

Original Article

Classification of non-allergic rhinitis based on inflammatory characteristics

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Abstract: The study aims to investigate nasal and lower airway inflammation in patients with non-allergic rhinitis (NAR), and to discuss a method of NAR classification based on inflammatory characteristics and its clinical significance. A total of 117 NAR patients admitted to our hospital from June 2010 to June 2011 were enrolled in this study, 162 healthy participants were employed as healthy controls. Nasal and lower airway inflammation were evaluated using the skin prick test, nasal and pulmonary visual analogue scale scoring, cell blood count, nasal douche, induced sputum assay, nasal provocation test, and bronchial provocation test. Compared to the healthy controls, NAR patients have significant higher levels of nasal douche eosinophils, more induced sputum eosinophils as well as blood eosinophils, and higher rates of nasal and bronchial provocation. Patient with high level of eosinophil in nasal douch tended to be with higher concentrations of eosinophils in induced sputum. Scores on the nasal and bronchial provocation tests are also correlated to each other. Among all NAR patients, 28 cases (23.9%) were with no abnormality detectable by eosinophil measurement or a provocation test, 39 cases (33.3%) were with elevated levels of eosinophils, and 50 cases (42.7%) exhibited a nasal provocation response. Based on this, all studied NAR cases were classified into 3 groups: non-specific type (group A, 28/117), increased eosinophil type (group B, 39/117), and hyper-reactive type (group C, 50/117). Some NAR cases may be considered as systemic inflammatory disease characterized by increased nasal eosinophil and nasal hyperreactivity.

Keywords: Non-allergic rhinitis, skin prick test, nasal provocation, bronchial provocation, nasal douche, induced sputum

Introduction

Rhinitis is usually classified into allergic rhinitis (AR) or non-allergic rhinitis (NAR), according to the skin-prick test (SPT) and/or the presence of serum-specific IgE [1]. Due to its high incidence globally and its close relationship with lower airway inflammation, AR is a hot topic and has been widely studied. NAR shares numerous traits with AR, i.e. the underlying inflammatory mechanism, nasal “entopy” [2] (a local inflammatory reaction mediated by IgE), autonomic nervous system dysfunction [3] (as manifested in sympathetic/parasympathetic nasal innervation), and sensory hypersensitivity [4]. However, relatively few studies were performed on NAR regarding the pathogenic mechanism, the characteristics of nasal inflammation, the associated effects on the lower airway, and the optimal

diagnosis approach as well as treatment approach.

Through a systemic study of the nasal, airway, and blood inflammatory indicators in NAR patients, the work aimed to figure out the primary inflammatory characteristics of NAR. A method for NAR classification was also proposed based on nasal inflammatory characteristics.

Methods

Patients

This study included 117 NAR patients, 59 males and 58 females, newly diagnosed in our outpatient clinic from June 2010 to June 2011. NAR was defined as having symptoms of rhinitis (≥ 2 of the following: sneezing, nasal itch, rhinorrhea, and nasal block) along with a negative

NAR classification on inflammatory characteristics

Table 1. Nasal inflammation in NAR patients

	Nasal provocation (%)	Nasal douche eosinophils (numbers)
NAR group (N = 117)	65.8 (77/117)	3.98±8.36
Control group (N = 162)	32.1 (52/162)	0.41±1.27
P value	< 0.0001	< 0.0001

Table 2. Inflammation of the lower airway and blood among NAR patients

	Bronchial provocation (%)	Induced sputum eosinophils (%)	Blood eosinophils (%)
NAR group (n = 117)	10.3 (12/117)	1.37±1.43	3.14±2.93
Control group (n = 162)	1.2 (2/162)	0.81±1.44	1.81±1.22
P value	0.001	< 0.0001	< 0.0001

SPT [5]. An additional 162 participants, 81 males and 81 females, were included as healthy controls. All subjects provided written informed consent.

The exclusion criteria for both groups are listed below: 1) with systemic disease, 2) with nasal sinus diseases including nasosinusitis, deviation of nasal septum, nasal polyp, and so on, 3) with airway inflammation diseases like asthma, bronchitis and so on, 4) with long-term/recent medication.

Testing method

Each rhinitis patient provided a case history, underwent the SPT, and scored their nasal and pulmonary symptoms with use of the visual analogue scale (VAS). Additional information was obtained in the form of a cell blood count and via the use of a nasal douche. Each patient was also made to undergo the induced sputum, nasal provocation, and bronchial provocation tests [6]. Patients were required to stop any use of antihistamines, glucocorticoids or cold medicine one week before the test.

SPT: SPT were performed with use of international standard allergens (Alutard® SQ, Denmark ALK-Abello) including 13 types of airborne allergens: house dust mites, dust mites, tropical mites, dog hair, cat hair, pollen group I, pollen group IV, German cockroach, American cockroach, felon herb, ragweed, mold group I, and mold group IV. The species included in pollen group I were the plane tree, poplar, willow, and elm. In pollen group IV, there were spider brake, ghee timothy, darnel, and pasture grass. In mold group I, there were *Alternaria tenuis*, *Chaetomium globosum*, mixed *Cladosporium*, and *Fusarium verticillioides*. In mold group IV, there were blue mold, *Penicillium expansum*, and *Penicillium notatum*. The prick test was performed in each case by a professional who adhered strictly to the protocol received with the ALK prick-test solution. The test results were obtained 15 min after the prick. It was considered positive if there was a pale yellow skin papule with erythema around it.

Nasal and pulmonary symptoms were scored according to the VAS. The score is ranged from 0 to 10, where 0 indicates lack of any symptom and 10 indicates the most severe extent of symptoms.

Nasal douche test: Saline nasal irrigation was performed with use of a syringe, and 10 ml of irrigation solution (warm 0.9% normal saline) were injected into the middle and lower nasal meatus (Irrigation was considered thorough if there was saline running out from the other nostril). The irrigation fluid was recollected with a funnel, and irrigation with the fluid in the funnel was repeated 3 times, such that the entire irrigation phase lasted approximately 5 minutes. Next, we drew out the irrigation fluid. After that, the patient was asked to blow his/her nose gently to make the remaining fluid completely collected in the funnel. Then, 5 ml of the irrigation fluid was used for testing. After filtration and centrifugation, the supernatant was collected and stored at -80°C for further use.

In this study, the cell sediments were stained by hematoxylin and eosin (H&E). For the test, 20 µl of the cell sediments were smeared onto a slide and the slide was observed with use of a microscope under a 200× magnification field. The total number of inflammatory cells (e.g., eosinophils and macrophages) was determined using the absolute value for eosinophil-related calculations.

Induced sputum test: The patient was asked to inhale ultrasonic nebulized 3% hypertonic saline for 10 minutes, and then cough forcefully to get sputum out. If failed, the patient continued the ultrasonic nebulization for another 5 minutes. A sample of sputum with high density and viscosity was obtained using flat tweezers,

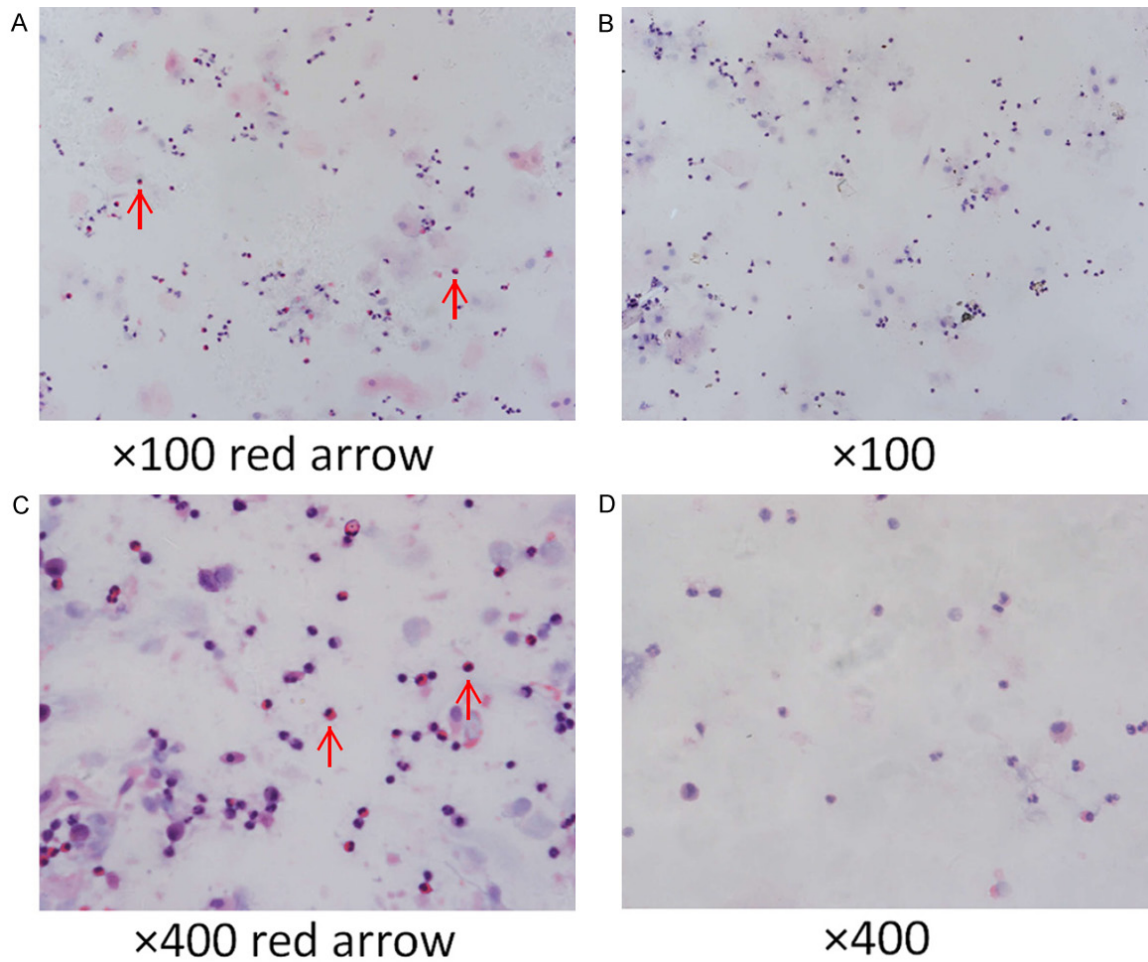


Figure 1. A. Nasal douche of NAR group (H&E staining $\times 200$); B. Nasal douche of control group; C. Induced sputum of NAR group; D. Induced sputum of control group; The arrows show eosinophils.

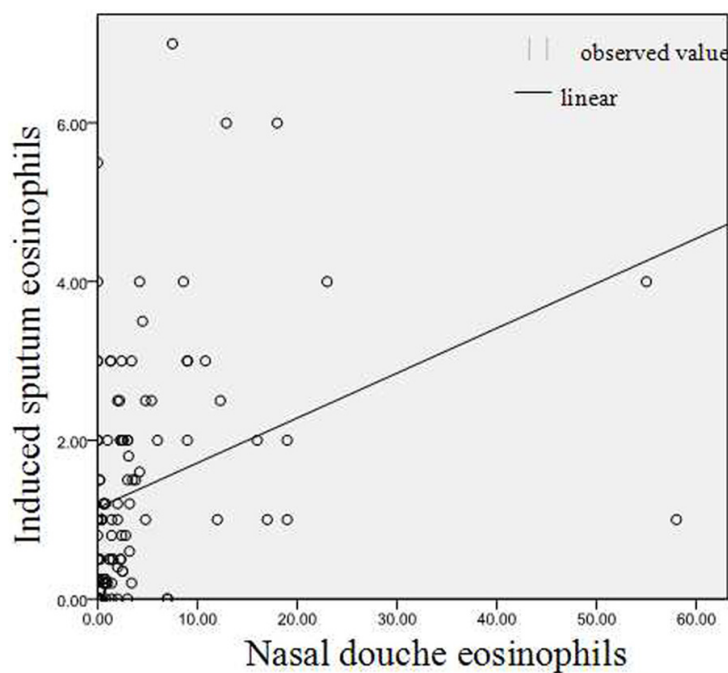


Figure 2. Correlation between nasal douche and induced sputum eosinophils.

and dissolved in solution of 0.1% DTT at a 1:5 volume ratio. Samples were shaken and then placed in 37°C water bath for 10 min. Impurities were filtered using a mesh screen. After centrifugation, the supernatant was collected and stored at -80°C for further use. The cell sediments were stained by H&E and smeared onto slides. The slides were read at 400 \times magnification. The proportion of each type of inflammatory cell (eosinophils, macrophages, lymphocytes, and neutrophils) in a 200-cell sample was determined.

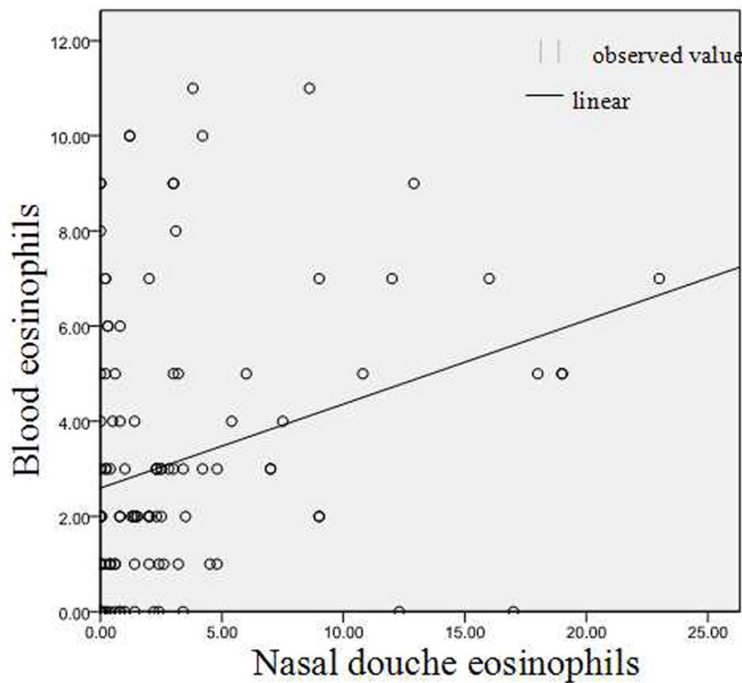


Figure 3. Correlation between numbers of eosinophils in nasal douche and blood.

Nasal provocation test: This test was performed to measure the resistance of the patient's bilateral nasal airway. A solution of 0.1 mg/ml histamine was sprayed onto both inferior turbinates, one spray on each side. The resistance encountered was measured and recorded. If nasal airway resistance had increased by $\geq 100\%$, the test was considered as positive and terminated. If not, solutions of increasing histamine concentration (0.2, 0.4, and 0.8 mg/ml) were administered. If a positive standard had not been reached with the highest concentration of 0.8 mg/ml, the nasal provocation test was judged as negative. The nasal provocation test results were assigned 5 grades according to the concentration that provoked a positive result. Total nasal resistance (TNR) was measured by calculating the resistance of each nostril separately (P/V): $TNR = \text{Right NR} \times \text{Left NR} / (\text{Right NR} + \text{Left NR})$, as previously described.

Bronchial provocation test: In order to measure basic pulmonary function, the patient was asked to inhale 0.1 μmol methacholine. If the final expiratory volume was reduced by $\geq 20\%$ of the basic value, the bronchial provocation test was judged as positive. If not, a series of increasing concentrations was administered, and the cumulative dosages administered were

0.4, 1.6, 3.2, 6.4, and 12.8 μmol . If the positive standard had not been achieved with a cumulative dosage of 12.8 μmol , the bronchial provocation test was judged as negative. The bronchial provocation test results were stratified into 6 groups.

Statistical analysis

All the data are shown as mean \pm standard deviation ($X \pm SD$). Data arrangements and analysis were done using the statistical software SPSS 13.0. The relationship between 2 vari-
ances was studied via relevance analysis, t-test was applied to compare the results of the 2 groups, Fisher's exact test was used to compare the positive rates, and one-way variance analysis was performed to compare the results among 3 groups.

A difference was considered statistically significant with a P value < 0.05 . All the statistical tests were two-sided.

Results

Nasal and lower airway inflammatory characteristics of NAR patients

The nasal douche eosinophil number, induced sputum eosinophil proportion, blood eosinophil proportion, nasal provocation positive ratio, and bronchial provocation positive ratio were all significantly higher in the NAR group than in the control group ($P < 0.05$), as presented in **Tables 1, 2** and **Figure 1**.

Relationship of nasal and lower airway inflammation in NAR patients

There was a positive correlation between nasal douche eosinophil numbers and the proportion of eosinophils in induced sputum ($r = 0.531$, $P < 0.0001$, **Figure 2**). However, there was no relationship between the number of nasal douche eosinophils and bronchial provocation ($P > 0.05$). There was a correlation between nasal provocation and bronchial provocation results ($r = 0.190$, $P = 0.040$).

Table 3. Relative nasal, lower airway, and blood inflammatory indicators in each NAR subgroup and in control group

	Blood eosinophils (%)	Nasal VAS	Pulmonary VAS	Induced sputum eosinophils (%)	Positive results on the bronchial provocation test (%)
Nonspecific NAR	1.86±2.09	5.00±1.83	0.61±1.69	0.92±0.93	7.14 (2/28)
High-eosinophil NAR	4.49±3.13	6.10±2.12	1.18±2.25	2.24±1.64	10.26 (4/39)
Hyperreactive NAR	2.80±2.79	6.27±2.01	0.82±2.24	0.94±1.17	12 (6/50)
Control group	1.81±1.22	-	-	0.81±1.44	1.2 (2/162)

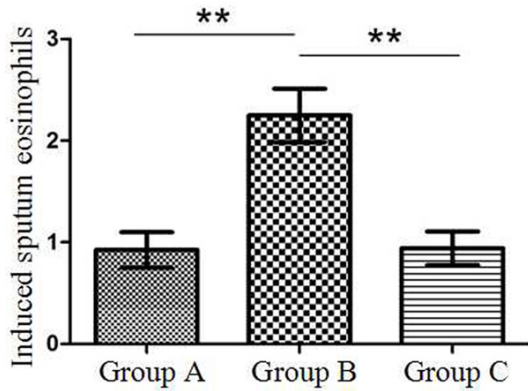


Figure 4. Comparison of induced sputum eosinophils among the groups (* $P < 0.05$, ** $P < 0.01$).

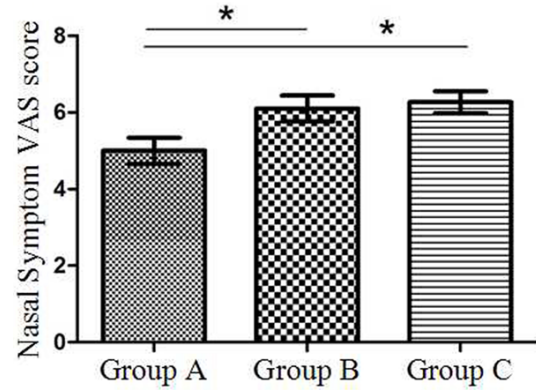


Figure 6. Nasal symptom VAS scores in all groups.

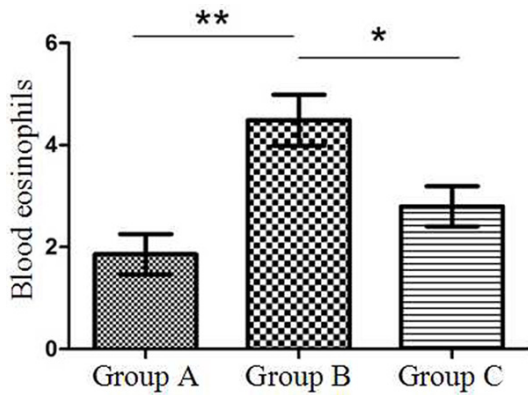


Figure 5. Comparison of blood eosinophil levels among the groups (* $P < 0.05$, ** $P < 0.01$).

Relationship between nasal douche and blood eosinophil levels

There was a positive correlation between the number of eosinophils in nasal douche and in the blood ($r = 0.334$, $P < 0.0001$, **Figure 3**). There was no relationship between nasal provocation test results and the proportion of eosinophils found in blood during the inflammatory response ($P > 0.05$).

NAR classification based on the characteristics of nasal inflammation

The characteristics of nasal inflammation varied among NAR patients. Some had increased eosinophil populations, some were hyperreactive, and others exhibited neither of these abnormalities. Therefore, we classify NAR patients into 3 groups: Group A, nonspecific NAR (nasal douche eosinophils are not increased and negative nasal provocation results); Group B, high-eosinophil NAR (increased nasal douche eosinophils and positive or negative nasal provocation results); and Group C, hyper-reactive NAR (no increase in nasal douche eosinophil levels; positive nasal provocation results).

The nasal, lower airway, and blood indicators in the control and 3 NAR groups are presented in **Table 3**. Compared to other groups, the high-eosinophil NAR group exhibited a larger proportion of eosinophils in the blood as well as in induced sputum ($P < 0.01$, **Figures 4, 5**). In this group, there was a correlation between the nasal and bronchial provocation test results ($r = 0.356$, $P = 0.026$). Nasal VAS scores were significantly lower in the nonspecific NAR group than in other 2 NAR groups ($P < 0.05$, **Figure 6**).

Discussion

Previous research has proved that NAR shared some common mechanism with AR, like the increased nasal mucosa IgE [7, 8]. Patients in one study can converted from NAR to AR [9]. In our study, another similar but different eosinophil changes between NAR and AR was revealed. Compared to AR characterized by high eosinophil levels, only 33.3% of NAR patients showed locally increased eosinophil. In addition, our previous study has proved that NAR differed from AR in the age and season of onset, clinical symptoms, number of nasal eosinophils, and total blood IgE [8]. These findings proved that NAR and AR differs in terms of pathogenesis.

Except for the nasal douch, the increased eosinophils in sputum and blood as well as the airway provocation indicates that NAR is also a systemic inflammatory disease involving the lower-airway. Hence, we speculated that both the eosinophil number and airway hyperactivity could be used to indicate NAR. Unlike the consistency of eosinophils in nasal, lower airway (sputum), and blood, the correlation between eosinophils and lower-airway hyperactivity was indefinite. Therefore, we classified the NAR into 3 groups: nonspecific NAR (Group A), high-eosinophil NAR (Group B), and hyper-reactive NAR (Group C).

Compared to the ARIA classifications according to pathological etiology, i.e. infectious, drug-induced, etc., our classification is based on pathological mechanism and facilitates the diagnosis and therapy. Group B, similar to AR, is the sub-type of allergic airway inflammation with or without hyperactivity, which could be treated by intranasal corticosteroids. Group C, a kind of airway neurogenic inflammation, is the sub-type of nasal cavity hyperresponsiveness without allergic airway inflammation, which could be treated by drugs reducing hyperresponsiveness. Group A is an atypical sub-type, which needs further investigation to elucidate the characteristics.

Although several study has showed that NAR is a risk factor for asthma and chronic bronchitis [10], the pathogenesis and its influence on the lower airway did not get full consideration before. The prospective design is also an advantage of the current study.

In summary, NAR is in some cases not a local inflammation of the nasal mucosa, but a systemic inflammatory disease. The classification of NAR based on eosinophil level and airway hyperactivity is helpful to diagnosis and treatment, although further study is needed to reveal the pathogenic mechanisms of non-specific NAR.

Disclosure of conflict of interest

None.

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References

- [1] Bousquet J, Fokkens W, Burney P, Durham SR, Bachert C, Akdis CA, Canonica GW, Dahlen SE, Zuberbier T, Bieber T, Bonini S, Bousquet PJ, Brozek JL, Cardell LO, Cramer R, Custovic A, Demoly P, van Wijk RG, Gjomarkaj M, Holland C, Howarth P, Humbert M, Johnston SL, Kauffmann F, Kowalski ML, Lambrecht B, Lehmann S, Leynaert B, Lodrup-Carlsen K, Mullol J, Niggemann B, Nizankowska-Mogilnicka E, Papadopoulos N, Passalacqua G, Schunemann HJ, Simon HU, Todo-Bom A, Toskala E, Valenta R, Wickman M and Zock JP. Important research questions in allergy and related diseases: non-allergic rhinitis: a GA2LEN paper. *Allergy* 2008; 63: 842-53.
- [2] Forester JP and Calabria CW. Local production of IgE in the respiratory mucosa and the concept of entopy: does allergy exist in nonallergic rhinitis? *Ann Allergy Asthma Immunol* 2010; 105: 249-55; quiz 256-8.
- [3] Jaradeh SS, Smith TL, Torrico L, Prieto TE, Loehr TA, Darling RJ and Toohill RJ. Autonomic nervous system evaluation of patients with vasomotor rhinitis. *Laryngoscope* 2000; 110: 1828-1831.
- [4] Silvers WS. The skier's nose: a model of cold-induced rhinorrhea. *Ann Allergy* 1991; 67: 32-36.
- [5] Shaaban R, Zureik M, Soussan D, Neukirch C, Heinrich J, Sunyer J, Wjst M, Cerveri I, Pin I, Bousquet J, Jarvis D, Burney PG, Neukirch F and Leynaert B. Rhinitis and onset of asthma: a longitudinal population-based study. *Lancet* 2008; 372: 1049-1057.
- [6] Zhang Y, Wang Q, Xie Y, Wang Z, Li D, Ma L, Pang X, Yu W, Zhong N. The normative value of inflammatory cells in the nasal perfusate of

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- Chinese adults: a pilot study. *J Thorac Dis* 2014; 6: 905-12.
- [7] Powe DG and Jones NS. Local mucosal immunoglobulin E production: does allergy exist in non-allergic rhinitis? *Clin Exp Allergy* 2006; 36: 1367-1372.
- [8] Wang ZY, Zhang Y and Wang QP. Comparison of clinical features of allergic rhinitis and non-allergic rhinitis. *Chin J Otorhinolaryngolgy-Skull Base Surgery* 2011; 17: 340-343.
- [9] Rondon C, Dona I, Torres MJ, Campo P and Blanca M. Evolution of patients with nonallergic rhinitis supports conversion to allergic rhinitis. *J Allergy Clin Immunol* 2009; 123: 1098-1102.
- [10] Hakansson K, von Buchwald C, Thomsen SF, Thyssen JP, Backer V and Linneberg A. Nonallergic rhinitis and its association with smoking and lower airway disease: A general population study. *Am J Rhinol Allergy* 2011; 25: 25-29.