ORIGINAL ARTICLE

Bronchial Hyperresponsiveness and Airway Inflammation in Patients With Seasonal Allergic Rhinitis

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Abstract

Background: Allergic rhinitis is a chronic inflammatory disease induced by an immunoglobulin (Ig) E–mediated reaction after exposure to an allergen. Many patients with allergic rhinitis and no clinical evidence of asthma show a heightened response to histamine.
Objectives: The aims of the study were to measure changes in markers of airway inflammation in patients with seasonal allergic rhinitis and estimate changes in bronchial reactivity before and during the pollen season.
Methods: The study sample comprised 22 patients sensitized to grass pollen and 10 healthy volunteers. Based on the results of the bronchial provocation test (BPT) during the pollen season, we divided patients into those with and without bronchial hyperresponsiveness (BHR). We determined changes in nitrite and pH in exhaled breath concentrate (EBC), fraction of exhaled nitric oxide (FE_{NO}), blood eosinophil count, and BPT results before and during the pollen season.
Results: In allergic rhinitis patients with BHR, we observed an increase in EBC nitrite (5.44 [2.33] vs 8.57 [3.35] nmol/mL, *P*=.02) and FE_{NO} (20.90 [13.68] vs. 43.40 [31.60] ppb, *P*=.02) and a decrease in EBC pH (7.07 [0.33] vs. 6.74 [0.28], *P*=.01) during the pollen season. In allergic rhinitis patients with BHR, the increase in BHR was negatively correlated with increased FE_{NO} and EBC nitrite and positively correlated with a decrease in EBC pH during the pollen season.
Conclusions: Our results revealed a relationship between increased BHR in patients with seasonal allergic rhinitis and changes in airway inflammation markers. EBC pH, EBC nitrite concentration, and FE_{NO} could act as prognostic markers for identifying patients at risk of developing asthma.

Key words: Allergic rhinitis. Bronchial hyperresponsiveness. Allergic inflammation. Exhaled breath condensate. Exhaled nitric oxide.

Resumen

Antecedentes: La rinitis alérgica (RA) es una enfermedad inflamatoria crónica inducida por una reacción mediada por inmunoglobulina (Ig) E tras la exposición a un alérgeno. Muchos pacientes con RA y sin pruebas clínicas de asma muestran una respuesta aumentada a la histamina.
Objetivos: Los fines del estudio fueron medir los cambios en marcadores de inflamación de las vías respiratorias en pacientes con RA estacional y estimar los cambios en la reactividad bronquial antes y durante la estación polínica.
Métodos: La muestra del estudio estuvo compuesta por 22 pacientes sensibilizados al polen de gramíneas y 10 voluntarios sanos. Sobre la base de los resultados de la prueba de provocación bronquial (PPB) durante la estación polínica, se dividió a los pacientes en aquellos con y sin hiperreactividad bronquial (HRB). Se determinaron los cambios en nitritos y pH del condensado de aire exhalado (CAE), la fracción de óxido nítrico exhalado (NOe), el recuento de eosinófilos en sangre y los resultados de la PPB antes y durante la estación polínica.
Resultados: En pacientes con RA con HRB se observó un aumento de nitritos del CAE (5,44 [2,33] frente a 8,57 [3,35] nmol/ml, $p=0,02$) y de la fracción de NOe (20,90 [13,68] frente a 43,40 [31,60] ppb, $p=0,02$) y una disminución del pH del CAE (7,07 [0,33] frente a 6,74 [0,28], $p=0,01$) durante la estación polínica. En los pacientes con RA con HRB, el aumento de la HRB presentó correlación negativa con un aumento de la fracción de NOe y de los nitritos del CAE, y correlación positiva con una disminución del pH del CAE durante la estación polínica.
Conclusiones: Los resultados revelaron una relación entre el aumento de la HRB en pacientes con RA estacional y los cambios en los marcadores de inflamación de las vías aéreas. El pH del CAE, la concentración de nitritos del CAE y la fracción de NOe podrían actuar como marcadores pronósticos para identificar a pacientes con riesgo de padecer asma.

Palabras clave: Rinitis alérgica. Hiperreactividad bronquial. Inflamación alérgica. Condensado de aire exhalado. Óxido nítrico exhalado.

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Introduction

Allergic diseases of the airways, which include seasonal rhinitis and asthma, are recognized as inflammatory disorders of the airway mucosa, but differ in the location of the inflammatory reaction and clinical manifestation of the disease [1,2].

Allergic rhinitis is a chronic inflammatory disease of the nasal mucosa induced by an immunoglobulin (Ig) E–mediated reaction that is characterized by nasal discharge, blockage, sneezing, and itching [1]. Many patients with allergic rhinitis and no clinical evidence of bronchial asthma show a heightened response to histamine. Several studies have suggested that patients with seasonal allergic rhinitis and nonspecific bronchial hyperresponsiveness (BHR) to histamine are at higher risk of developing asthma [3]. Allergic rhinitis is a risk factor for asthma, but the relationship between these disorders is not clear, and the mechanisms underlying BHR in allergic rhinitis are not fully understood [4,5].

Airway inflammation can be studied using invasive techniques (flexible bronchoscopy with bronchoalveolar lavage, bronchial biopsy) and noninvasive techniques (induced sputum, measurement of exhaled nitric oxide, fraction of exhaled nitric oxygen [FE_{NO}] and inflammatory mediators in exhaled breath condensate [EBC]).

EBC collection is useful for the assessment of nitrogen oxides (NO_x) such as nitrite and nitrate and can be used to assess airway acidification (pH), which may be an additional feature of the airway inflammatory process [4,6-9].

NO is a biological messenger produced from L-arginine by NO synthase enzymes. It has been shown to play an important role in the physiological regulation and pathophysiology of airway diseases. FE_{NO} was recently shown to have a role as a noninvasive marker for measuring inflammation and oxidative stress in the lung [10]. Previous studies have shown increased concentrations of NO in patients with allergic rhinitis during the pollen season [11].

Nitrite is present in healthy individuals in respiratory tract lining fluid. Increased nitrite levels have been detected in EBC from patients with asthma [12]. In an acidic environment, nitrite is converted to NO [13].

The aims of this study were to measure changes in EBC pH and nitrites, eosinophil count, and FE_{NO} in patients with allergic rhinitis and to estimate the changes observed in relation to bronchial reactivity before and during the pollen season.

Material and Methods

Patients

The study population comprised 22 patients with allergic rhinitis and a control group of 10 healthy volunteers. Allergic rhinitis was diagnosed on the basis of the clinical history and the results of a skin prick test. Patients had recurrent symptoms of sneezing, rhinorrhea, and nasal stuffiness or itching. None of the participants had had common cold during the proceeding year and none had ever received immunotherapy or topical corticosteroids. Skin prick tests with commonly encountered aeroallergens (house dust mite, trees, weeds, grasses, cat, *Alternaria*, and *Cladosporium*) revealed that the 22 patients with allergic rhinitis were sensitized to grass pollen. Patients sensitized to other aeroallergens were excluded.

The study was performed before and during the pollen season in 2008. Preseasonal measurements were taken between January 5 and March 3. Seasonal measurements were taken between June 2 and June 16. The pollen grass count was 100-200 grains/m^3 during the study period.

None of the patients had had acute exacerbations or respiratory tract infections during the 2 months before the start of the study. Allergic rhinitis was controlled using oral antihistamines as required. All patients were free of asthma symptoms according to the Global Initiative for Asthma (GINA) 2006 document [2].

Before and during the pollen season, FE_{NO} and spirometry parameters were measured, EBC was collected, and all patients underwent a bronchial provocation test (BPT) with histamine.

Antihistamines were not allowed for 10 days before BPT. All rhinitis patients had a negative BPT result with histamine before the pollen season. Based on the BPT results during the pollen season, we divided patients into those with and without nonspecific BHR (allergic rhinitis with BHR and allergic rhinitis without BHR). Patients with clinical evidence of asthma during the pollen season according to GINA 2006 were excluded from the study.

The control group comprised 10 healthy volunteers, all of whom underwent FE_{NO}, spirometry, skin prick tests with common aeroallergens, EBC collection, and bronchial provocation test with histamine. Forced expiratory volume in the first second of expiration (FEV_1) was >80% predicted.

Both patients and controls were nonsmokers and had not been passive smokers during the previous year.

The study protocol was approved by the Ethics of Research Committee of the Medical University of Bialystok (agreement number, 3-06730 P). Informed consent was obtained from all study participants.

Measurements

FE_{NO} was measured in all participants using the chemiluminescence technique (Sievers 280i NO Analyzer, Boulder, Colorado, USA). The measurements were performed at an expiratory flow of 50 mL/s [14]. The duration of exhalation had to be at least 6 seconds to produce a stable NO level for 3 seconds. Three FE_{NO} measurements were taken for each participant. Repeated measurements were performed until the 3 values agreed within 10% of the mean. The mean value of the 3 measurements was recorded as the final FE_{NO} level.

Baseline spirometry was performed using a MasterScreen Pneumo PC spirometer (Jaeger, Höchberg, Germany) according to American Thoracic Society standards [15]. FEV_1 was evaluated. Before the examination, the patients did not take any medications that could affect the spirometry results. The highest value from 3 technically satisfactory attempts was recorded.

A nonspecific BPT with histamine was carried out according to the method described by Ryan et al [16]. Provocation was performed using a De Vilbiss nebuliser 646 (Viasys Healthcare GmbH, Höchberg, Germany) linked to a

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Rosenthal-French dosimeter (Baltimore, Maryland, USA) at an air pressure of 0.15 mPa. The results were presented as the concentration of histamine capable of causing a 20% decrease in FEV_1 over baseline (PC_{20}). A concentration of histamine <8 mg/mL was taken as positive BPT result.

EBC was collected using a condensing chamber (EcoScreen, Erich Jaeger GmbH, Höchberg, Germany). Exhaled air entered and left the chamber through 1-way valves at the inlet and outlet, thus keeping the chamber closed. A low temperature around the chamber allowed the condensate to be collected. The temperature of collection was around 0°C [17]. Patients were instructed to breathe tidally for 10 minutes with a nose clip in place. The respiratory rate ranged from 15-20 breaths/min. Participants were asked to swallow their saliva periodically and to temporarily discontinue collection if they needed to cough. In addition, participants were instructed to refrain from food, drink, and exercise during the 4 hours prior to sample collection. At the end of collection, the total amount of condensate (1.5 to 3.5 mL) was divided into 0.5-mL aliquots, transferred to Eppendorf tubes, and immediately frozen. Samples were stored at –80°C [18,19]. pH and nitrate were analyzed within 4 weeks of collection.

Serum total IgE and sIgE (pollen grass) concentrations were measured using ImmunoCAP technology (Pharmacia Diagnostics, Uppsala, Sweden). The eosinophil count was measured using a hematologic analyzer (Coulter Electronics GmbH, Miami, Florida, USA).

Nitrite was measured using the Griess reaction. Briefly, EBC samples were diluted 4-fold with distilled water and deproteinized by adding 1/20th volume of zinc sulfate (300 g/L) to give a final concentration of 15 g/L. After centrifugation at 10 000*g* for 5 minutes at room temperature (or l000*g* for 15 minutes), 100 µL of EBC was applied to a microtiter plate well, followed by 100 µL of Griess reagent (1 g/L sulfanilamide, 25 g/L phosphoric acid, and 0.1 g/L N-[1-naphthyl] ethylenediamine). After 10 minutes of color development at room temperature, absorbance was measured on a microplate reader (Tecan Austria GmbH, Grödig, Austria) at a wavelength of 540 nm. Each sample was assayed in duplicate wells. Background values were obtained by treating samples as described but using 25 g/L phosphoric acid instead of complete Griess reagent. Calibration curves were constructed with sodium nitrite in deionized water (linear range 0-100 µmol/L). The detection limit of the assay was approximately 1.5 µmol/L in deionized water [20].

EBC pH was measured using a CP-401 pH meter (ELMETRON, Zabrze, Poland) with an ERH-13-6 electrode (HYDRONET, Gliwice, Poland) after deaeration and decarbonation by bubbling with argon (300 mL/min for 10 minutes), with an accuracy of ±0.01, as previously described [21]. In order to avoid contamination with ambient O_2 and CO_2, EBC samples were tightly capped during measurement of pH. Calibration was performed using buffers of pH 3, 5, 7, 9, and 11 before measuring the samples.

Table. Characteristics of the Study Participants[a]

Characteristics	Allergic Rhinitis Patients With BHR	Allergic Rhinitis Patients Without BHR	Differences Between Rhinitis Patients With and Without BHR	Controls
Before Pollen Season				
Number of patients	10	12		10
Sex, F/M	4/6	5/7		5/5
Age, y	31.20 (7.70)	35.95 (7.92)	*P*=.15	29.42 (10.50)
Duration of symptoms, y	4.20 (3.53)	4.92 (2.84)	*P*=.48	
Eosinophil count, cells/mm^3	107.50 (64.60)	93.70 (47.82)	*P*=.57	54.20 (24.10)[b,c]
Serum total IgE, kU_A/L	106.40 (66.75)	119.13 (85.36)	*P*=.7	64.80 (26.64)
sIgE (pollen grass), kU_A/L	29.41 (23.96)	25.92 (26.41)	*P*=.75	<0.35
Log PC_{20}, % predicted	1.41 (0.16)	1.51 (0.00)	*P*=.69	
FEV_1, % predicted	108.40 (10.59)	113.83 (13.42)	*P*=.2	107.40 (11.04)
FE_{NO}, ppb	20.90 (13.68)	26.41 (14.40)	*P*=.37	13.70 (6.70)
pH	7.07 (0.33)	7.14 (0.19)	*P*=.58	7.10 (0.19)
Nitrate, nmol/mL	5.44 (2.32)	4.72 (1.94)	*P*=.43	4.69 (1.15)
During Pollen Season				
Log PC_{20}, mg/mL	0.08 (0.82)	1.49 (0.05)	*P*<.001	
FEV_1, % predicted	106.80 (11.43)	110.58 (12.01)	*P*=.46	106.30 (9.11)
FE_{NO}, ppb	43.40 (31.60)	35.75 (20.78)	*P*=.19	11.64 (8.92)
pH	6.74 (0.29)	7.04 (0.29)	*P*=.02	7.08 (0.12)[b]
Nitrate, nmol/mL	8.57 (3.35)	5.13 (1.39)	*P*=.004	4.72 (1.22)[b,c]

Abbreviations: BHR, bronchial hyperresponsiveness; FE_{NO}, fraction of exhaled nitric oxygen; FEV_1, forced expiratory volume in 1 second; Ig, immunoglobulin; PC_{20}, provocative concentration of histamine that causes a 20% fall in FEV_1.

[a]Data are presented as mean (SD)

[b]Values significantly different from allergic rhinitis patients with BHR (*P*<.05)

[c]Values significantly different from allergic rhinitis patients without BHR (*P*<.05)

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Statistical Analysis

Statistical significance was established using analysis of variance followed by a *t* test with a Bonferroni correction to determine statistically significant differences. All values were expressed as mean (SD); *P* values <.05 were considered significant. PC_{20} values were logarithmically transformed for analysis. The relationship between the parameters studied was assayed by correlation. The Pearson linear correlation coefficient was applied.

Results

The characteristics of patients and controls are presented in the Table.

All participants had a negative nonspecific BPT result before the pollen season. During the pollen season, 10 patients had a positive result and 12 a negative result.

The eosinophil count was statistically significantly higher in both groups of rhinitis patients than in the healthy volunteers.

There were no statistically significant differences between any parameters in either group of allergic rhinitis patients before the pollen season.

BHR to histamine in allergic rhinitis patients with BHR was significantly higher during the pollen season than before the pollen season (log PC_{20}, 0.07 [0.82] vs 1.41 (0.16)] mg/mL, *P*=.0009) (Figure 1). We observed a statistically significant decrease in EBC pH (7.07 [0.33] vs 6.74 [0.28], *P*=.01) (Figure 2) and a increase in FE_{NO} (20.90 [13.68] vs 43.40 [31.60] ppb, *P*=.02) (Figure 3) and EBC nitrite (5.44 nmol/mL [2.33] vs 8.57 [3.35], *P*=.02) (Figure 4) in allergic rhinitis patients with BHR during the pollen season. There was no significant difference in FEV_1 before or during pollen season (data not shown).

The decrease in log PC_{20} showed a negative correlation with increased FE_{NO} levels (r=–0.8, *P*=.005) or EBC nitrite levels (r=–0.73, *P*=.01) and a significant positive correlation with the decrease in EBC pH (r=0.73, *P*=.01) in allergic rhinitis patients with BHR. We also observed a statistically significant positive correlation between the increase in FE_{NO} during the pollen season and an increase in EBC nitrite (r=0.85, *P*=.001) and a negative correlation between the increase in FE_{NO} and a decrease in EBC pH (r=–0.71, *P*=.02) in this group of patients. Such correlations were not observed in the allergic rhinitis patients without BHR.

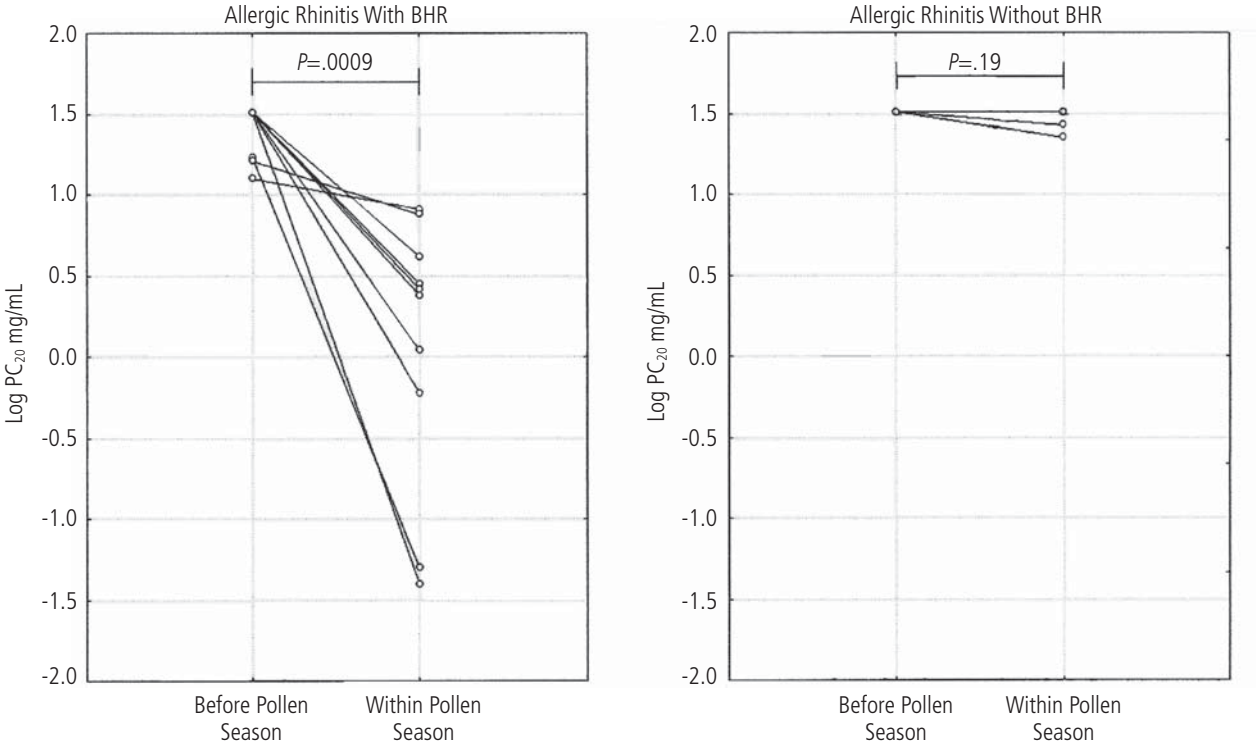

Figure 1. Changes in BHR to histamine before and during the pollen season. BHR indicates bronchial hyperresponsiveness; FEV_1, forced expiratory volume in 1 second; PC_{20}, provocative concentration of histamine that causes a 20% fall in FEV_1.

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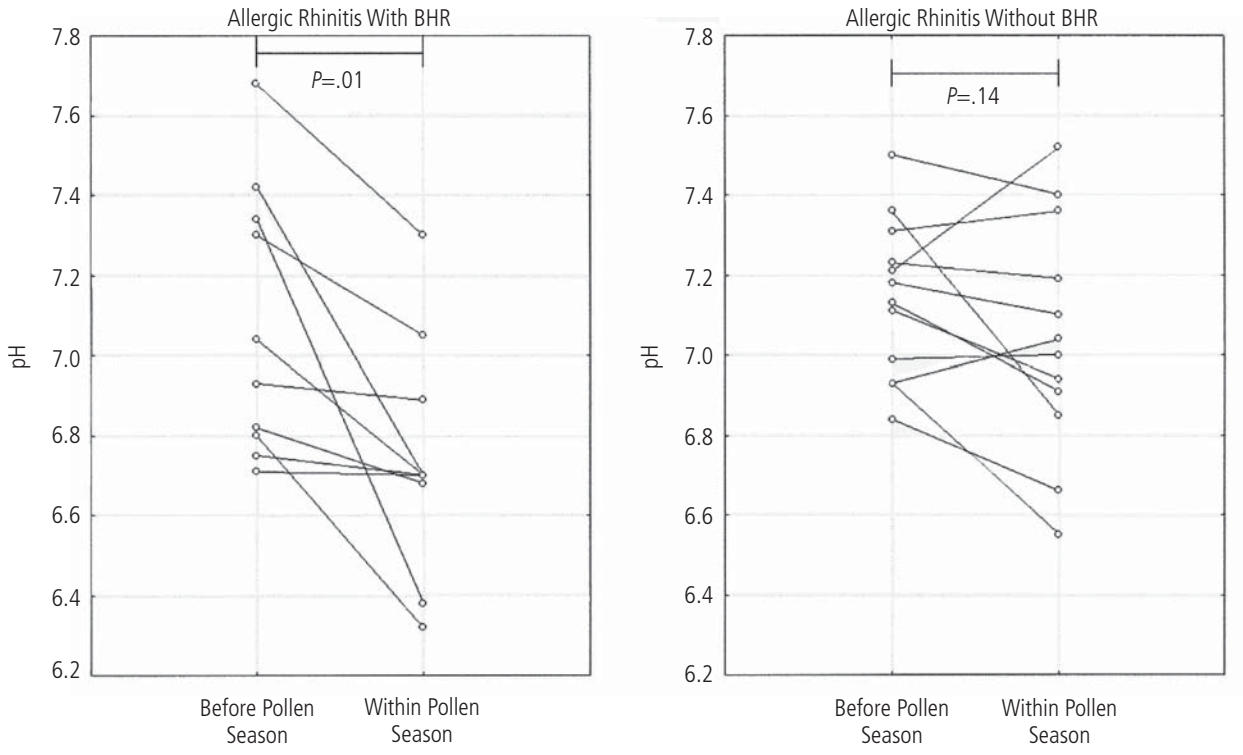

Figure 2. Changes in pH in exhaled breath condensate before and during the pollen season. BHR indicates bronchial hyperresponsiveness.

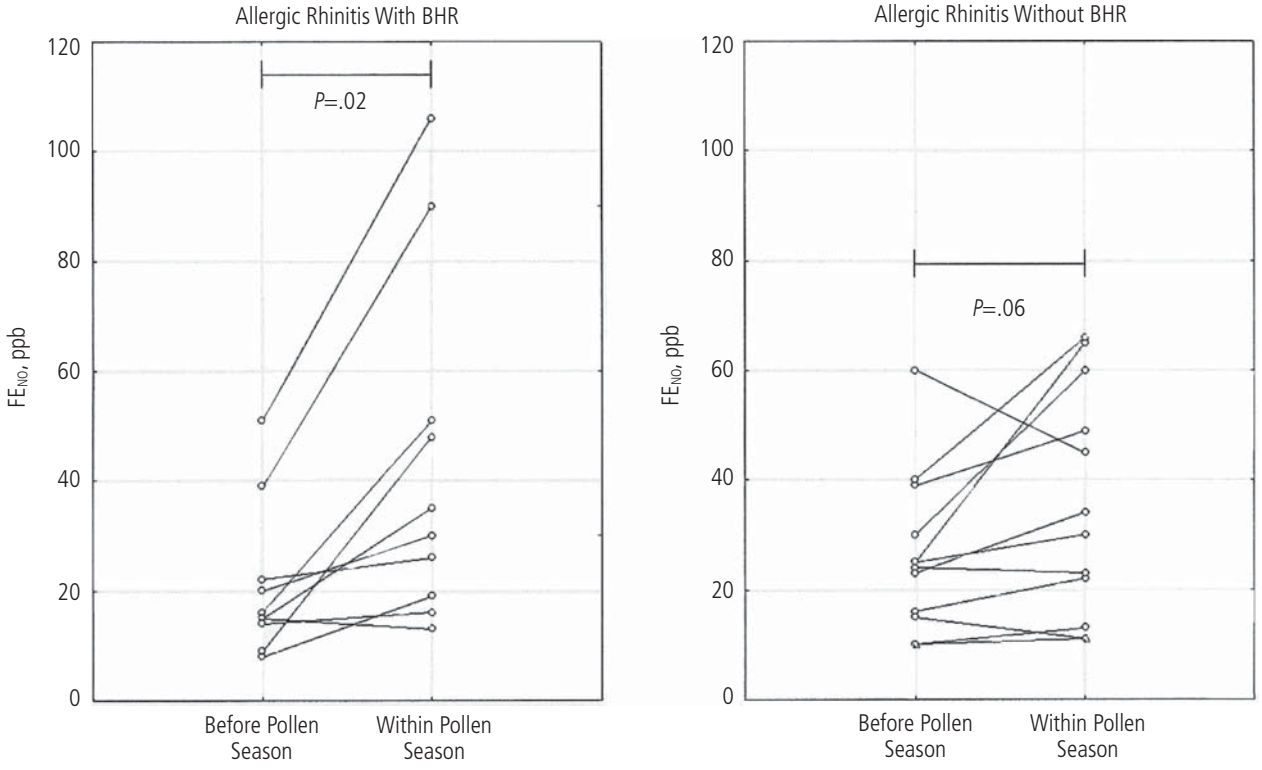

Figure 3. Changes in FE_{NO} before and during the pollen season. BHR indicates bronchial hyperresponsiveness; FE_{NO}, fraction of exhaled nitric oxygen.

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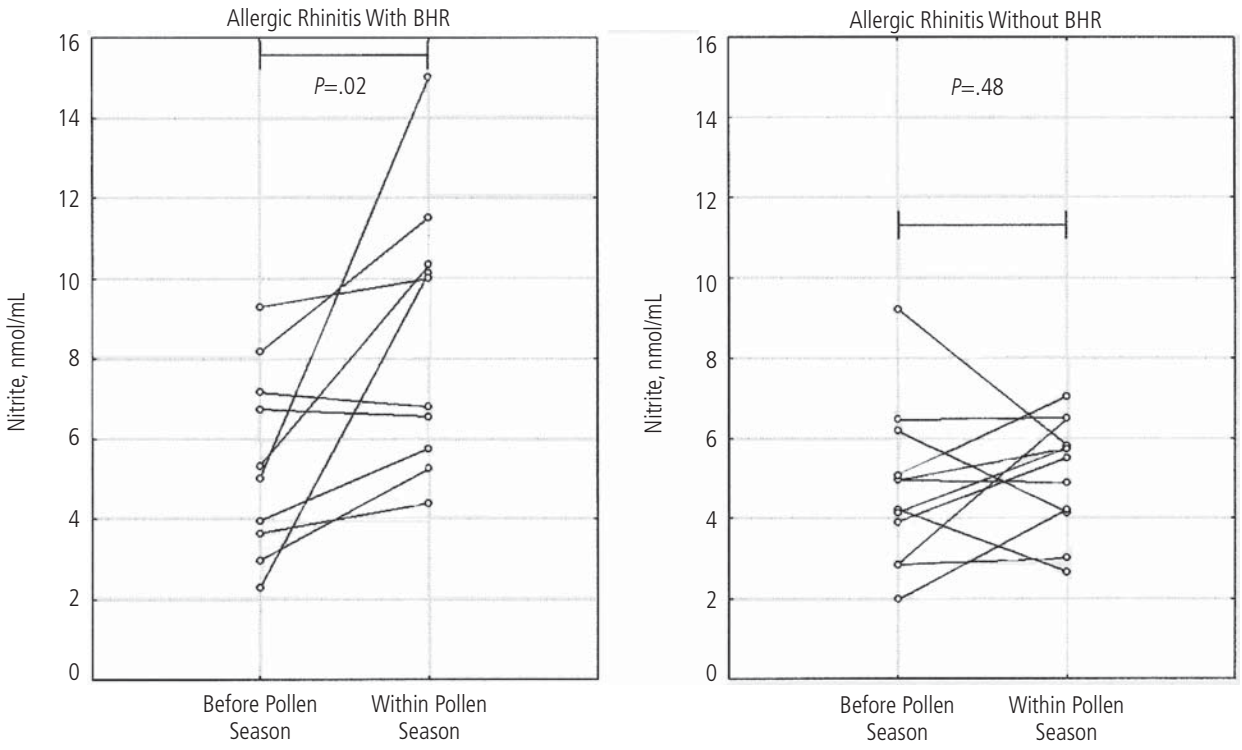

Figure 4. Concentration of nitrite in exhaled breath condensate before and during the pollen season.

Discussion

We studied a homogeneous group of grass pollen–sensitized patients with allergic rhinitis. We divided the patients into 2 groups according to BHR to histamine in the pollen season, namely, allergic rhinitis patients with BHR and allergic rhinitis patients without BHR. There were no statistically significant difference between any parameters in either group before the pollen season. In the allergic rhinitis patients with BHR, we observed a statistically significant increase in inflammatory parameters in EBC and FE_{NO} during the pollen season.

FE_{NO} has many potential cellular sources within the respiratory tract, although airway epithelium is thought to be the primary source, because of its proximity to the air space and its large surface area [6]. Healthy individuals produce low levels of FE_{NO}, which presumably originates from constitutive nitric oxide synthase (NOS) activity [7]. Inducible NOS is induced by numerous mediators released during allergic inflammation, and its expression has been correlated with exhaled NO [22]. In their study of the effect of allergen exposure on atopic asthma, Roberts et al [7] suggested that increased FE_{NO} concentration was associated with an increase in the mean pollen count during the preceding week. In our study, we observed that atopic nonasthmatic patients with allergic rhinitis had increased concentrations of FE_{NO} during the pollen season, despite a decrease in FEV_1. This finding is consistent with those of Roberts et al [7].

The mechanisms leading to airway acidification and pH homeostasis are unclear. Changes in EBC pH may be caused by overproduction and release of acids in the airways, downregulation of buffer systems, or both. pH homeostasis is maintained mainly by airway and lung epithelial cells that produce ammonia from glutamine in a reaction catalyzed by glutaminase. Ammonia buffers endogenous and exogenous acids in the human airway [23]. The activity of glutaminase is downregulated by inflammatory cytokines such as interferon (INF) γ or tumor necrosis factor (TNF) α [23].

EBC pH values in adults [24] and children [25] with asthma are lower than those in healthy individuals and decrease during exacerbations [8,9]. EBC pH values are higher in asthmatic children under treatment with inhaled corticosteroids than in those who are not, presumably via suppression of eosinophilic inflammation and inducible NOS [23]. Kostikas et al [9] found a positive correlation between the amount of hydrogen ions and eosinophil count in the induced sputum of asthmatic patients. Acidification enhances oxidative and nitrosative stress [8]. No data are available on EBC pH in rhinitis patients.

We revealed a statistically significant negative correlation between pH and BHR or FE_{NO}. Dysregulation of the airway acid–base equilibrium may have a role in the pathophysiology of BHR by triggering capsaicin-sensitive neurons [26] and releasing tachykinins [27], leading to bronchospasm and airway reactivity.

We observed elevated levels of FE_{NO} during the pollen season in allergic patients. A possible explanation could be that many inflammatory markers (TNF-α, interleukin (IL) 1β, IL-13, IFN-γ) are able to modulate expression of inducible nitric oxide synthase [28] in cultures of human airway epithelial cells, alveolar epithelial cells, and nonpulmonary cells [29]. Asthma

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patients also have higher arginase activity than healthy individuals [30]. Arginase I is strongly induced by type 2 helper T-cell cytokines associated with the atopic phenotype [31]. Arginase expression and activity are a consequence of allergen-induced gene activation. The alteration of arginine metabolism observed in asthma [30] can lead to increased FE_{NO} levels in atopic patients during the pollen season. In our study, we observed a decrease in EBC pH during the pollen season. Hunt et al [8] showed that acidification of the airway can induce conversion of endogenous nitrite to nitric oxide (FE_{NO}). Gaston et al [32] confirmed that metabolism of NO_x was pH-dependent and that FE_{NO} decreased after buffer inhalation in patients with asthma.

An interesting observation in our study was the correlation between nitrite concentration in EBC and nitric oxide (FE_{NO}). Hunt et al [8] showed that, in patients with asthma exacerbation and an acidic airway environment (EBC pH, 5.23), the preferred pathway is by formation of NO from nitrite. EBC pH in patients with asthma is generally acidic, although it is higher in stable patients than in those who suffer acute exacerbations. In our study, we found that the pH of EBC was slightly acid (pH, 6.74) during the pollen season and that it was probably not sufficient to cause NO to progress from endogenous nitrite. Gratziou et al [33] also showed elevated levels of EBC nitrite/nitrate and FE_{NO} in patients with allergic rhinitis during the pollen season.

Determination of FE_{NO} is a widely accepted method of monitoring inflammation in the airway. We observed an increase in FE_{NO} during the pollen season in almost all allergic rhinitis patients. FE_{NO} tends to increase in allergic rhinitis patients without BHR (P=.06). We also observed an increase in EBC nitrite and a decrease in EBC pH in this group, although the difference was much smaller (pH, P=.14; nitrite, P=.48) than in the allergic rhinitis with BHR group during pollen season (pH, P=.01; nitrite, P=.02).

We hypothesized that nitrites and EBC pH were better than FE_{NO} for differentiating between allergic rhinitis patients who are at risk of BHR. The pathophysiology of allergic inflammation leading to development of BHR is complicated and unclear. In our opinion, measurement of the acid–base equilibrium and pH-dependent nitrite production leading to BHR is a better diagnostic tool than the widely accepted and clinically useful FE_{NO}.

The epidemiology and causes of bronchial hyperresponsiveness in patients with allergic rhinitis have yet to be elucidated. Some reports reveal that a significant proportion of allergic rhinitis patients without asthma have BHR to methacholine, which increases during a period of natural exposure [34]. On the other hand, Spanevello et al [35] showed that BHR was undetectable in patients with seasonal allergic rhinitis outside the exposure period. The 22 patients in our study, who were sensitized only to pollen grass and had no symptoms of asthma, did not show BHR to histamine before the pollen season. However, during the season, 10 patients had positive BPT results and 12 had negative BPT results.

One explanation for our results could be a possible association between elevated levels of recognized inflammatory markers and BHR. NO, hydrogen ions, and reactive nitrogen species may damage the airways and cause BHR by a series of mechanisms. Subclinical inflammatory changes within the lower airway have been observed in patients with allergic rhinitis [8].

The airway acid–base equilibrium and nitrogen metabolite concentration in EBC is a new area of research. Our study revealed a relationship between increased BHR and increased nitrite and FE_{NO} and decreased pH in patients with seasonal allergic rhinitis. This association can be assessed using a noninvasive, safe, and easy method. Further studies are needed to standardize the method and to determine its clinical usefulness in distinguishing between asthmatic patients, atopic patients, and healthy patients. The technique could help identify patients who are at risk of developing asthma.

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