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ORIGINAL PAPER **Clinical Mechanisms in Allergic Disease**

Inflammatory and oxidative stress biomarkers in allergic rhinitis: the effect of smoking

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Summary

Background Accumulating evidence confirms the presence of pan-airway inflammation in allergic rhinitis patients. Smoking is known to affect the asthmatic airway inflammation. However, no study has evaluated the impact of smoking on airway inflammation of allergic rhinitis patients.

Objective The aim of the present study was to evaluate the impact of smoking on inflammatory and oxidative stress biomarkers in patients with seasonal allergic rhinitis, using non-invasive methods for sample collection.

Methods Forty patients with seasonal allergic rhinitis (20 smokers and 20 non-smokers) and 30 healthy subjects (15 smokers and 15 non-smokers) were recruited for the study during pollen season. All subjects were submitted to measurement of the fraction of exhaled NO (FeNO), exhaled breath condensate (EBC) collection, nasal lavage collection, pre- and post-bronchodilation spirometry and metacholine bronchial challenge testing. pH, leukotriene B_4 (LTB_4) and 8-isoprostane were determined in EBC and nasal lavage samples.

Results Patients with allergic rhinitis presented higher LTB_4 and 8-isoprostane levels in nasal lavage ($P < 0.0001$ for both comparisons), with no significant differences between smokers and non-smokers. Patients with allergic rhinitis also presented higher LTB_4 levels and lower pH in EBC ($P < 0.001$ and $P = 0.004$, respectively), with prominent differences between smokers and non-smokers ($P < 0.0001$ and $P = 0.003$, for LTB_4 and pH, respectively). A significant correlation between nasal lavage and EBC LTB_4 values was observed ($r_s = 0.313$, $P = 0.048$).

Conclusions Patients with allergic rhinitis present increased LTB_4 and 8-isoprostane in their nasal cavity, however, with no significant differences between smokers and non-smokers. In contrast, smokers with allergic rhinitis present higher LTB_4 levels and lower pH in EBC, suggesting that these patients may be more susceptible to the deleterious effects of smoking, compared with non-smokers.

Keywords allergic rhinitis, biomarkers, exhaled breath condensate, inflammation, smoking
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Introduction

Allergic rhinitis is a common disease that is reported to affect 10–40% of the global population and its prevalence is increasing both in adults and in children [1]. Previous studies have documented that allergic rhinitis and asthma co-exist in a great proportion of atopic subjects and have revealed that they share common pathogenetic mechanisms and inflammatory characteristics. Although the underlying pathways of airway inflammation in the lower respiratory tract of allergic rhinitis patients are not yet fully elucidated, several studies using invasive and non-invasive methods have demonstrated the presence of lower bronchial inflammation in this context. It has been shown that allergic rhinitis patients present bronchial hyperresponsiveness [2, 3], higher values of exhaled biomarkers, indicating atopic inflammation [such us exhaled nitric oxide (NO) [4] and leukotrienes [5]] as well as enhanced lower airway inflammation in induced sputum [3], bronchoalveolar lavage [6] and bronchial biopsies [7, 8], even in patients without asthmatic symptoms.

Smoking is known to affect the asthmatic airway inflammation, the asthmatic patients' response to treatment and the expression of genes pre-disposing to asthma [9].

Smoking induces oxidative stress [10] and is strongly related to neutrophilic inflammation [11]. Leukotriene B_4 (LTB_4), a neutrophilic chemoattractant, and 8-isoprostane, a product of free-radical catalyzed lipid peroxidation of arachidonic acid [12], have been associated with the inflammatory and oxidative burden caused by smoking [13–16]. Endogenous airway acidification, as evaluated by the pH in exhaled breath condensate (EBC), has also been related to neutrophilic inflammation and oxidative stress in patients with chronic obstructive pulmonary disease (COPD) [17] and asthma [18]. Although accumulating evidence confirms the presence of pan-airway inflammation in allergic rhinitis patients, many of the underlying mechanisms and characteristics remain unclear. Moreover, to our knowledge, no study has evaluated the impact of smoking on airway inflammation in patients with allergic rhinitis.

The aim of the present study was to evaluate the differences in the inflammatory and oxidative stress status in the nasal cavity and lower respiratory tract of allergic rhinitis patients compared with healthy controls, by assess ing LTB_4, 8-isoprostane and pH in nasal lavage and EBC. The impact of smoking on the inflammatory and oxidative stress biomarkers was further investigated. We tested the hypothesis that patients with allergic rhinitis would present higher nasal and lower respiratory tract levels of LTB_4 and 8-isoprostane, and lower pH values, compared with controls. We additionally hypothesized that these differences would be more prominent in smokers with allergic rhinitis, suggesting that these patients may be more susceptible to the deleterious effects of smoking, compared with non-smokers.

Materials and methods

Study subjects

Forty patients with seasonal allergic rhinitis (20 current smokers and 20 non-smokers) were included in the present study during pollen season. Allergic rhinitis subjects had no other history of respiratory disease, no symptoms related to asthma and no signs of respiratory infection during the last 4 weeks before their inclusion in the study. All had a normal spirometry and did not present significant increase in spirometry values after a bronchodilation test. Thirty healthy individuals (15 smokers and 15 non-smokers) served as controls. All smokers had a smoking habit <20 pack-years, in order to exclude patients with COPD. The study protocol was approved by the Ethical Committee of the University Hospital of Larissa and all participants provided written informed consent.

Study design

Allergic rhinitis subjects were enrolled during pollen season and were included in the study while they were symptomatic and before they received any medication (including topical corticosteroids, decongestants or oral antihistamines and anti-leukotrienes). Allergic rhinitis was defined based on compatible symptoms (nasal obstruction, rhinorrhea, sneezing, itching of the nose and/or postnasal drainage, with or without ocular symptoms) in patients with atopy. The classification of the disease severity was performed according to the ARIA guidelines [12]. All patients were suffering from moderate to severe allergic rhinitis. After their recruitment, all subjects were submitted to exhaled NO measurement, EBC collection, nasal lavage collection and finally a pre- and post-bronchodilation spirometry, in the aforementioned order. Blood was drawn for IgE measurements and skin prick tests were performed on a separate day for the evaluation of atopic status.

Evaluation of atopic status

Skin prick tests were performed on the volar surface of the forearm and the reaction was measured and recorded. The allergens used were dermatophagoides mix, birch, parietaria, cypress, sycamore, olive, alternaria, cladosporium, cat epithelium and dog epithelium. The negative control was glycerol/buffer diluent and the positive control was histamine (1 mg/mL). The reaction was evaluated 20 min after the administration and was characterized as positive if it was >3 mm greater than the negative control. Levels of total IgE were measured in the serum of all subjects by means of particle-enhanced immunonephelometry (Dade Behring, Marburg GmbH, Germany).

Fraction of exhaled nitric oxide measurement

Exhaled NO was measured with the use of a commercially available analyser (NiOX MiNO, Aerocrine, Sweden) as previously described [19].

Exhaled breath condensate collection

EBC samples were collected using a commercially available device (Ecoscreen, Viasys, Germany). Subjects were asked to rinse their mouth with distilled water and to perform tidal breathing through a mouthpiece for 15 min while wearing a nose clip. Samples of EBC (approximately 1.5–2 mL) were stored at $-70\,^{\circ}C$ immediately after the collection for no more than 3 months before the measurements.

Nasal lavage collection and processing

Nasal lavage was collected using a 12F bladder catheter EVO Enterprises, Malaysia with the distal tip cut-off. The subjects were asked to place their face up, the catheter was placed in the anterior cavity of each nostril and air was inflated until the catheter was secured and the entrance of the nostril was sealed. Then the subjects were asked to bend their head down and 4 mL of normal saline at a temperature of $37\,^{\circ}C$ were instilled in and aspirated from

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each nasal cavity three times before the sample was collected. The samples were further processed as previously described [20]. Briefly, samples were centrifuged at 400×*g* at 4 °C for 10 min and the supernatants were extracted and aliquoted in 0.5 mL samples and stored at −80 °C for subsequent measurements. Two slides were prepared for each patient and were stained with May–Giemsa. Differential cell counts were performed based on microscopy, where 400 cells were counted on each slide. The eosinophil and neutrophil count in nasal lavage was expressed as a percentage of the total cell count.

Pulmonary function and bronchial challenge testing

All measurements were carried out using a dry spirometer (Masterscreen®Body, Viasys, Hoechberg, Germany). Spirometry was performed according to the ATS recommendations [21]. Bronchodilation reversibility was evaluated according to the GINA guidelines [22]. A metacholine challenge test was performed in all rhinitis subjects to exclude concomitant asthma, using a commercially available system (Aerosol Provocation System, APS, Viasys, Germany). The APS provided a cumulative dose of 2000 μg metacholine in five increments following an initial dose of 0.9% NaCl. The cumulative dose that caused a 20% decline in baseline FEV_1 (PD_{20}) was determined by interpolation of the last two points on the dose–response plot. Patients with a PD_{20} >800 μg metacholine were excluded from further analysis.

pH measurements

pH was measured as previously described immediately after the collection of condensate and nasal lavage. Stable pH in EBC samples was achieved in all cases after deaeration of the condensate with an inert gas (argon, 350 mL/min for 10 min) and was measured in all samples using a pH meter (Jenway, model 3510, Gransmore Green, UK) [17].

Leukotriene B_4 and 8-isoprostane measurements

EBC and nasal lavage LTB_4 and 8-isoprostane were determined using commercially available ELISA kits (R&D Systems, Minneapolis, MN, USA, for LTB_4; Cayman Chemical, Ann Arbor, MI, USA, for 8-isoprostane), according to the manufacturers' protocols. A procedure of solid-phase extraction of the EBC and nasal lavage samples was performed before the LTB_4 measurements, according to the manufacturers' protocols. Briefly, the samples were acidified and centrifuged at 1600×*g* for 1 min in order to remove any precipitate. Then they were applied on a C18 reverse column (R&D Systems) with a flow rate of approximately 0.5 mL/min. Afterwards the column was washed with 10 mL of cold deionized water, followed by 10 mL of 15% ethanol and 10 mL of hexane. The samples were eluted from the column by the addition of 10 mL of ethyl acetate. Finally, the samples were evaporated under a stream of nitrogen and reconstituted with 250 μL of Calibrator Diluent RD5-44 (R&D Systems, Minneapolis, MI). All samples were stored <4 weeks before the measurements (median 2.4 weeks).

Statistical analysis

Normally distributed data are presented as mean±standard deviation (SD), whereas skewed data are presented as median (interquartile ranges, IQR), with the exception of percentage differential cell counts in nasal lavage presented as median (ranges). Normality of distribution was examined with Shapiro–Wilk's test. Comparisons between two groups were performed with unpaired *t*-tests or Mann–Whitney *U*-tests (for normally distributed and skewed data, respectively). Comparisons among three or more groups were performed with one-way ANOVA for normally distributed data or with Kruskall–Wallis tests for skewed data, with appropriate *post hoc* tests. Correlations were examined using Spearman's rank correlation coefficient (r_s), derived from the actual (not transformed) data. Significance was defined as a *P*-value <0.05.

Results

Demographic characteristics of the study subjects are summarized in Table 1. The two study groups were matched for age, gender and smoking habits. All allergic rhinitis subjects presented elevated serum total IgE and were highly sensitive to pollen allergens, whereas none of them was positive to other tested allergens. In contrast, healthy controls presented low levels of serum total non-specific IgE and presented no reaction to the tested allergens. All rhinitis patients presented a PD_{20} to metacholine >800 μg (985 [871.3, 1149] μg; 953.0 [862.5, 1035.0] μg vs. 1085 [876.3, 1263.5] μg for non-smokers and smokers, respectively).

Differential cell counts in nasal lavage

The percentage of eosinophils (median [range]) was higher in the nasal lavage of allergic rhinitis patients compared with controls (4 [0–68]% vs. 0 [0–2]%, respectively, $P<0.0001$); no significant differences were observed in either of the two groups between smokers and non-smokers. No differences in the percentage of neutrophils were observed between allergic rhinitis patients and controls (15.5 [0–64]% vs. 21.9 [0–80]%, respectively, $P=0.33$); no significant differences were observed in either of the two groups between smokers and non-smokers.

Leukotriene B_4 in nasal lavage and exhaled breath condensate

LTB_4 concentrations (median [IQR]) in the nasal lavage of allergic rhinitis patients were significantly higher than

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Table 1. Demographic characteristics of study participants

	Healthy controls ($n = 30$)	Allergic rhinitis ($n = 40$)
Age (years)		
All	31.8±5.9	32.8±7.2
Non-smokers	31.7±5.5	33.5±8.9
Smokers	32.0±6.4	32.3±5.3
Smokers	15 (50%)	20 (50%)
Pack-years	10.9±4.5	8.6±6.4
Sex (female/male)	16/14	22/18
FEV_1 (%pred.)		
All	100.3±6.3	98.8±9.1
Non-smokers	102.5±6.6	99.9±10.7
Smokers	98.2±6.5	97.8±7.1
FVC (%pred.)		
All	102.8±7.8	103.5±10.8
Non-smokers	104.5±7.4	102.9±12.1
Smokers	101.1±8.1	104.0±9.8
FEV_1/FVC		
All	89.1±4.3	85.9±3.5
Non-smokers	90.2±4.8	86.1±3.1
Smokers	88.1±3.6	85.7±3.8
Serum total IgE (IU/mL)*		
All	4.8 [4.8, 19.0]	179.0 [132.3, 210.0]
Non-smokers	4.8 [4.8, 18.0]	188.0 [120.5, 206.0]
Smokers	9.0 [4.8, 22.0]	166.0 [143.8, 210.0]

FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity.
*Normally distributed data are presented as mean±SD, whereas skewed data are presented as median [interquartile ranges].

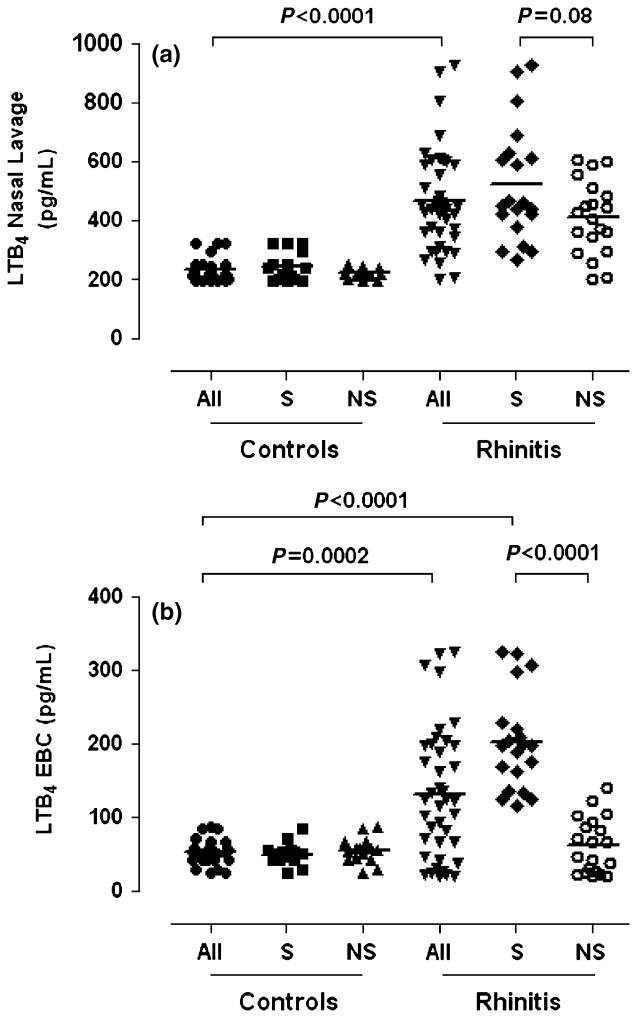


Fig. 1. Comparisons of leukotriene B_4 (LTB_4) levels between healthy subjects (smokers and non-smokers) and patients with allergic rhinitis (smokers and non-smokers) (a) in nasal lavage and (b) in exhaled breath condensate (EBC). Horizontal bars represent mean values.

those of controls (445.2 [351.4, 590.4] pg/mL vs. 220.0 [201.6, 247.2] pg/mL, respectively, $P < 0.0001$) (Fig. 1a). There was a trend for higher LTB_4 concentrations in the nasal lavage of smokers, but this did not reach statistical significance (457.7 [389.4, 623.5] pg/mL vs. 416.4 [310.3, 506.2] pg/mL, respectively, $P = 0.08$).

The levels of LTB_4 in the EBC in the whole group of allergic rhinitis patients were significantly higher compared with those of healthy subjects (132.2±88.7 pg/mL vs. 52.8±16.3 pg/mL, respectively, $P = 0.0002$) (Fig. 1b). The levels of LTB_4 in EBC did not differ between smoking and non-smoking control subjects (50.3±14.9 pg/mL vs. 55.2±17.7 pg/mL, respectively, P = ns). However, LTB_4 concentrations were significantly higher in the EBC of smoking compared with non-smoking allergic rhinitis patients and controls (202.4±65.9 pg/mL vs. 61.9±37.6 pg/mL, $P < 0.0001$; and 202.4±65.9 pg/mL vs. 52.8±16.3 pg/mL, $P < 0.0001$, respectively) (Fig. 1b). In contrast, LTB_4 levels in the EBC of non-smokers with allergic rhinitis did not differ from controls.

8-Isorostanen nasal lavage and exhaled breath condensate

Allergic rhinitis patients presented a significantly higher concentration of 8-isoprostane in nasal lavage than healthy subjects (26.9±8.6 pg/mL vs. 17.4±3.4 pg/mL, respectively, $P < 0.0001$) (Fig. 2a). However, no differences were found between smokers and non-smokers in both study groups (Fig. 2a).

No differences were found between the study groups in the levels of 8-isoprostane in EBC of controls and allergic rhinitis patients, despite a trend for higher levels in the latter (9.3±2.1 pg/mL vs. 10.5±3.4 pg/mL, respectively, $P = 0.09$) (Fig. 2b). No differences were observed between smokers and non-smokers in either group.

pH in nasal lavage and exhaled breath condensate

No significant difference was found in the pH values of nasal lavage between controls and patients with allergic rhinitis (6.77±0.13 vs. 6.69±0.27, respectively, $P = 0.09$). Additionally, no difference was found between smokers

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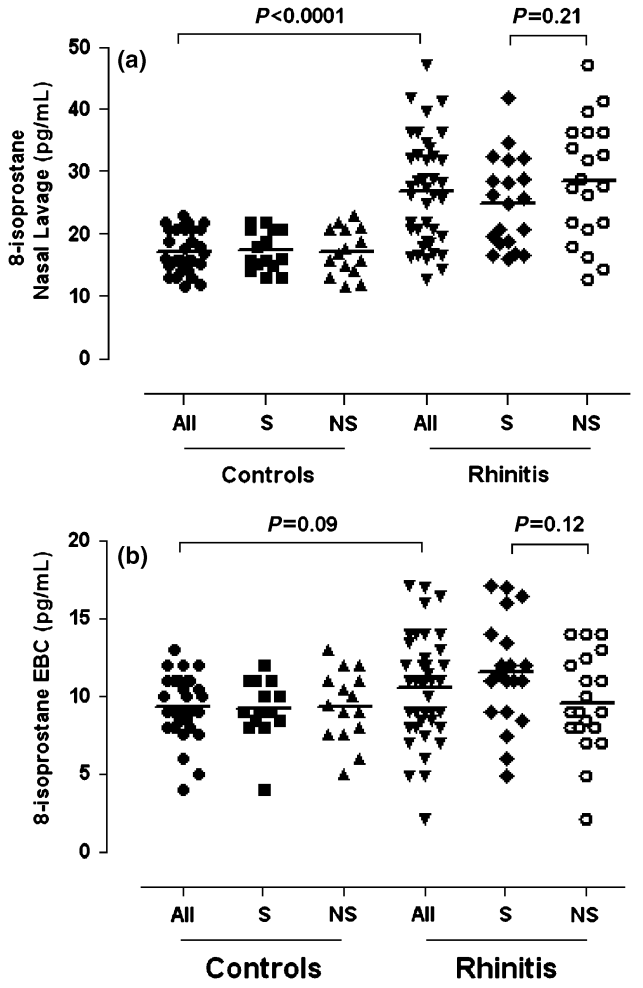


Fig. 2. Comparison of 8-isoprostane levels between healthy subjects (smokers and non-smokers) and patients with allergic rhinitis (smokers and non-smokers) (a) in nasal lavage and (b) in exhaled breath condensate (EBC). Horizontal bars represent mean values.

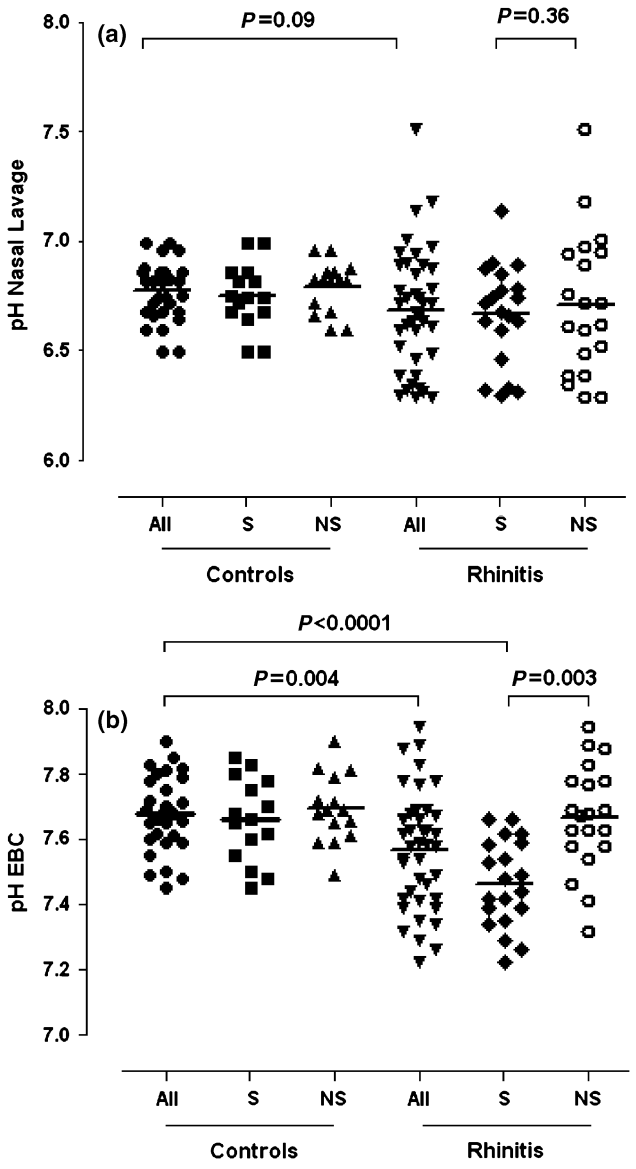


Fig. 3. Comparison of pH between healthy subjects (smokers and non-smokers) and patients with allergic rhinitis (smokers and non-smokers) (a) in nasal lavage and (b) in exhaled breath condensate (EBC). Horizontal bars represent mean values.

and non-smokers with allergic rhinitis (6.67±0.23 vs. 6.71±0.32, respectively, $P = 0.36$) (Fig. 3a).

Allergic rhinitis subjects presented significantly lower EBC pH values compared with healthy controls (7.56± 0.18 vs. 7.67±0.11, respectively, $P = 0.004$) (Fig. 3b). This difference was due to a significant difference between smokers and non-smokers with allergic rhinitis (7.47± 0.13 vs. 7.67±0.16, respectively, $P = 0.003$) (Fig. 3b). Indeed, smokers with allergic rhinitis presented lower EBC pH values compared with controls ($P < 0.0001$), whereas EBC pH levels did not differ between non-smokers with rhinitis and controls ($P = 0.85$) (Fig. 3b).

Fraction of exhaled nitric oxide

The levels of fraction of exhaled NO (FeNO) of healthy controls were significantly lower than those of allergic rhinitis patients (9.5 [8.0, 12.0] ppb vs. 20.5 [12.5, 33] ppb, $P < 0.0001$) (Fig. 4). Smokers with allergic rhinitis presented significantly lower values of FeNO than non-smokers (15.0 [8.0, 23.8] vs. 29.0 [18.0, 55.0], $P = 0.009$) (Fig. 4). Both smokers and non-smokers with allergic rhinitis presented higher FeNO levels compared with healthy controls ($P = 0.01$ and $P < 0.0001$, respectively) (Fig. 4).

Correlations

LTB_4 levels in nasal lavage presented a significant correlation with LTB_4 levels in EBC in patients with allergic rhinitis ($r_s = 0.313$, $P = 0.048$; Fig. 5). LTB_4 levels in EBC of subjects with allergic rhinitis presented statistically significant negative correlations with EBC pH and PD_{20}

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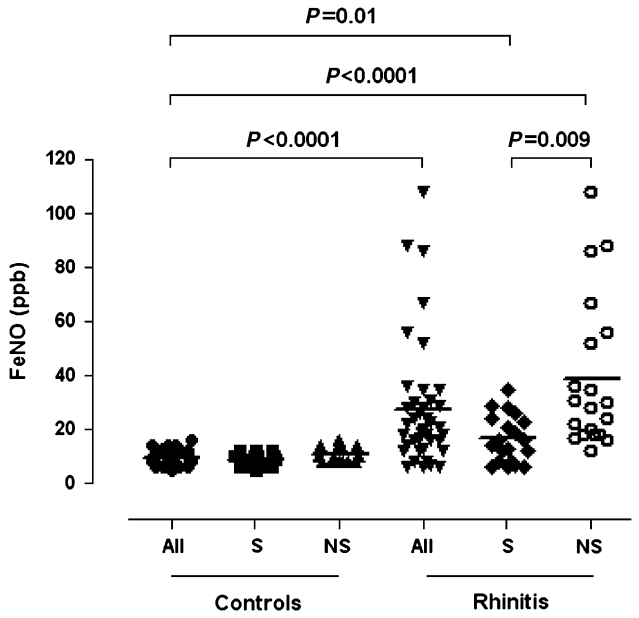


Fig. 4. Comparison of the fraction of exhaled nitric oxide (FeNO) between healthy subjects (smokers and non-smokers) and patients with allergic rhinitis (smokers and non-smokers). Horizontal bars represent mean values.

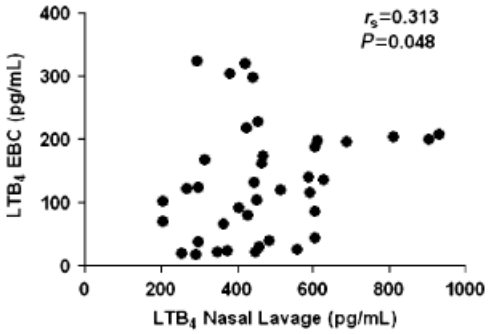


Fig. 5. Correlation between the levels of leukotriene B_4 (LTB_4) in nasal lavage and exhaled breath condensate (EBC) in patients with allergic rhinitis.

metacholine ($r_s = -0.416$, $P = 0.008$ and $r_s = -0.339$, $P = 0.03$, respectively). No other statistically significant correlations were found between the specific biomarkers and any clinical parameters of the study subjects. FeNO values in allergic rhinitis patients did not correlate with any of the measured biomarkers.

Discussion

In the present cross-sectional study, we examined the impact of smoking on inflammatory and oxidative stress biomarkers in samples collected non-invasively from the nasal cavity and lower respiratory tract of patients with seasonal allergic rhinitis. The principal findings of our study are as follows: (1) nasal lavage and EBC LTB_4 concentrations were significantly higher in allergic rhinitis patients than in controls, and EBC LTB_4 levels were higher in smokers with allergic rhinitis compared with non-smokers; (2) patients with allergic rhinitis presented increased local oxidative stress, as expressed by 8-isoprostane levels in nasal lavage, compared with controls; (3) EBC pH values were lower in smokers with allergic rhinitis compared with non-smokers; and (4) allergic rhinitis patients presented higher FeNO levels compared with controls, with smoking patients presenting lower FeNO compared with non-smokers. This is the first study to our knowledge that has evaluated the impact of smoking on airway inflammation of patients with seasonal allergic rhinitis.

LTB_4 is produced by neutrophils, macrophages, mast cells and dendritic cells, constituting a potent chemoattractant for neutrophils with a substantial role in inflammatory pathways [23]. Previous studies have shown that cysteinyl leukotrienes are elevated in EBC of allergic rhinitis patients [24, 25]. Cap et al. [24] have additionally shown that during pollen season, cysteinyl leukotrienes and LTB_4 were elevated in the EBC of allergic rhinitis patients and remained elevated during the off-pollen season, although with lower levels, compared with healthy subjects. In our study, non-smoking allergic rhinitis patients did not differ in terms of exhaled LTB_4 levels from healthy subjects, which is in accordance with another study that reports no difference in LTB_4 and LTE_4 concentrations in EBC of healthy children compared with atopic non-asthmatic children [26]. In the present study, we have further shown that LTB_4 levels were higher in the EBC of smokers than in non-smokers with allergic rhinitis, a difference that was not evident in control subjects. Asthma and active cigarette smoking interact to aggravate asthma symptoms and decline in lung function and to cause impaired therapeutic response to corticosteroids [27]. The exposure to smoke affects the production of cytokines and mediators in asthmatics, leading to an alteration of the inflammatory pattern towards a more neutrophilic type of inflammation [28]. Our data suggest that also smokers with allergic rhinitis without asthma are more susceptible in developing lower airway inflammation, thus being more prone in developing asthma in the future. A recent large population study has shown that cigarette smoking represents an important independent risk factor for the development of new asthma cases in patients with allergic rhinitis [29], and our study provides evidence that the development of asthma may involve an LTB_4-mediated inflammatory process, as LTB_4 has been previously shown to be an important inflammatory mediator in asthmatic smokers [14]. The significant negative correlations between EBC LTB_4 and EBC pH and PD_{20} further support a possible mechanism for the subsequent development of asthma, involving airway acidification and bronchial hyperresponsiveness.

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Higher concentrations of LTB_4 were additionally detected in nasal lavage samples of patients with allergic rhinitis during pollen season, which is in accordance with previous studies that have demonstrated elevation of LTB_4 in nasal lavage fluids after nasal provocation of atopic patients [30, 31]. The significant correlation between the levels of LTB_4 in the nasal lavage and EBC in allergic rhinitis patients additionally supports the one-airway hypothesis, suggesting a possible pathogenetic mechanism for the concomitant development of asthma in some of the patients with allergic rhinitis.

8-Isoprostane is a marker of oxidative stress and lipid peroxidation that is proven to be elevated in EBC of asthmatic patients, proportionally to the severity of the disease, reflecting the underlying airway redox imbalance [32–34]. Moreover, 8-isoprostane production has been associated with smoking, which is a causative factor of oxidative stress [16]. In a previous study, Van Hoydonck et al. showed that 8-isoprostane concentrations in EBC of healthy smokers presented low concentration and could be detected in a small proportion of the study participants [35]. In the present study, 8-isoprostane was detectable in all EBC and nasal lavage samples, with levels comparable with those of previous studies using the same immunoassay kit [15, 16, 36]. In our study, 8-isoprostane was not elevated in the EBC of patients with allergic rhinitis and did not differ between healthy smokers and non-smokers. The discrepancy between our results and those of Montuschi et al. [16] could be explained by the fact that healthy subjects included in that study had a greater smoking habit, in terms of pack-years, than our control subjects. A study by Kinnula et al. [10] demonstrated elevated levels of 8-isoprostane in sputum of healthy smokers. The differences in epithelial lining fluid sampling between the two techniques may account for the discrepancy between our findings and the latter study. Interestingly, 8-isoprostane was elevated in the nasal lavage of patients with allergic rhinitis compared with healthy subjects, suggesting the presence of increased local oxidative stress in the nasal cavity of allergic rhinitis patients. This is the first study, to our knowledge, that has evaluated the nasal oxidative stress of allergic rhinitis patients, providing evidence for a possible deleterious effect of smoking in the nasal cavity. Longitudinal studies are needed in order to evaluate the evolution of nasal inflammation in smokers.

There is some evidence that airway acidification, as expressed by lower pH in EBC, is present in atopic children [18], whereas another study has shown that endogenous airway acidification was not present in children with allergic rhinitis, but only for asthma and atopic dermatitis [37]. In our study, EBC pH did not differ between the whole group of allergic rhinitis patients and healthy control subjects. Nevertheless, smoking patients with allergic rhinitis presented lower EBC pH than non-smokers with rhinitis and healthy smokers, suggesting that smoking induces lower airway acidification, only in smokers with allergic rhinitis. Interestingly, the smokers with allergic rhinitis also present subclinical neutrophilic inflammation, as assessed indirectly by the presence of elevated levels of LTB_4. However, no significant correlation between EBC pH and LTB_4 was observed in that subgroup. Finally, nasal lavage pH did not present any differentiation between the study groups; therefore, it may not represent a potential biomarker for the evaluation of nasal inflammatory changes in allergic rhinitis patients.

In the present study, we have shown differences in oxidative stress and inflammatory biomarkers in the nasal lavage between patients with allergic rhinitis and healthy controls. In contrast with the findings in EBC, in nasal lavage we have failed to show any impact of smoking in nasal inflammation, with the exception of a trend for higher levels of LTB_4 in smokers with allergic rhinitis, which did not reach statistical significance. This finding is in contrast to a previous study with nasal biopsies, showing that smokers with normal lung function have nasal inflammation, characterized by eosinophilic infiltration [38]. The different sampling methods and the smaller smoking habit of our patients may account for the discrepancy with our findings and longitudinal studies are needed to clarify this issue.

Finally, our findings regarding the FeNO are in accordance with several previous studies that have shown elevated levels of FeNO in allergic rhinitis patients in a similar way to asthmatics [4, 19, 39, 40], and a reduction of FeNO in smokers [19, 41, 42]. In contrast, a recent study has shown that patients with atopic asthma have higher exhaled NO concentrations than non-asthmatic patients with allergic rhinitis, and the different study design may account for those differences [43]. Finally, FeNO levels in our study did not correlate with any of the other assessed biomarkers, suggesting that they sample different aspects of airways inflammation, in addition to the attenuating effect of smoking habit in exhaled NO.

In conclusion, patients with allergic rhinitis present increased LTB_4 and 8-isoprostane in their nasal cavity, however, with no significant differences between smokers and non-smokers. In contrast, smokers with allergic rhinitis present higher LTB_4 levels and lower pH in EBC, suggesting that these patients may be more susceptible to the deleterious effects of smoking, compared with non-smokers. A significant correlation between nasal lavage and EBC LTB_4 suggests a possible mechanism for the development of asthma in some of the patients with allergic rhinitis. Further studies are needed to elucidate the exact pathophysiologic mechanisms that lead to this enhanced reaction to the smoke exposure by the allergic rhinitis patients.

Acknowledgements

Conflicts of interest: All authors declare that they have no conflicts of interest related to the present study.

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References

1 Bousquet J, Van Cauwenberge P, Khaltaev N. Allergic rhinitis and its impact on asthma. *J Allergy Clin Immunol* 2001; **108**:S147–334.
2 Ciprandi G, Cirillo I, Tosca MA, Vizzaccaro A. Bronchial hyperreactivity and spirometric impairment in patients with seasonal allergic rhinitis. *Respir Med* 2004; **98**:826–31.
3 Foresi A, Leone C, Pelucchi A *et al*. Eosinophils, mast cells, and basophils in induced sputum from patients with seasonal allergic rhinitis and perennial asthma: relationship to methacholine responsiveness. *J Allergy Clin Immunol* 1997; **100**:58–64.
4 Gratziou C, Rovina N, Lignos M, Vogiatzis I, Roussos C. Exhaled nitric oxide in seasonal allergic rhinitis: influence of pollen season and therapy. *Clin Exp Allergy* 2001; **31**:409–16.
5 Failla M, Crimi N, Vancheri C. Exhaled bronchial cysteinyl leukotrienes in allergic patients. *Curr Opin Allergy Clin Immunol* 2007; **7**:25–31.
6 Becky Kelly EA, Busse WW, Jarjour NN. A comparison of the airway response to segmental antigen bronchoprovocation in atopic asthma and allergic rhinitis. *J Allergy Clin Immunol* 2003; **111**:79–86.
7 Braunstahl GJ, Overbeek SE, Fokkens WJ *et al*. Segmental bronchoprovocation in allergic rhinitis patients affects mast cell and basophil numbers in nasal and bronchial mucosa. *Am J Respir Crit Care Med* 2001; **164**:858–65.
8 Chanez P, Vignola AM, Vic P *et al*. Comparison between nasal and bronchial inflammation in asthmatic and control subjects. *Am J Respir Crit Care Med* 1999; **159**:588–95.
9 Hylkema MN, Sterk PJ, de Boer WI, Postma DS. Tobacco use in relation to COPD and asthma. *Eur Respir J* 2007; **29**:438–45.
10 Kinnula VL, Ilumets H, Myllarniemi M, Sovijarvi A, Rytila P. 8-Isoprostane as a marker of oxidative stress in nonsymptomatic cigarette smokers and COPD. *Eur Respir J* 2007; **29**:51–5.
11 Baraldo S, Turato G, Badin C *et al*. Neutrophilic infiltration within the airway smooth muscle in patients with COPD. *Thorax* 2004; **59**:308–12.
12 Montuschi P, Barnes PJ, Roberts LJ II Isoprostanes: markers and mediators of oxidative stress. *FASEB J* 2004; **18**:1791–800.
13 Montuschi P, Kharitonov SA, Ciabattoni G, Barnes PJ. Exhaled leukotrienes and prostaglandins in COPD. *Thorax* 2003; **58**:585–8.
14 Kostikas K, Gaga M, Papatheodorou G, Karamanis T, Orphanidou D, Loukides S. Leukotriene B4 in exhaled breath condensate and sputum supernatant in patients with COPD and asthma. *Chest* 2005; **127**:1553–9.
15 Kostikas K, Papatheodorou G, Psathakis K, Panagou P, Loukides S. Oxidative stress in expired breath condensate of patients with COPD. *Chest* 2003; **124**:1373–80.
16 Montuschi P, Collins JV, Ciabattoni G *et al*. Exhaled 8-isoprostane as an in vivo biomarker of lung oxidative stress in patients with COPD and healthy smokers. *Am J Respir Crit Care Med* 2000; **162**:1175–7.
17 Kostikas K, Papatheodorou G, Ganas K, Psathakis K, Panagou P, Loukides S. pH in expired breath condensate of patients with inflammatory airway diseases. *Am J Respir Crit Care Med* 2002; **165**:1364–70.
18 Brunetti L, Francavilla R, Tesse R *et al*. Exhaled breath condensate pH measurement in children with asthma, allergic rhinitis and atopic dermatitis. *Pediatr Allergy Immunol* 2006; **17**:422–7.
19 Kostikas K, Papaioannou AI, Tanou K, Koutsokera A, Papala M, Gourgoulianis KI. Portable exhaled nitric oxide as a screening tool for asthma in young adults during pollen season. *Chest* 2008; **133**:906–13.
20 Salib RJ, Lau LC, Howarth PH. Nasal lavage fluid concentrations of eotaxin-1 (CCL11) in naturally occurring allergic rhinitis: relationship to disease activity, nasal luminal eosinophil influx, and plasma protein exudation. *Clin Exp Allergy* 2005; **35**:995–1002.
21 American Thoracic Society Standardization of spirometry, 1994 update. *Am J Respir Crit Care Med* 1995; **152**:1107–36.
22 Bateman ED, Hurd SS, Barnes PJ *et al*. Global strategy for asthma management and prevention: GINA executive summary. *Eur Respir J* 2008; **31**:143–78.
23 Montuschi P, Sala A, Dahlen SE, Folco G. Pharmacological modulation of the leukotriene pathway in allergic airway disease. *Drug Discov Today* 2007; **12**:404–12.
24 Cap P, Pehal F, Chladek J, Maly M. Analysis of exhaled leukotrienes in nonasthmatic adult patients with seasonal allergic rhinitis. *Allergy* 2005; **60**:171–6.
25 Failla M, Biondi G, Provvidenza Pistorio M *et al*. Intranasal steroid reduces exhaled bronchial cysteinyl leukotrienes in allergic patients. *Clin Exp Allergy* 2006; **36**:325–30.
26 Mondino C, Ciabattoni G, Koch P *et al*. Effects of inhaled corticosteroids on exhaled leukotrienes and prostanoids in asthmatic children. *J Allergy Clin Immunol* 2004; **114**:761–7.
27 Thomson NC, Chaudhuri R, Livingston E. Asthma and cigarette smoking. *Eur Respir J* 2004; **24**:822–33.
28 Chalmers GW, MacLeod KJ, Thomson L, Little SA, McSharry C, Thomson NC. Smoking and airway inflammation in patients with mild asthma. *Chest* 2001; **120**:1917–22.
29 Polosa R, Knoke JD, Russo C *et al*. Cigarette smoking is associated with a greater risk of incident asthma in allergic rhinitis. *J Allergy Clin Immunol* 2008; **121**:1428–34.
30 Kumar NS, Schaefer PA, Lark G, Frieri M. Late phase response during nasal challenge: effect of astemizole on leukotriene B4 levels. *Allergy Asthma Proc* 1996; **17**:93–9.
31 Frieri M, Therattil J, Chavarria V *et al*. Effect of mometasone furoate on early and late phase inflammation in patients with seasonal allergic rhinitis. *Ann Allergy Asthma Immunol* 1998; **81**:431–7.
32 Montuschi P, Corradi M, Ciabattoni G, Nightingale J, Kharitonov SA, Barnes PJ. Increased 8-isoprostane, a marker of oxidative stress, in exhaled condensate of asthma patients. *Am J Respir Crit Care Med* 1999; **160**:216–20.
33 Zanconato S, Carraro S, Corradi M *et al*. Leukotrienes and 8-isoprostane in exhaled breath condensate of children with stable and unstable asthma. *J Allergy Clin Immunol* 2004; **113**:257–63.
34 Shahid SK, Kharitonov SA, Wilson NM, Bush A, Barnes PJ. Exhaled 8-isoprostane in childhood asthma. *Respir Res* 2005; **6**:79.
35 Van Hoydonck PG, Wuyts WA, Vanaudenaerde BM, Schouten EG, Dupont LJ, Temme EH. Quantitative analysis of 8-isoprostane and hydrogen peroxide in exhaled breath condensate. *Eur Respir J* 2004; **23**:189–92.
36 Carpagnano GE, Kharitonov SA, Resta O, Foschino-Barbaro MP, Gramiccioni E, Barnes PJ. 8-Isoprostane, a marker of oxidative stress, is increased in exhaled breath condensate of patients with obstructive sleep apnea after night and is reduced by continuous positive airway pressure therapy. *Chest* 2003; **124**:1386–92.

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37 Profita M, La Grutta S, Carpagnano E *et al*. Noninvasive methods for the detection of upper and lower airway inflammation in atopic children. *J Allergy Clin Immunol* 2006; **118**: 1068–74.
38 Vachier I, Vignola AM, Chiappara G *et al*. Inflammatory features of nasal mucosa in smokers with and without COPD. *Thorax* 2004; **59**:303–7.
39 Travers J, Marsh S, Aldington S *et al*. Reference ranges for exhaled nitric oxide derived from a random community survey of adults. *Am J Respir Crit Care Med* 2007; **176**: 238–42.
40 Lopuhaa CE, Koopmans JG, Jansen HM, van der Zee JS. Similar levels of nitric oxide in exhaled air in non-asthmatic rhinitis and asthma after bronchial allergen challenge. *Allergy* 2003; **58**: 300–5.
41 Verleden GM, Dupont LJ, Verpeut AC, Demedts MG. The effect of cigarette smoking on exhaled nitric oxide in mild steroid-naive asthmatics. *Chest* 1999; **116**:59–64.
42 Malinovschi A, Janson C, Holmkvist T, Norback D, Merilainen P, Hogman M. Effect of smoking on exhaled nitric oxide and flow-independent nitric oxide exchange parameters. *Eur Respir J* 2006; **28**:339–45.
43 Rolla G, Guida G, Heffler E *et al*. Diagnostic classification of persistent rhinitis and its relationship to exhaled nitric oxide and asthma: a clinical study of a consecutive series of patients. *Chest* 2007; **131**:1345–52.

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