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Lower airway inflammation before and after house dust mite nasal challenge: An age and allergen exposure-related phenomenon

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Exhaled nitric oxide

Summary
Background: Upper and lower airways allergic disease is currently considered unitarily. Allergic inflammation in one site can extend to other sites of the respiratory tract.
Objective: To evaluate bronchial inflammation before and after allergen-specific nasal challenge (ASNC) in rhinitic and asthmatic children, considering the different levels of allergen exposure, i.e. summer (low) and winter (high).
Methods: Fourteen children with rhinitis and 15 with rhinitis and asthma, all monosensitized to mites and 10 healthy controls were studied. Nasal IgE were measured before ASNC in summer and in winter season. Nasal clinical score, eosinophil cationic protein (ECP), nasal tryptase, bronchial clinical score, FEV_1, PEF, sputum ECP, sputum tryptase and exhaled nitric oxide (eNO) were evaluated before and after ASNC in summer and winter season.
Results: Nasal scores significantly increased after ASNC in rhinitic and asthmatic children in both seasons. Nasal IgE were significantly higher in summer compared to winter. Bronchial symptoms, FEV_1 and PEF showed no mean differences in rhinitic and asthmatic children after ASNC, with an increase of bronchial symptoms and a decrease of FEV_1 and PEF occurring in 3/15 asthmatic children. In both groups nasal tryptase and ECP after ASNC significantly increased in summer and winter, while sputum tryptase was undetectable before or after ASNC in both groups. Sputum ECP and eNO at baseline were significantly higher in patients than in controls (summer $P = 0.002$, winter $P = 0.001$). Sputum ECP

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significantly increased after ASNC in 3/15 asthmatics in summer and in 11/15 in winter, as well as in 3/14 rhinitics in summer and in 4/14 in winter. eNO significantly increased after ASNC in 3/15 asthmatics in summer and in 10/15 in winter, and in 1/14 rhinitics in summer and in 4/14 in winter. A significant median increase of sputum ECP ($P = 0.0007$) and eNO ($P = 0.0012$) after ASNC in asthmatic and of eNO ($P = 0.013$) in rhinitic children was also found in winter.

Conclusions: Basal sputum ECP and eNO values, significantly higher before ASNC in rhinitic patients compared to control subjects, confirm the inflammatory link of upper and lower airways. The more frequent detection of inflammatory changes induced by ASNC in winter suggests that allergen exposure favours the transfer of nasal inflammation to lower airways.

Introduction

In the ARIA document, a position paper recently published by WHO, a new classification of allergic rhinitis was presented, which underlines the importance to consider unitarily upper and lower airway disease.[1]

To support the relationship among different sites of the respiratory tract in clinical conditions such as rhinosinusitis, nasal poliposis and asthma, recent studies have demonstrated that inflammation in one site can extend to other sites of the respiratory tract.[2,3]

Analysis of blood samples and biopsies before and after allergen segmental bronchial challenge (ASBC) in patients with allergic rhinitis showed a significant eosinophil increase in pulmonary tract biopsies distant from the site of challenge and also in nasal epithelium.[4,5]

In a recent study on rhinitic patients monosensitized to grass pollen out of season, a significant increase of eosinophils, bronchial symptoms and a significant decrease of FEV_1 after allergen-specific nasal challenge (ASNC) was observed.[6] Moreover, several studies on adult rhinitic patients have evidenced the presence of eosinophils after ASNC in induced sputum.[7–10] By contrast, another study reported that nasal provocation with house dust mite did not cause a decrease in FEV_1 and concomitant wheezing in any of the 15 rhinitic patients studied and only in 3/5 rhinitics with a previous history of asthma.[11] As to effect of anti-inflammatory drugs, old studies,[12–14] subsequently not confirmed,[15] showed that nasal corticosteroid therapy achieved a reduction of bronchial symptoms and bronchial hyperreactivity in patients with allergic rhinitis and asthma to grass pollen.

In order to better understand the link between upper and lower airways interaction, we evaluated bronchial inflammation before and after ASNC in rhinitic and asthmatic patients allergic to mite, in two different periods of low (summer) and high (winter) allergen exposure.

Material and methods

Fifteen children (age range 6–10 years) with rhinitis and episodic asthma, 16 children with isolated rhinitis, all monosensitized to dust mites, and 10 healthy non-atopic controls were enrolled in the study. Diagnosis of asthma was defined according to clinical history, clinical evaluation during acute symptoms and FEV_1 increase after inhaled salbutamol. Asthma severity was classified as level 1 according to the GINA international guidelines.[16] In both groups the rhinitis was mild or moderate persistent, according to ARIA document.[1] Exclusion criteria included local or systemic anti-inflammatory therapy (corticosteroids, DSCG, anti-leukotrienes, H1-anti-histamines) 2 months prior to the study, specific immunotherapy, malformations or chronic inflammatory diseases of airways and severe systemic diseases. Both groups were instructed to enclose mattresses and pillows in non-allergenic covers. During the follow-up period patients could occasionally use oral cetirizine for nasal symptoms and salbutamol and oral metilprednisone for acute asthma. The study was approved by the ethical committee of the University of Perugia and all children's parents gave their written informed consent.

In patients skin prick tests (SPT) and serum-specific IgE were evaluated at the beginning (July) of the study. Nasal-specific IgE were measured before ASNC in summer and in winter season. Nasal and sputum tryptase, nasal and sputum eosinophil cationic protein (ECP), nasal and bronchial clinical scores, spirometry and exaled nitric oxide (eNO) were evaluated in all patients before and after ASNC at the beginning of the study in summer (low exposure) and at the end in winter (high exposure). At baseline, nasal-specific IgE, nasal and sputum tryptase, nasal and sputum ECP, spirometry and eNO were evaluated also in the control group. The coefficient of variation (CV) of sputum ECP and eNO was evaluated twice in 120 asthmatic patients. In the study patients tryptase, ECP and eNO after ASNC were considered significant if they showed an increase of 50% compared to basal values before ASNC.

Skin prick test

SPT were performed with commercial glycerinated extracts (Alk-Abellò, Lainate, Italy). The standard panel of allergens included *Phleum pratensis*, *Parietaria judaica*, *Olea europaea*, *Dermatophagoides pteronyssinus*, *Alternaria tenuis*, cat, and dog epithelium, plus a positive (histamine 10 mg/mL) and a negative (normal saline) control. Wheals were contoured with a soft pen and transferred onto sticky tape. Skin reactivity was measured as the mean of the major diameter of the wheal plus its orthogonal and graded from 0 = negative to ++++ = highly positive, compared to

hystamine (considered +++−). A skin reaction of 3 mm was considered the threshold for positivity.

Serum IgE

Specific serum IgE were measured with UNICAP SYSTEM IGE FEIA method (Pharmacia, Uppsala, Sweden) for the same allergens tested in SPT.

Nasal-specific IgE

Allergen-specific nasal IgE were measured using a previously described method,[17] modified as follows. Briefly, seven allergen covalently coated paper disks plus a negative control disk with chicken albumin (Sigma Aldrich, Milan, Italy) were fixed on two sides of a plastic applicator (6 cm length and 0.6 cm width). The allergens were the same used for the prick test: *Phleum pratensis*, *Parietaria judaica*, *Olea europaea*, *D. ptironyssinus, A. tenuis*, cat epithelium, and dog epithelium Alk-Abellò). The applicator carrying the disks was covered with a permeable polyamide monofilament membrane (03-64/45-SEFAR Heiden, Switzerland). The prepared device was inserted into a nostril of the subject and left inside for 20 min. The applicator was then removed from the nostril, disks were put into a tube containing a buffer solution (NaCl 0.9%, Tween 0.1% and NaN_3 0.05%) and stored at 4 °C. All assays were performed at the same time. After 10 min washing, the allergen-coated disks were incubated for 30 min with a biotinate goat IgG anti-human IgE (VECTOR Laboratories, Peterborough, England) then washed and incubated for 30 min with Streptavidine-β-galattosidase (Boehringer Ingelheim, Milan, Italia), washed again and incubated for 10 min with the fluorimetric development kit (CAP System FEIA Pharmacia) and determined, as compared to a standard reference curve (CAP System FEIA IgE Pharmacia) performed with goat IgG anti-human IgE-coated paper disks (VECTOR Laboratories, Peterborough, England). Values equal or greater than 0.35 kU/L were considered positive.

Nasal ECP and tryptase

Tryptase and ECP levels were determined by ELISA (UniCAP Tryptase System FEIA and UniCAP ECP System FEIA, Pharmacia) adapted for mucosal sampling.[18] For ASNC the measurement of tryptase was made 30 min after and that of ECP 24 h after. Briefly, the sponges of the CAP System, coated with anti-tryptase or anti-ECP antibodies, were washed with physiologic solution and then mounted on an appropriate plastic stick and fixed by a permeable envelope. The plastic stick carrying the sponges was then inserted into a nostril and incubated in situ for 10 min fixed with a tape externally on the nose. The sponges were then removed and frozen at −20 °C in buffered (NaCl plus NaN3 0.1%) vials. At the end of the trial, all sponges were washed 3 times for 10 min with NaCl 0.9% and Tween 0.1%, reinserted into CAPs and run at the same time with Unicap System FEIA Pharmacia. The concentration of tryptase and ECP was expressed in μg/L according to a calibration curve.[19]

Sputum ECP and tryptase

Sputum was induced by having the subject inhaled hypertonic saline solution 4%.[20] Briefly, prior to sputum induction, all subjects inhaled a β_2-agonist to avoid hypertonic saline solution-induced bronchoconstriction. The subjects were asked to rinse their mouth and throat every 5 min and then to try to cough sputum into a sterile plastic container. The nebulization was continued for at least 10 min and stopped after 20 min or earlier if a $\geqslant$2 mL sputum sample of good quality was obtained. The collected sample was weighted, mixed with an equal volume of a solution containing dithiothreitol (DTT) 0.1%, and then centrifuged at 2000*g* for 20 min. The supernatant was aspirated and ECP and tryptase were measured in duplicate using the UniCAP Tryptase System FEIA and UniCAP ECP System FEIA (Pharmacia).

Spirometry

Spirometry was performed with a Microloop Micro Medical spirometer (Micro Medical, Rochester, England). The subjects were asked to perform subsequently three times a maximum expiratory effort immediately following a maximum inspiration, with a rapid start, and the best FEV_1 was registered.

Exhaled NO

Exhaled NO was measured using a chemoluminescence NO analyzer (Eco Physics CLD 77 AM, Eco Physics, Switzerland) with a detection limit of 0.02 parts per million (ppm). The zero signal was calibrated with an air filter (Sievers AFL 01410-01) and the measurement scale was calibrated with a gas containing 100 ppm of NO in nitrogen (MESSER, Turin, Italy). Subjects at rest, sitting down and wearing a mouthpiece, performed a single exhalation from total lung capacity without breath-holding and were encouraged to maintain a constant expiration throughout the manoeuver.

ASNC, nasal and bronchial clinical scores

ASNC was performed by spraying the vehicle into a nostril followed by increasing concentrations of house dust mite extract: 2, 4, and 8 BU/mL (Alk-Abellò). These concentrations correspond respectively to about 0.016, 0.032, and 0.064 μg per puff of the major allergen.

Nasal symptoms were evaluated attributing an arbitrary score to itching, sneezing, rhinorrhea and blockage as follows: 0 = absent, 1 = mild, 2 = moderate, 3 = severe. Asthma symptoms considered were: coughing (asthma related), wheezing and breathing difficulty. Severity of asthma symptoms was evaluated attributing an arbitrary score to each symptom as follows: 0 = absent, 1 = mild, 2 = moderate, 3 = severe. A total score increase of at least 3, between basal and after ASNC values, was used to define the threshold dose for the positive challenge.

Statistical analysis

Sputum ECP and eNO were evaluated twice in 120 patients and within-subjects standard deviation was also used for computation of the CV. The Wilcoxon signed rank test for paired data was used to detect differences between basal and after-ASNC values of nasal and sputum tryptase, nasal and sputum ECP and eNO. Mann-Whitney test was used for intergroup analysis of the same values and for basal IgE values, evaluated during maximal and minimal exposure period in the two groups. A *P*-value lower than 0.05 was considered significant.

Results

In non-atopic healthy controls nasal-specific IgE and tryptase were negative, nasal ECP showed a median value of 7.6 mcg/L (range <2–22) (Table 1). In these children sputum tryptase was negative, sputum ECP had a median value of 5.31 mcg/L (range <2–11.25) and eNO showed a median of

Table 1 Normal values in 10 non atopic controls.

Non-atopic controls	Nasal IgE (kU/L)	Nasal tryptase (mcg/L)	Nasal ECP (mcg/L)
Mean	<0.35	<1	7.6
Range	<0.35−< 0.35	<1−<1	<2−22
Non-atopic controls	Sputum tryptase (mcg/L)	Sputum ECP (mcg/L)	eNO (ppm)
Mean	<1	5.31	8.47
Range	<1−<1	<2−11.25	3.88−16.42

Table 2 Clinical scores, FEV_1 and PEF.

Season	Baseline	After challenge	Baseline vs. after (*P*-value)
Nasal clinical scores			
Summer			
Rhinitis	2.56±2.92	6.78±3.49	0.014
Asthma	2.18±2.13	6.63±3.20	0.005
Winter			
Rhinitis	1.56±2.37	5.67±3.53	0.012
Asthma	2.18±2.67	5.65±3.67	0.027
Bronchial clinical scores			
Summer			
Rhinitis	1.13±1.45	2.47±2.13	NS
Asthma	2.28±2.56	3.03±3.34	NS
Winter			
Rhinitis	2.09±2.47	2.98±3.32	NS
Asthma	2.54±2.45	4.25±3.46	NS
FEV_1			
Summer			
Rhinitis	91±3.3	90±2.7	NS
Asthma	88.1±3.2	88±3.5	NS
Winter			
Rhinitis	89.7±5.2	87.6±2.3	NS
Asthma	88.2±4.3	87.3±2.9	NS
PEF			
Summer			
Rhinitis	87.4±6.8	92.1±4.8	NS
Asthma	86±4.6	86.9±3.4	NS
Winter			
Rhinitis	88.2±3.9	86.7±4.9	NS
Asthma	87.4±3.9	85.3±2.4	NS

8.47 ppm (range 3.88–16.42) (Table 1). Sputum ECP evaluated twice in 120 patients showed a CV of 33.6%; eNO evaluated twice in 120 patients showed a CV of 24%. For the aim of our study, tryptase, ECP and eNO were considered significant after ASNC if they showed an increase >50% compared to values before ASNC. Two rhinitic children were not evaluated, the first because he did not complete the study, the second because had persistent high values of sputum ECP (>200 mcg/L) with no variation in the different evaluations. Thus, the number of studied children with rhinitis was 14.

All patients reported occasional ingestions of oral cetirizine during winter. 12/15 asthmatic children occasionally took salbutamol and 5/15 oral methylprednisolone (mean assumption: 35 mg/month) in the last 2 months of winter. 13/15 asthmatics and 5/14 rhinitics reported to have used enclosed mattresses and pillows, as we suggested at the beginning of the study.

Table 3 Nasal tryptase, ECP and IgE.

Season	Baseline	After challenge	Basel vs. after (*P*-value)
Nasal tryptase (mcg/L)			
Summer			
Rhinitis	3.50±6.88	40.02±61.29	0.017
Asthma	19.25±29.48	28.67±36.59	0.021
Winter			
Rhinitis	12.03±26.75	57.77±70.50	0.012
Asthma	24.73±39.29	51.74±56.25	0.004
Nasal ECP (mcg/L)			
Summer			
Rhinitis	20.33±25.64	77.50±84.63	0.028
Asthma	47.59±62.05	95.34±68.68	0.003
Winter			
Rhinitis	17.44±35.33	73.88±77.73	0.011
Asthma	45.9±51.78	112.35±68.07	0.021
HDM nasal-specific IgE (KU/l)			
Summer			
Rhinitis	1.28±2.15		Summer vs. winter
Asthma	1.88±3.25		
Winter			
Rhinitis	6.14±8.16		0.038
Asthma	3.16±3.39		0.033

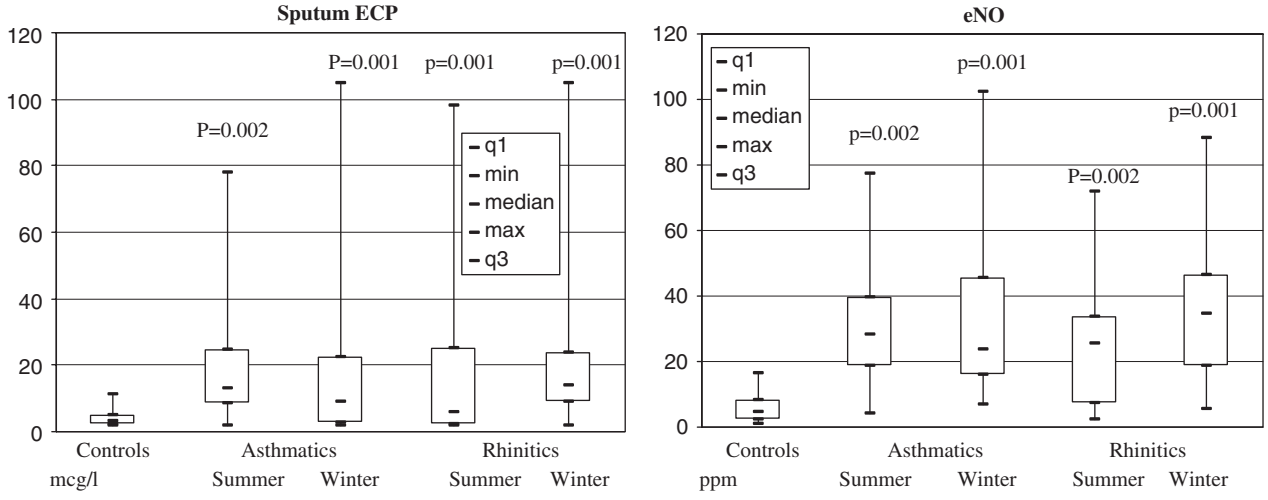

Figure 1 Comparison between baseline values of sputum ECP and eNO in patients and controls.

Rhinitic and asthmatic children monosensitized to mites showed a significant increase of nasal symptoms after ASNC in summer (rhinitics: $P = 0.014$, asthmatics: $P = 0.005$) and in winter (rhinitics: $P = 0.012$; asthmatics: $P = 0.027$) (Table 2).

In rhinitic patients symptoms of bronchial obstruction and significant decrease of FEV_1 or PEF after ASNC were not observed in both periods (Table 2). Three of 15 asthmatic patients showed symptoms of bronchial obstruction and a decrease of FEV_1 after ASNC in winter season, but in these patients a significant mean increase of bronchial symptoms or a decrease of spirometric values after ASNC was not apparent (Table 2). House dust mite nasal-specific IgE showed a significant increase in winter when compared to summer both in rhinitic ($P = 0.038$) and in asthmatic ($P = 0.033$) patients (Table 3).

Nasal tryptase in rhinitic and asthmatic patients was higher than in healthy controls and showed a significant increase after ASNC in summer (rhinitic $P = 0.017$, asthmatic $P = 0.021$) and in winter (rhinitic $P = 0.012$, asthmatic $P = 0.004$) (Table 3).

Nasal ECP in rhinitic and asthmatic patients was more elevated compared to healthy controls and showed a significant increase after ASNC in summer (rhinitic $P = 0.028$, asthmatic $P = 0.003$) and in winter (rhinitic $P = 0.011$, asthmatic $P = 0.021$) (Table 3).

Sputum tryptase before and after ASNC was not detectable in most cases and was not evaluated (data not reported).

The median basal values of sputum ECP in rhinitic patients was significantly more elevated than in control subjects in winter ($P = 0.001$) and in summer ($P = 0.001$) (Fig. 1). In these children sputum ECP after ASNC showed a significant increase ($>50\%$) in 3/14 cases in summer and in 4/14 in winter, when compared to values before ASNC (Fig. 2).

In asthmatic children, basal sputum ECP was significantly higher than in control subjects in winter ($P = 0.001$) and in summer ($P = 0.002$), but not significantly different from rhinitic patients (Fig. 1). In these asthmatic children sputum ECP showed a significant increase after ASNC only in 3/15 cases in summer and in 10/15 cases in winter (Fig. 2), but the global difference in these two groups was not significant.

In rhinitic children the median basal value of eNO was higher than in control subjects in winter ($P = 0.001$) and in summer ($P = 0.002$) (Fig. 1). In these children eNO showed a significant increase ($>50\%$) after ASNC in 1/14 cases in summer and in 4/14 cases in winter (Fig. 2).

In asthmatic children basal eNO was higher than in control subjects in summer ($P = 0.002$) and in winter ($P = 0.001$) period (Fig. 1). In these children eNO showed a significant increase after ASNC in 3/15 cases in summer and in 10/15 in winter (Fig. 2).

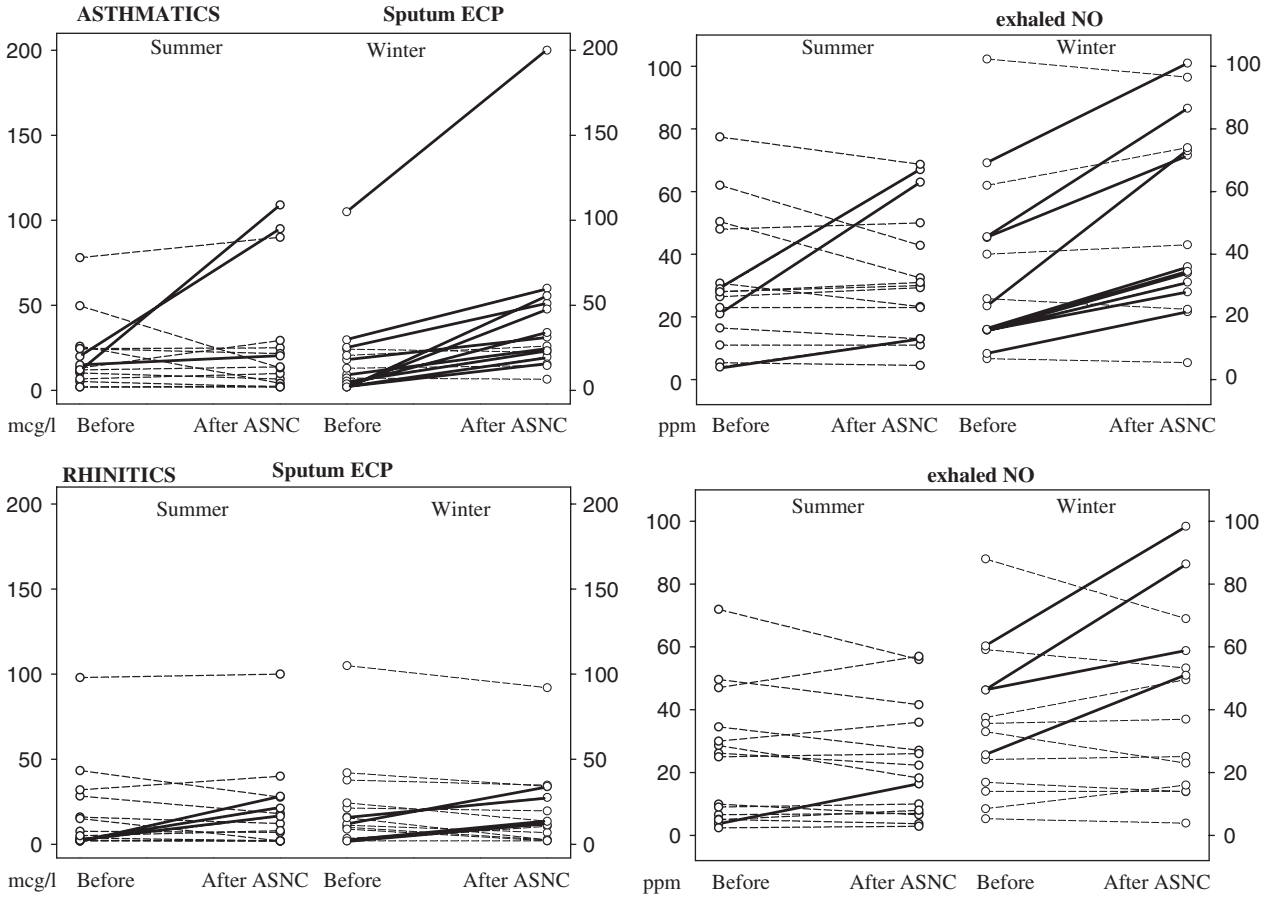

Figure 2 Sputum ECP and eNO in asthmatic and rhinitics in both periods, before and after ASNC. Continuous line = increase $\geqslant 50\%$, dotted line = increase $<50\%$.

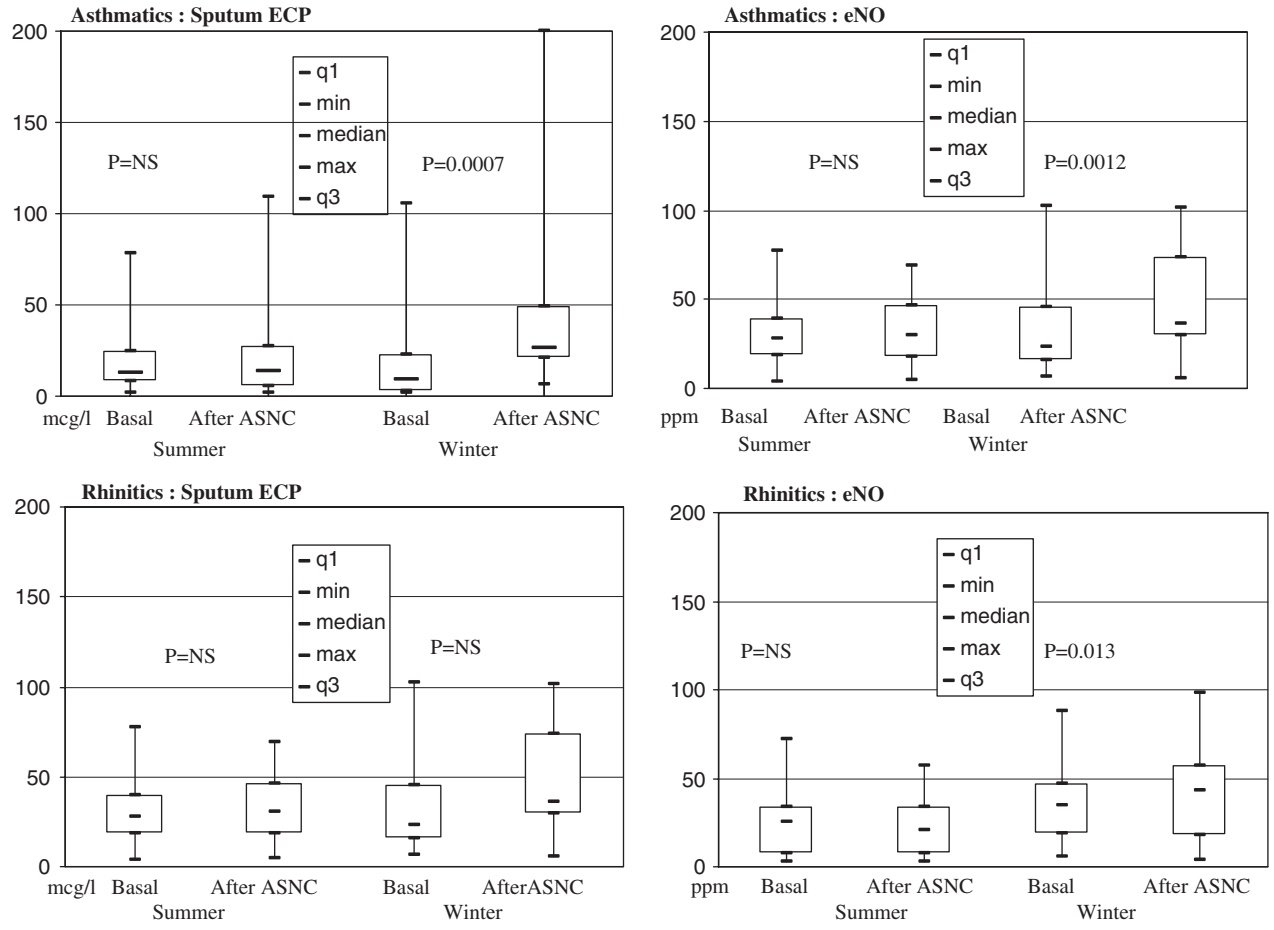

Figure 3 Median value of sputum ECP and eNO in asthmatic and rhinitic groups.

The median increase of sputum ECP and eNO after ASNC was significant in asthmatic group in winter season (respectively $P = 0.0007$ and $P = 0.0012$) (Fig. 3). In rhinitic group a significant median increase of eNO ($P = 0.013$) after ASNC was also observed in winter (Fig. 3).

Discussion

In recent years several mechanisms have been suggested to support the clinical evidence of a link between upper and lower airways.[2,3] Until now the most reliable mechanism is that allergic inflammation, which originates in a site of the respiratory tract, through bone marrow stimulation and cellular homing, is transferred to a different site of the respiratory tract.[4,5] The aim of our study was to evaluate bronchial inflammation before and after ASNC in asthmatic and rhinitic children monosensitized to mites in two different periods of low (summer) and high (winter) allergen exposure.

Our rhinitic and asthmatic patients showed before ASNC in both seasons a median basal sputum ECP value significantly higher than in control subjects. The basal sputum ECP data were also confirmed by median basal eNO which was more elevated in patients compared to control subjects (Fig. 1). These results, observed also in rhinitic children, support the concept of the inflammatory link between upper and lower airways. Similarly to a previous study[21] a significant difference of basal sputum ECP and eNO between rhinitic and asthmatic patients (all with episodic asthma) was not observed. This may be also due to the different compliance to environmental measures and use of oral corticosteroid therapy we observed. In fact, in 13/15 asthmatics but only in 5/14 rhinitics the enclosing of mattresses and pillows was performed and 5/15 asthmatics reported occasional oral methylprednisolone therapy. Differently from a previous study conducted in patients allergic to grass pollen out of season exposure,[6] our young patients monosensitized to mites showed after ASNC a significant increase of sputum ECP and eNO, more frequent in winter compared to summer season (Fig. 2). These data were also confirmed by the results of the significant median increase of sputum ECP ($P = 0.0007$) and eNO ($P = 0.0012$) after ASNC in the group of asthmatic patients in winter and of eNO (0.013) in the group of rhinitic patients in the same season (Fig. 3). A higher exposure to mites during winter, described in previous studies,[21,22] was indirectly confirmed in our study by the significant increase of nasal IgE in this period, compared to summer values (Table 3). Considering all these data, differently from other studies, we did not evidence significant variations in these mediators in the lower exposure period.

The difference between our data and Braunstahl study[6] may be explained by the different population enrolled in the two studies. In fact, we studied children with mild or

moderate persistent rhinitis and children with episodic, classified as GINA level 1, asthma, between 6 and 10 years of age, in which the link between nasal and bronchial inflammation is probably lower compared to adult patients with a long-lasting disease. Braunstahl patients were adults with at least 2 years of symptoms due to grass pollen. This hypothesis of a lower inflammation level in our children is supported by clinical scores and respiratory functional values observed in our patients before and after ASNC. In fact, unlike the above study,[6] we found symptoms of bronchial obstruction and variations in spirometric values after ASNC only in 3/15 asthmatic children. Our data are in agreement with three previous studies in which rhinitic patients did not show bronchial symptoms or spirometric changes after ASNC.[23–25] Furthermore, in a recent report nasal challenge with house dust mite did not elicit either a fall in FEV_1 or wheezing in any of the 15 rhinitic patients studied and only in 3/5 rhinitics with a previous history of asthma.[11] We should consider that the occurrence of wheezing and a fall of FEV_1 in rhinitic patients after ASNC in the study by Braunstahl et al. on adults could be due to the presence of an undiagnosed asthma condition, as supposed by the same authors.[6]

In a recent study, performed in adult patients allergic to mites, an increase of sputum ECP after bronchial allergen challenge was observed in asthmatic and in rhinitic subjects, but a significant increase of neutrophil, interleukin-8 (IL-8) and mieloperoxidase (MPO) was evident only in asthmatic patients.[21] These findings suggest that such specific immunologic modifications in lower airways predispose to the development or persistence of asthmatic symptoms in allergic subjects.[21] Our data following ASNC, as well as the absence of bronchial symptoms and lack of increase of sputum ECP and eNO in more allergic patients, should be explained with the hypothesis that the implication of neutrophil inflammation, reported in severe asthma,[26,27] is likely to be negligible in children with intermittent asthma. More asthmatic children in fact showed a further recovery of bronchial symptoms, that might be due to spontaneous immunological tolerance to inhaled antigen.[28]

In conclusion, the results of basal sputum ECP and eNO before ASNC, significantly more elevated in asthmatic and also in rhinitic patients compared to control subjects, confirm the inflammatory link of upper and lower airways. Differently from previous studies in adults, in our children the bronchial inflammation after ASNC is more apparent in winter. This observation, concerning not only the asthmatic but also some rhinitic children, support the concept that specific bronchial immunologic modifications,[21] induced by chronic disease, are needed to transfer nasal inflammation following ASNC to lower airways. The increase in sputum ECP and eNO after ASNC in rhinitic children without wheezing could suggest the use of these mediators as positive predictive markers of evolution towards asthma, but further prospective studies are necessary to confirm this hypothesis.

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