

Eosinophil Count in Nasal Mucosa Is More Suitable than the Number of ICAM-1-Positive Nasal Epithelial Cells to Evaluate the Severity of House Dust Mite-Sensitive Allergic Rhinitis: A Clinical Correlation Study

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Key Words

House dust mites · Adhesion molecule-1, intercellular · Epithelium, nasal · Provocation tests, nasal · Perennial allergic rhinitis

Abstract

Background: House dust mite (HDM)-sensitive allergic rhinitis is a perennial rhinitis with persistent nasal inflammation. Currently, there are no reliable parameters to monitor the severity of perennial allergic rhinitis. The purpose of this study was to evaluate correlations between clinical and laboratory parameters in patients with HDM-sensitive allergic rhinitis. **Methods:** We measured nasal symptoms, did the *Dermatophagoides pteronyssinus* (Der P) skin prick test (SPT), evaluated the Der P allergen nasal challenge threshold, and laboratory parameters [(1) inflammatory cell count from nasal mucosal scraping specimens: eosinophils and neutrophils and (2) immunocytochemistry: ICAM-1 expression on nasal epithelial cells] in 20 cases of HDM-sensitive allergic rhinitis and performed correlation tests between all parameters. **Results:** The wheal diameter induced by Der P SPT was significantly correlated with the Der P allergen nasal challenge threshold ($p = 0.001$). The number of eosinophils from

nasal mucosal scraping specimens was correlated with the ICAM-1 expression on nasal epithelial cells ($p = 0.039$), the number of neutrophils from nasal mucosal scraping specimens ($p = 0.001$), and nasal stuffiness ($p = 0.037$) but did not correlate with total nasal symptom scores. **Conclusion:** Clinical symptoms of HDM-sensitive allergic rhinitis showed a poor correlation with inflammatory parameters. The eosinophil count in nasal mucosa is correlated with ICAM-1 expression and more suitable than ICAM-1 levels to evaluate the severity of HDM-sensitive allergic rhinitis. This study also supports the role of the SPT in the diagnosis of nasal allergy to HDM.

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Introduction

Allergic rhinitis is a common allergic disease worldwide [1, 2]. It is not only a bothersome condition but also contributes to various complications such as sinusitis, otitis media, and asthma [3–5]. In tropical countries, perennial allergic rhinitis (PAR) is more common than the seasonal type. Physicians considered PAR to be more difficult to treat and the level of patient satisfaction with the treatment of PAR was low [6]. There is evidence that the

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inflammatory process in PAR is more severe than that of seasonal allergic rhinitis (SAR) [7]. Persistent minimal inflammation was demonstrated in both asymptomatic cases of PAR and SAR [8, 9]. The threshold of bronchial hyperresponsiveness in PAR was lower than that in the seasonal counterpart [10, 11]. Clinical features of PAR vary from nearly asymptomatic to a classical allergic response [12]. Therefore, clinical symptoms alone are not sensitive enough to evaluate the severity of allergic inflammation taking place in the nose.

Currently, there is no gold standard to evaluate the severity of PAR. A uniform grading system to assess the severity of rhinitis symptoms has not been standardized [13]. Most correlation data between clinical and laboratory findings were collected in SAR and the results are still controversial. A study showed that skin prick tests (SPT) had a better positive predictive value in the diagnosis of respiratory atopic diseases compared to serum IgE levels and Phadiatop [14]. It was demonstrated that the nasal peak inspiratory flow rate correlated significantly with nasal symptom scores after 2 and 4 weeks of treatment of SAR as well as a downward trend of nasal nitric oxide (NO) [15, 16]. In patients with SAR, the symptom scores of patients allergic to birch and grass pollen were correlated with the results of basophil histamine release, specific IgE, eosinophil counts, total IgE, serum and plasma eosinophil protein X, and serum eosinophil cationic protein (ECP). However, the relationship between various allergic inflammatory markers and the symptom score could explain only half of the total variation in the patients' symptoms [17]. In a study of long-term patients with allergic rhinitis, the nasal histamine challenge was associated with a concomitant change in skin and nasal mucosal reactivity to allergens but was not closely related to symptom severity [18]. Correlation data in perennial allergens are less evident and far from conclusive. In PAR, a positive nasal provocative test was found to correlate with a positive SPT in the diagnosis of PAR due to *Dermatophagoides pteronyssinus* (Der P) [19]. In the study in asymptomatic adult Pacific Islanders, the wheal size of house dust mite (HDM) SPT was associated with exhaled NO levels and nasal NO levels in HDM-sensitive subjects [20]. Previous studies showed that nasal smear eosinophilia represented a specific test for the diagnosis of allergic rhinitis, when patients with nasal polyposis and aspirin sensitivity and/or negative skin tests had been excluded [21, 22]. The relationship between nasal hyperreactivity, mediators and eosinophils in patients with perennial rhinitis showed that nasal hyperreactivity tested by histamine challenge was correlated with

the percentage of eosinophils in nasal lavage fluid [23]. Furthermore, eosinophilia in nasal secretions was found to correlate with the SPT and nasal provocative test with specific antigen in the diagnosis of nasal allergy [24]. Evaluation of nasal eosinophilia in children suffering from PAR demonstrated that nasal eosinophilia decreased during treatment with intranasal beclomethasone dipropionate [25].

Recently, roles of adhesion molecules in allergic rhinitis have been widely investigated. A number of CD4-positive cells was found to correlate with the intensity of ICAM-1 expression, especially in the superficial portion of nasal lamina propria [26]. Nasal sICAM-1 levels in patients with PAR were correlated with levels of soluble ECP and were higher than those in patients with seasonal rhinitis outside the pollen season and nonallergic patients. Therefore, nasal soluble ICAM-1 levels have been proposed as a hallmark for clinical severity and follow-up evaluation in PAR [27]. Apart from ICAM-1, levels of serum soluble vascular cell adhesion molecule-1 (sVCAM-1) in patients with severe PAR were significantly higher than those in both the mild rhinitis group and the control group. In addition, the serum levels of sVCAM-1 were significantly correlated with the nasal symptoms in the rhinitis group [28]. However, one study failed to demonstrate the elevation of sICAM-1 in nasal secretion of patients with SAR compared to the control group. In contrast, the elevated levels of sICAM-1 were found to be significant in patients with chronic nonallergic sinusitis [29]. So far, no study has compared the number of nasal eosinophils and ICAM-1 expression in terms of clinical relevance.

HDM allergy is a common cause of PAR in tropical climate [30–33]. In Thailand, 72% of patients with chronic allergic rhinitis were sensitized to the HDM allergen proved by SPT [34]. The present study was designed to study correlations between clinical symptoms, SPT, the in vivo provocation test, and laboratory parameters in HDM-sensitive allergic rhinitis patients.

Materials and Methods

Patients

Patients visiting the Allergy Clinic of the King Chulalongkorn Memorial Hospital were entered into the study. Twenty patients with PAR who were HDM-sensitive proved by a positive SPT and a corresponding history of HDM allergy were enrolled. All patients were between 15 and 65 years old and had had symptoms of allergic rhinitis for at least 1 year with moderate to severe symptoms (average daily nasal symptom scores at least 7 base on a 4-point scale system). Nasal symptoms recorded were sneezing, nose itching, rhinorrhea

and nasal obstruction. Scoring was according to an arbitrary 4-point rating scale (0 = absent, 1 = mild, 2 = moderate, 3 = severe). A total symptom score was calculated as the sum of single symptom scores.

Patients with respiratory tract infections within the preceding 2 weeks, with anatomical nasal abnormalities, or who had previously received allergen immunotherapy were excluded. Pregnant or lactating women were not eligible for the study. All patients were non-smokers and had no concurrent significant illnesses. Antihistamines and systemic/intranasal/inhaled corticosteroids were discontinued for at least 2 and 6 weeks before the study, respectively. All patients had given their written consent. The study was approved by the hospital ethical committee.

Study Design

The design of the study was cross-sectional.

Operations and Measurement

Patients enrolled in the study had been asked for their average nasal symptom scores in the past year and the symptom scores on the day of the study. Skin wheal diameters positive by the prick method with Der P allergen extract were recorded. After that, nasal mucosal scrapings for epithelial cells and inflammatory cells on nasal mucosa were done, followed by nasal provocation with Der P allergen extract.

Skin Prick Test

The skin test had been performed on the volar surface of the forearm by pricking with an injection needle No. 26 with Der P allergen extract [10,000 allergic unit (AU)/ml]. Skin wheal diameters ≥ 5 mm and $\geq$ size of 10 mg/ml histamine hydrochloride control were defined as a positive response.

Nasal Scrapings

Nasal scrapings were obtained at baseline before Der P allergen extract nasal challenge. Specimens obtained by nasal scrapings were examined for ICAM-1 expression on nasal epithelial cells and for cytological assessment.

Nasal Provocative Test

Before starting the nasal challenges the patients were asked to wait for 30 min in order to allow the nasal mucosa to become acclimatized. A control solution [phosphate-buffered saline (PBS) containing human serum albumin 0.03% and benzalkonium chloride 0.05%] had 15 min later been sprayed into each nostril with a metered dose spray delivering a fixed dose of 0.125 ml solution per puff. After provocation with a control solution, 0.125 ml of increasing concentrations of Der P allergen extract (25, 100, 250, and 500 AU/ml; Greer Laboratories, Lenoir, N.C., USA.) was applied to both nostrils at 15-min intervals until a positive nasal response was determined (total nasal symptom score ≥ 7) or the highest dose of Der P allergen extract (500 AU/ml) was reached.

Cytological Assessment

Nasal scrapings had been performed as previously reported by Ciprandi et al. [35] with some modifications. In brief, mucosal specimens were scraped from the surfaces of the middle thirds of the inferior turbinate in both nostrils with rhinoscopes (Allertec, Samutprakarn, Thailand) after nasal lavage. Specimens were placed in PBS solution (2 ml) to allow cell elution. The suspension was cytospun and slides were prepared using standard techniques (200 μ l of fluid,

800 g for 5 min). The specimens had been stored at -20°C until assayed.

The differentiation of inflammatory cells was made by May-Grünwald Giemsa staining. The slides had been read using a light microscope (Olympus BH2, objective 40, eyepiece 10 $\times$, field No. 26). The number of inflammatory cells (neutrophils and eosinophils) was considered as a total number of any cell type/each microscope field: the data were expressed as a mean of 10 fields. Levels of ICAM-1 expression were evaluated by immunocytochemistry in all 20 patients and the differentiation between eosinophils and neutrophils from nasal scraping specimens could be made by Giemsa staining in 19/20 patients. Slides were examined by a pathologist masked to the identity of the samples.

Immunocytochemistry

The sensitive immunoenzymatic alkaline phosphatase-monoclonal antialkaline phosphatase (APAAP) complex procedure had been employed but modified from Cordell et al. [36].

Specimens were submitted to 1:50 dilution of purified ICAM-1 mAb (1 mg/ml, 84H10, IgG1-Immunotech, Marseille, France). After washing in PBS pH 7.6, samples were incubated with rabbit anti-mouse Ig followed by APAAP complex. Afterwards, specimens were incubated in substrate solution containing basic new fustin, naphthol biphosphate and levamisole as an inhibitor of endogenous alkaline phosphates (Sigma, St. Louis, Mo., USA). In control samples either mAb or anti-mouse Ig were omitted. As negative isotype control for ICAM-1 staining on epithelial cells, an anti-T lymphocyte (CD3) mAb OKT3 and IgG1 (DAKO, Milan, Italy) at 1:20 dilution of the stock solution were used. The dilution was established on the basis of previous titration experiments. All preparations were counterstained with Harris' hematoxylin and examined by a pathologist. The identity of the samples was masked. Percentages of ICAM-1 expression on nasal epithelial cells had been scored.

Statistical Analysis

A statistical analysis of the results was performed by Spearman's test to evaluate correlations between average daily nasal symptom scores, nasal symptom scores on the day of the study, ICAM-1 expression on nasal epithelial cells and the number of inflammatory cells in nasal mucosa. $p < 0.05$ was considered statistically significant. The analysis was performed using SPSS 9.05 for Windows (SPSS, Chicago, Ill., USA).

Results

Clinical Data and Cytological Data

Twenty patients with HDM-sensitive allergic rhinitis (7 men and 13 women) were enrolled in this study. The patients' characteristics are listed in table 1. The duration of the symptoms was 7 years (range 1–22 years). Average daily nasal symptom scores were 10 (range 7–12).

On the day of the study, nasal symptom scores were 4 (range 0–8). The wheal diameter induced by Der P SPT was 7.25 mm (range 5–15.5 mm). The median Der P nasal challenge threshold was 100 AU/ml (range 25–500 AU/ml).

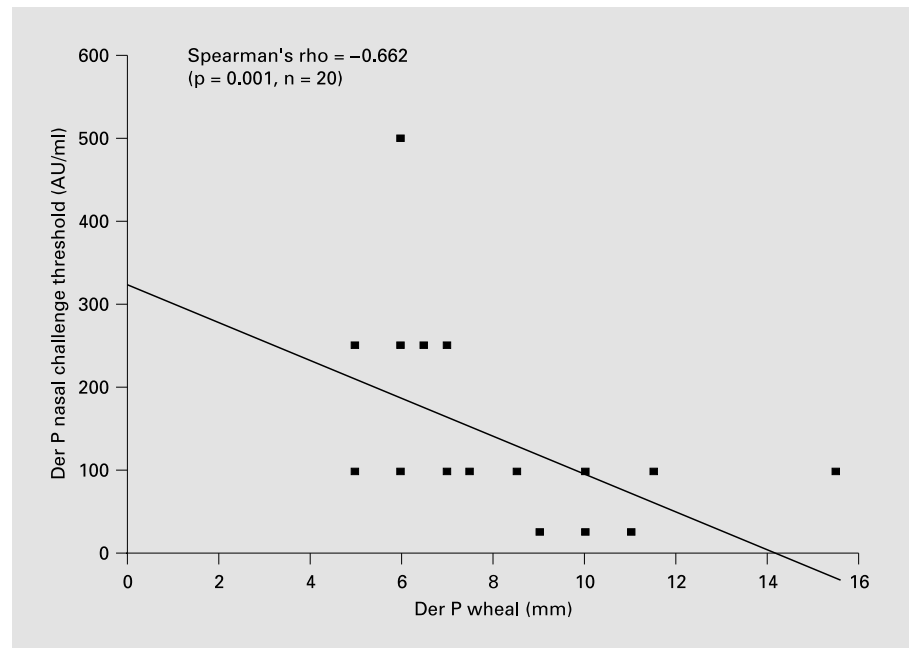


Fig. 1. Correlation between Der P wheal diameter SPT and Der P allergen nasal challenge threshold.

Table 1. Characteristics of 20 patients with HDM-sensitive allergic rhinitis (n = 20; M/F = 7/13)

	Median
Age, years	26 (16–48)
Duration of rhinitis, years	7 (1–22)
Average nasal symptom scores	10 (7–12)
Nasal symptom scores on the day of the study	4 (0–8)
Der P wheal, mm	7.25 (5.0–15.5)
Der P allergen nasal challenge threshold, AU/ml	100 (25–500)
ICAM-1 expression on nasal epithelial cells, %	33.5 (0.5–58)
Eosinophils in nasal scraping specimens, cells/ 10HPF	12.5 (1–436)
Neutrophils in nasal scraping specimens, cells/10 HPF	2 (0–44)

Figures in parentheses represent range.

The ICAM-1 expression on nasal epithelial cells was 33.5% (range 0.5–58%). The number of eosinophils and neutrophils from nasal scraping specimens was 12.5 (range 1–436) and 2 (range 0–44) cells/10 high-power fields, respectively.

Correlation Data

The wheal diameter induced by Der P SPT had a significant inverse correlation with the Der P nasal challenge threshold (p = 0.001, n = 20) (fig. 1).

The number of eosinophils from nasal mucosal scraping specimens of HDM-sensitive patients with allergic rhinitis was shown to correlate with ICAM-1 expression

on nasal epithelial cells (p = 0.039, n = 19) (fig. 2) and the number of neutrophils from nasal mucosal scraping specimens (p = 0.001, n = 19) (fig. 3). The number of eosinophils and neutrophils were both correlated with nasal stuffiness (p = 0.037, n = 19 and p = 0.010, n = 19, respectively) (fig. 4).

We did not find any correlation between the total nasal symptom scores on the day of the study and either the number of eosinophils in nasal scraping specimens (p = 0.142, n = 19) or the level of ICAM-1 expression on nasal epithelial cells (p = 0.602, n = 20) (data not shown).

Average daily nasal symptom scores in the preceding year or total nasal symptom scores on the day of the study

Fig. 2. Correlation between number of eosinophils in nasal scraping specimens and ICAM-1 expression on nasal mucosal epithelial cells.

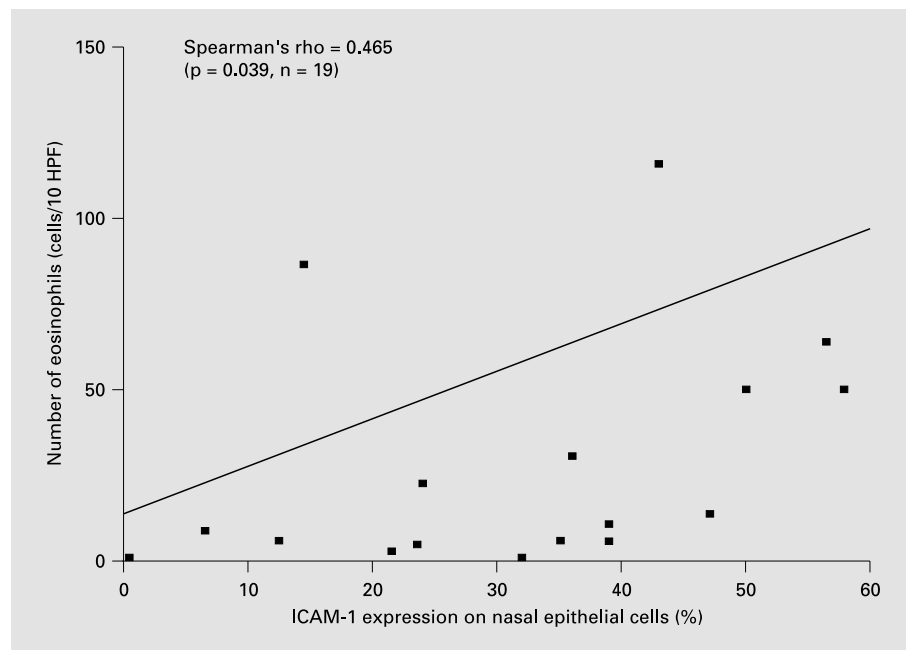
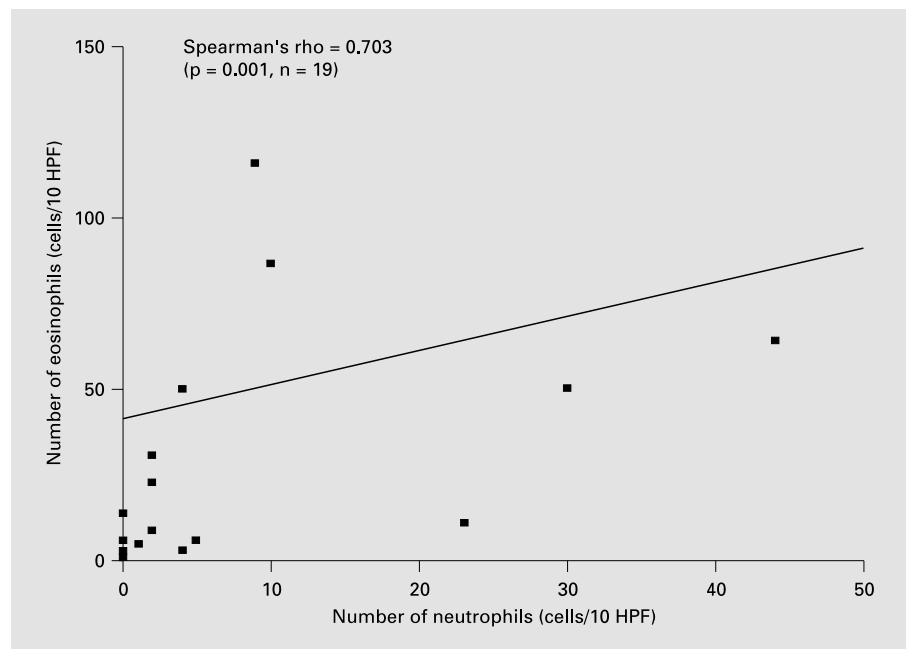


Fig. 3. Correlation between number of eosinophils and number of neutrophils in nasal scraping specimens.



did not demonstrate any correlations with the wheal diameter induced by Der P SPT, Der P nasal challenge threshold, ICAM-1 expression on nasal epithelial cells, or the number of inflammatory cells from nasal scraping specimens ($p > 0.05$, data not shown).

Discussion

PAR is very common in tropical climate. Prevalence of allergic rhinitis varies from country to country and ranges from 1.4 to 39.7% in schoolchildren [2]. Its symptoms are sneezing, nasal pruritus, rhinorrhea, stuffy nose, and nasal hyperresponsiveness. Serious complications such as otitis media, sinusitis, and asthma can be the consequence of

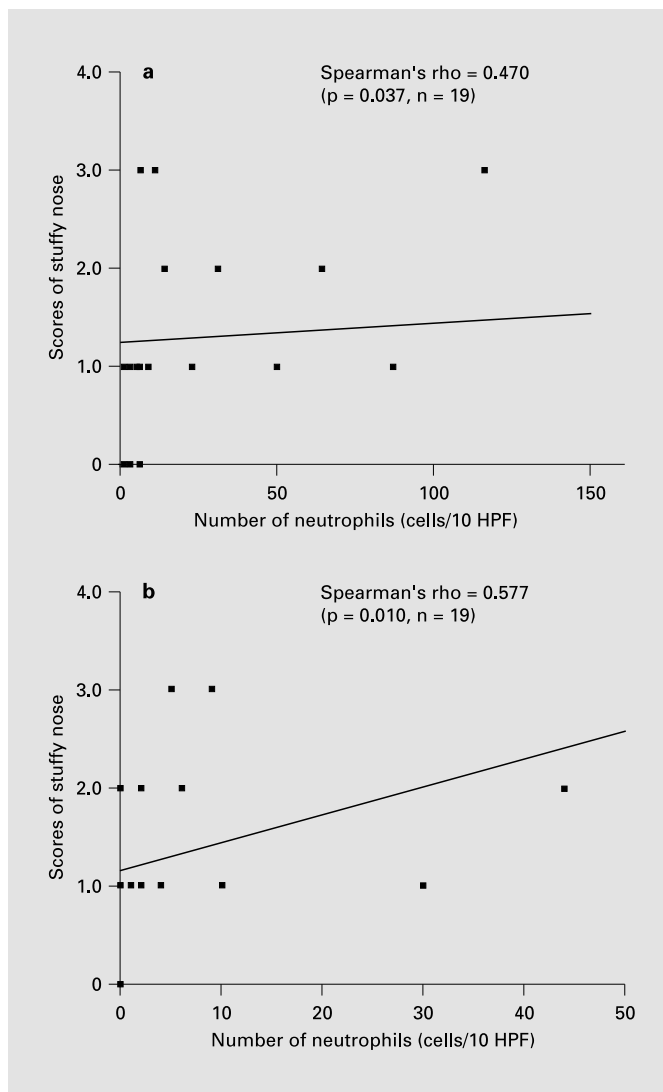


Fig. 4. Correlation between number of inflammatory cells and severity of stuffy nose. **a** Eosinophils. **b** Neutrophils.

chronic rhinitis. Because of its chronicity, however, many patients tolerate very well that they are nearly asymptomatic [12]. To date, there is no standard parameter to determine the severity of PAR [37].

This study recruited 20 patients with HDM-sensitive allergic rhinitis as a model to study PAR. Since both acute early phase and late phase of allergic reactions contribute to nasal symptom scores, clinical manifestations alone may not truly reflect the degree of allergic inflammation and vice versa. It had been previously demonstrated that minimal persistent inflammation could be detected on nasal mucosa in asymptomatic patients with HDM sensitivity [8]. Our study showed that symptom scores were

not correlated with any inflammatory markers and were unreliable to assess the severity of PAR.

The wheal diameter induced by Der P SPT was clearly demonstrated as a good marker to evaluate allergic nasal response [14]. It was very well inversely correlated with the dose of Der P allergen challenged into the nostrils. Our data confirmed a previous report that the wheal diameter of a positive SPT was strongly correlated with the nasal challenge threshold and is a good screening test for the diagnosis of HDM-sensitive allergic rhinitis [38].

Some of the laboratory parameters presented interesting results. Nowadays, the level of ICAM-1 expression on nasal epithelial cells has been used to evaluate nasal mucosa inflammation. ICAM-1 was mentioned as an early inflammatory marker in allergic rhinitis [39, 40]. Surprisingly, our study showed that the level of ICAM-1 expression on nasal epithelial cells was neither correlated with symptoms of PAR nor the nasal challenge threshold. The result indicated that the ICAM-1 expression might not be an appropriate parameter to monitor allergic inflammation in chronic cases such as PAR.

Eosinophil infiltration is the cornerstone of allergic inflammation. The number of eosinophils on nasal mucosa has been known as a marker of allergic inflammation in the nose for many years [21, 22, 24, 41–50]. Mediators from eosinophils, particularly eosinophil cationic protein, play a pivotal role in the pathogenesis of allergic rhinitis and are under intensive investigation [51–54]. The levels of inflammatory products of eosinophils and basophilic cells (ECP, histamine and tryptase) in nasal secretions showed a direct association with the circadian variation in nasal reactivity to methacholine and symptoms of allergic rhinitis [55]. Our data showed that the number of eosinophils from nasal mucosa in patients with HDM-sensitive allergic rhinitis was correlated with levels of ICAM-1 expression on nasal epithelial cells, a stuffy nose, and the number of neutrophils from nasal mucosa, whereas ICAM-1 expression on the nasal mucosal epithelial cells did not correlate with any parameters except the number of eosinophils. This study showed correlations between nasal stuffiness and the number of eosinophils and neutrophils. Nasal stuffiness is a prominent symptom in PAR, which probably relates to cellular recruitment from the late phase of inflammation in the nose.

According to this study, ICAM-1 expression is not more sensitive than the number of eosinophils to evaluate PAR. Since the number of eosinophils was correlated to the level of ICAM-1 expression on nasal epithelial cells and eosinophil count with the Giemsa stain is more feasible in routine clinical practice than ICAM-1 analysis by

immunocytochemistry, we conclude that the number of eosinophils from nasal mucosa is more cost-effective and more applicable than ICAM-1 expression on nasal epithelial cells to monitor allergic inflammation in PAR. Laboratory markers with a good correlation to severity of PAR are yet to be explored.

In summary, the present study suggested that the eosinophil count in nasal mucosa is one of the most appropriate parameters to measure the severity of nasal

allergic inflammation. The result also supports the role of the SPT in the diagnosis of HDM-sensitive allergic rhinitis.

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