</i>	46		39	
<i>Mild</i>	53	-	54	-
<i>Moderate</i>	1		7	
<i>Strong</i>	0		0	

Table 2. NAL biomarkers levels in Paris study (n=91)

Biomarkers	Healthy children n= 49		Asthmatic /allergic children n=42	
	Mean \pm SD	Median [1 st –3 rd quartile]	Mean \pm SD	Median [1 st –3 rd quartile]
Total cells x 10 ³ /ml	178.3 \pm 359.7	81.6 [26.7-155.2]	178.4 \pm 230.6	79.7 [11.2-23.0]
Epithelial cells x 10 ³ /ml	97.9 \pm 306.4	48.6 [17.8-75.1]	54.6 \pm 66.1	31.2 [5.3-89.0]
Neutrophils x 10 ³ /ml	47.9 \pm 84.9	6.3 [0-53.8]	52.0 \pm 90.6	20.2 [0.3-65.6]
Eosinophils x 10 ³ /ml	20.7 \pm 40.1	3.1 [0.0-22.9]	36.6 \pm 57.5	8.4 [0.1-36.6] †
Albumin (mg/L)	59.0 \pm 55.8	35.7[23.8-77.5]	65.9 \pm 117.3	26.8 [13.8-84.4]
Uric acid ($\mu\text{mol}/\text{L}$)	10.9 \pm 6.3	10.1[7.0-15.1]	13.4 \pm 7.9	12.1 [7.8-17.0]
Urea ($\mu\text{mol}/\text{L}$)	666 \pm 309	591 [443-837]	682 \pm 295	598 [452-883]
α 1-antitrypsin ($\mu\text{g}/\text{L}$)	1471 \pm 1448	980[514-1915]	1442 \pm 1583	916 [389-2049]
Elastase ($\mu\text{g}/\text{L}$)	7.1 \pm 8.4	3.4 [1.8-8.8]	9.8 \pm 19.2	2.3 [0.8-9.2]
IL-6 (ng/L)	42.9 \pm 96.1	6.6 [2.6-30.2]	28.5 \pm 36.8	8.1 [1.7-51.3]
IL-8 (ng/L)	1043 \pm 721	868 [538-1380]	1098 \pm 1157	680 [258-1540]

Abbreviation: SD: standard deviation; † : p<0.05