

The Use of Specific Immunoglobulin E in Nasal Secretions for the Diagnosis of Allergic Rhinitis

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Objective: Although local specific immunoglobulin (sIgE) has been employed as a diagnostic criterion for allergic rhinitis (AR), the use of local sIgE has not been fully evaluated in the diagnosis of allergic rhinitis. The aim of this study was to evaluate the use of nasal secretion sIgE in the diagnosis of allergic rhinitis.

Methods: A total of 51 patients (27 males and 24 females, mean age = 33.3 years) with rhinitis symptoms were enrolled consecutively from the allergy-rhinology clinic of Beijing TongRen Hospital (Beijing, People's Republic of China). Questionnaires were completed by each participant at recruitment to record demographic data, nasal symptom severity, and physician-diagnosed comorbid asthma. The severity of nasal obstruction, rhinorrhea, sneezing, and nasal/eye itching was recorded according to a visual analogue scale (VAS) of 10 cm. Sensitization to *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, mugwort, *Candida albicans*, *Penicillium notatum*, *Alternaria*, *Cladosporium*, and *Aspergillus* were assessed according to presence of sIgE antibodies to these allergens in serum and nasal secretions.

Results: VAS scores of the participants ranged from 11 to 25 (mean = 17), and 14 (27.5%) patients had comorbid asthma. Based on serum sIgE, 31 (60.8%) patients were allergic to mugwort, 14 (27.5%) to *Dermatophagoides*, and three (5.9%) to fungal allergens. Based on nasal secretion sIgE, 32 (62.7%) patients were allergic to mugwort, 12 (23.5%) to *Dermatophagoides*, and three (5.9%) to fungal allergens. The local mugwort sIgE level and serum mugwort sIgE level were positively correlated with each other and with VAS scores.

Conclusion: sIgE level in nasal secretions of subjects with rhinitis is a reliable noninvasive alternative to serum sIgE for diagnosis of allergic rhinitis.

Key Words: Allergic rhinitis, nasal secretion, local specific IgE, serum specific IgE, diagnosis, aeroallergen, mugwort, *Dermatophagoides*, fungi, local allergic rhinitis.

Level of Evidence: 4.

Laryngoscope, 00:000–000, 2018

INTRODUCTION

Rhinitis is one of the most common airway diseases; it has significant morbidity and is a considerable financial burden.¹ As the most important subtype of rhinitis, allergic rhinitis (AR) is thought to be an underestimated inflammatory disorder of the nasal mucosa, which is characterized by nasal congestion, rhinorrhea, sneezing, and itching.² Allergic rhinitis has a high prevalence and affects around 400 million people worldwide.³ Several

studies have shown that AR is associated with other airway inflammatory disorders of mucosa, such as rhinosinusitis and asthma,^{3,4} and adversely affects social life, study, and work productivity of affected individuals.⁴

The diagnosis of AR is generally based on history of symptoms, endoscopic examination, and serum immunoglobulin IgE levels. Whereas IgE detected in the serum isolated from peripheral blood of the patients is one of the most important features of AR for distinguishing the allergic from other forms of this airway disorder,³ and is normally employed to indicate the presence of systemic atopy, some studies have shown that there was no significant correlation between the prevalence of atopy and the level of serum IgE, and that about 50% of AR patients had serum IgE levels in the normal range.^{5–7} Thus, assessment of serum IgE raises the possibility of a considerable number of AR patients to be misdiagnosed as normal nonrhinitis patients or to be misdiagnosed as patients with other common types of rhinitis, such as nonallergic rhinitis with eosinophilia syndrome (NARES) or vasomotor rhinitis (VMR).

Over 4 decades ago, Huggins et al.⁸ first demonstrated that allergen-specific IgE (sIgE) could be detected in nasal secretions of AR patients. Several studies have since demonstrated local production of IgE antibodies in the nasal secretion and nasal mucosa of patients with AR,^{9–11} which led to the concept of *entopy*

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Editor's Note: This Manuscript was accepted for publication on January 9, 2018.

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This work was supported by grants from National Key R&D Program of China (2016YFC20160905200); the program for Changjiang Scholars and Innovative Research Team (IRT13082); National Science Fund for the Major International Joint Research Program (81420108009); National Natural Science Foundation of China (81630023, 81100704, 81441029, 81441031, 81570894, 81400444, and 81470678); and the Capital Health Development Foundation (2016-1-2052). The authors have no other funding, financial relationships, or conflicts of interest to disclose.

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DOI: 10.1002/lary.27120

to describe this phenomenon.^{5,9–12} Although local production of IgE antibodies in the absence of systemic atopy is also considered as a diagnostic criterion for local AR,¹² the use of local IgE in the diagnosis of AR has not been evaluated. We hypothesized that local sIgE could be used for the diagnosis of allergic rhinitis instead of serum sIgE, and thus aimed to compare nasal secretion sIgE levels with serum sIgE levels in AR patients.

MATERIALS AND METHODS

Study Design and Patients

This prospective study was conducted from August 1, 2017, to August 31, 2017, in a single center. The study participants suspected to have rhinitis, based on the presence of common symptoms of nasal congestion, rhinorrhea, sneezing, and nasal itching, were recruited consecutively from the allergy-rhinology clinic of Beijing TongRen Hospital (Beijing, People's Republic of China). Questionnaires were completed by each participant at recruitment to record demographic data, nasal symptom severity, and physician-diagnosed comorbidities (allergic conjunctivitis and asthma), and were assessed for sensitization to relevant aeroallergens by measurement of both serum sIgE and nasal secretion sIgE. Diagnosis of AR was confirmed based on the presence of symptoms, as noted above, and clinically relevant serum sIgE results.

The exclusion criteria for the study included pregnancy or lactation; incidence of any immunological disease; chronic rhinosinusitis and/or nasal polyposis, as defined by the European position paper on rhinosinusitis and nasal polyps¹³; any respiratory infection in the previous 4 weeks; and a positive CT scan. Also excluded were patients who had taken systemic corticosteroids over the past 3 months, intranasal corticosteroids over the past 4 weeks, antihistamines over the past 2 weeks, and vasoconstrictors over the past 1 week.

The study was conducted in full accordance with Declaration of Helsinki and approved by the Medical Ethics Committee of Beijing TongRen Hospital. All patients also provided written informed consent prior to entry into the study and collection of data.

Visual Analogue Scale

The severity of ocular and nasal symptoms, including nasal obstruction, rhinorrhea (watery, mucous, and purulent), sneezing, and nasal/eye itching, was recorded using a visual analogue scale (VAS) of 10 cm. Each symptom was categorized as mild (VAS: 0–3 cm), moderate (VAS: >3–7 cm), or severe (VAS > 7 cm).

Serum sIgE Examination

Serum sIgE levels to common aeroallergens were determined using a fluoroenzyme immunosorbent assay (UniCAP, Uppsala, Sweden), with a value for serum sIgE ≥ 0.35 kU/L regarded as positive. The sIgE examination was performed with a panel of allergens including *Dermatophagoides pteronyssinus* (Der p), *Dermatophagoides farina* (Der f), mugwort, *Candida albicans*, *Penicillium notatum*, *Alternaria*, *Cladosporium*, and *Aspergillus*.

Local sIgE Examination

Local sIgE examination was performed in nasal secretions, which were collected and processed as described previously.¹⁴

Briefly, nasal secretions were obtained by inserting a postoperative sinus sponge pack in each nostril for 5 minutes. The quantity of secretions collected in each sponge was determined by comparing the weight of the sponge before and after insertion into the nasal cavity, and the secretion was extracted from the sponge by addition of 2 mL of 0.9% sodium chloride solution. All sponges were stored at 4°C for 2 hours and then transferred to a 5-mL BD syringe (Becton, Dickinson and Company, Franklin Lakes, NJ). The bulk of the nasal secretion was forced out of the sponges using the piston of the syringe, and centrifuged at 1,500 g for 15 minutes at 4°C. The supernatants were separated and stored in aliquots at –20°C until analysis for the presence of sIgE was assessed using a panel of aeroallergens for serum. Again, a value for sIgE ≥ 0.35 kU/L was regarded as positive.

Statistics Analysis

Correlations between local nasal sIgE, serum sIgE, and VAS scores were analyzed by Spearman's rank order test, and *P* values < 0.05 were regarded as statistically significant. Sector graphs and correlation analysis were performed with GraphPad Prism 7.0 software (GraphPad Software, Inc., La Jolla, CA).

RESULTS

A total of 51 subjects, including 27 males and 24 females aged from 8 years to 53 years (mean = 33.3 years), were recruited to this study. Table I shows the demographic characteristics, asthma history, nasal symptoms VAS scores, and the sIgEs against aeroallergens detected in serum and nasal secretions of individual patients. Overall, 14 patients (27.5%) had comorbid asthma, and the VAS scores ranged from 11 to 25 (mean = 17). Thirty-six (70.6%) patients were allergic to only one kind of aeroallergen; seven (13.7%) patients were allergic to more than one kind of aeroallergen; and eight (15.7%) patients were not found to be allergic to any of the aeroallergens tested. The serum sIgE levels ranged from 0.35 kU/L to 189 kU/L, the local nasal sIgE levels from 0.35 kU/L to 17.5 kU/L, and the total concentration in undiluted nasal secretion collected s from 0.5 kU/L to 26.2 kU/L (Table I).

Based on serum sIgE, 31 (60.8%) patients were found to be allergic to mugwort, 14 (27.5%) patients allergic to *Dermatophagoides*, and three (5.9%) patients allergic to fungi (Fig. 1a). Similarly, based on local nasal sIgE, 32 (62.7%) patients were found to be allergic to mugwort, 12 (23.5%) patients allergic to *Dermatophagoides*, and three (5.9%) patients allergic to fungi (Fig. 1b).

Correlation analysis demonstrated local nasal mugwort sIgE level to be positively correlated with serum mugwort sIgE level ($r^2 = 0.70$) (Fig. 2a). Further, mugwort local nasal sIgE and serum mugwort sIgE levels were correlated with VAS scores ($r^2 = 0.36$, and $r^2 = 0.46$, respectively) (Figs. 2b and 2c).

DISCUSSION

Allergic rhinitis poses a big challenge to public health,¹⁵ affecting around 400 million people worldwide, especially in the industrialized countries.³ Recent studies have indicated that the prevalence of self-reported allergic rhinitis in China has increased from 11.1% to

TABLE I.
The Demographic Characteristics and sIgE Detected in Serum and Nasal Secretions of Individual Patients.

No.	Age	Gender (M/F)	Asthma	VAS	Serum IgE	Local IgE	TCLgE	Allergen	Diagnosis
1	33	M	Y	25	189/35.6	17.5/–	26.2/–	Mg/D	AR
2	30	F	Y	25	62.8/17.4	16.9/–	25.7/–	Mg/D	AR
3	30	M	N	21	51.5	17.5	27.1	D	AR
4	23	M	Y	18	< 0.35	< 0.35	< 0.35	–	IR
5	36	F	N	15	< 0.35	< 0.35	< 0.35	–	IR
6	34	M	N	17	17.9	7.8	10.9	Mg	AR
7	29	F	N	16	0.95	7.6	10.3	Mg	AR
8	24	F	N	17	0.7/0.7	7.8/0.7	11.0/1.05	Mg/D	AR
9	47	F	N	18	17.5	3	4.6	Mg	AR
10	34	M	N	16	3.6	2.9	4.06	Mg	AR
11	14	F	N	11	0.35/–	2.9/0.35	4.6/0.56	D/F	AR
12	21	M	N	16	0.35	2.9	4.93	D	AR
13	32	F	N	15	0.7	2.1	3.2	Mg	AR
14	53	M	N	16	0.37	1.9	2.9	Mg	AR
15	37	M	N	18	4.1	1.3	2.2	D	AR
16	43	F	N	16	<0.35	3.5	6.0	F	LAR
17	28	M	N	17	0.7	0.5	0.9	Mg	AR
18	26	M	N	15	0.37	0.45	0.63	Mg	AR
19	50	F	N	11	4.1	0.4	0.7	Mg	AR
20	27	M	Y	20	< 0.35	< 0.35	< 0.35	–	IR
21	26	M	N	21	50.1/0.84/0.7	17/1.02/–	26.0/1.4/–	Mg/D/F	AR
22	26	M	Y	25	59	15.8	22.1	Mg	AR
23	40	F	Y	17	17.5	14.6	22.0	Mg	AR
24	42	F	Y	15	< 0.35	< 0.35	< 0.35	–	IR
25	37	F	N	16	< 0.35	< 0.35	< 0.35	–	IR
26	35	M	N	18	< 0.35	< 0.35	< 0.35	–	IR
27	36	M	N	15	20.8	7.2	13.0	Mg	AR
28	35	M	Y	17	17.6	11.2	16.8	Mg	AR
29	27	M	Y	18	3.5/3.5/3.5	5.5/–/–	7.2/–/–	Mg/D/F	AR
30	43	F	N	13	3.6	0.7	1.1	Mg	AR
31	37	M	N	15	0.35	0.7	1.2	D	AR
32	33	F	N	15	0.35	0.6	1.0	D	AR
33	37	M	N	13	< 0.35	2.9	4.6	Mg	LAR
34	18	M	Y	16	59/0.7	15.4/0.35	21.6/0.5	Mg/D	AR
35	34	F	N	18	17.9	8	12.0	D	AR
36	28	F	N	18	27.6	5.8	9.9	Mg	AR
37	42	M	N	21	< 0.35	< 0.35	< 0.35	–	IR
38	25	F	Y	19	17.5	4.9	6.9	F	AR
39	8	F	Y	17	23.5	6.3	10.7	Mg	AR
40	40	M	N	16	17.6	7.5	11.3	Mg	AR
41	26	M	N	18	17.9	3	5.0	Mg	AR
42	22	F	N	21	0.74	3	4.9	D	AR
43	35	M	N	18	19.1	17.4	22.6	Mg	AR
44	41	F	Y	16	19.6	9.5	17.1	Mg	AR
45	42	F	Y	17	< 0.35	< 0.35	< 0.35	–	IR
46	30	M	N	15	21.4	6	9.6	Mg	AR
47	33	F	N	18	< 0.35	0.35	0.5	D	LAR
48	34	M	N	18	0.45	0.7	1.1	Mg	AR
49	47	F	N	16	17.5	0.7	1.1	Mg	AR
50	34	M	N	18	0.36	0.35	0.6	Mg	AR
51	47	F	N	18	0.35	0.35	0.5	Mg	AR

AR = allergic rhinitis; D = *Dermatophagoides* (*Der p* and *Der f*); F = female; Fu = fungi (*Candida albicans*, *Penicillium notatum*, *Alternaria*, *Cladosporium*, *Aspergillus*); IgE = immunoglobulin E; IR = idiopathic rhinitis; LAR = local allergic rhinitis; M = male; Mg = mugwort; N = no (without asthma); TCLgE = total concentration of local sIgE; VAS = visual analog score (nasal congestion + rhinorrhea + sneezing + nasal/eye itching); Y = yes (with asthma).

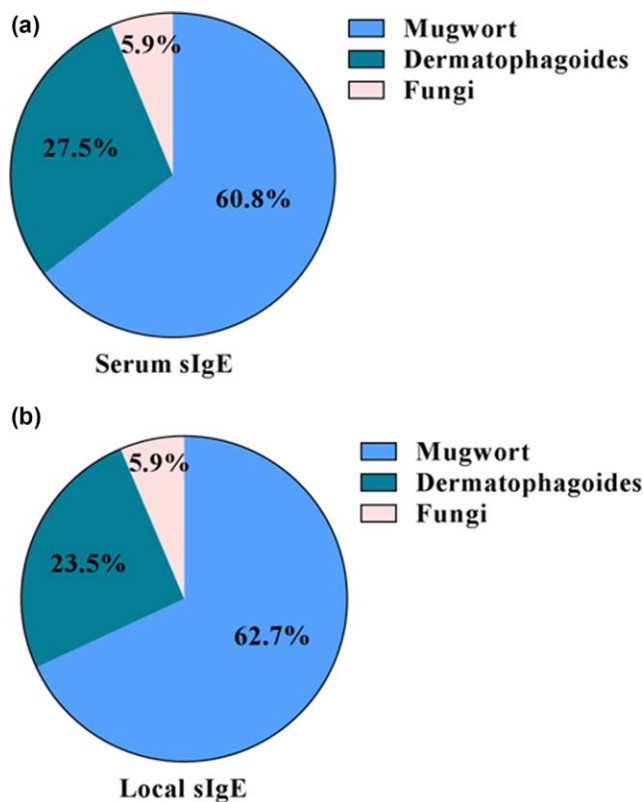


Fig. 1. The distribution of aeroallergens detected as (a) serum sIgE and (b) local nasal sIgE. specific immunoglobulin = sIgE. [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

17.6% during the past 6 years.^{16,17} Because allergic rhinitis imposes a considerable burden on society and negatively impacts the quality of life of affected individuals,¹⁸ the detection of the sensitizing aeroallergen/s has become an integral component of the strategy for diagnosis and management of patients with allergic rhinitis.

In the present study, we measured the serum sIgE levels to a panel of common aeroallergens shown to trigger AR symptoms in Chinese patients and showed that 40 (78.4%) of the patients had positive serum sIgE levels, ranging from 0.35 kU/L to 189 kU/L. The most prevalent sensitizing aeroallergen in this cohort was mugwort (60.8%), followed by *Dermatophagoides* (27.5%) and fungal allergens (5.9%). Combined with medical history, nasal symptoms, and the endoscopic examination, 39 patients were diagnosed to have allergic rhinitis. These findings are in accordance with the findings of Lou et al.,¹⁸ who demonstrated that mugwort and Der f were the most prevalent sensitizing aeroallergens for AR throughout China. In accordance with the findings of other studies,^{1,3,4} the present study also demonstrated that 25% of the allergic rhinitis patients had comorbid asthma.

Several studies have shown that sIgE can be detected in the nasal mucosa and nasal secretions.^{3,5,8} In the present study, we investigated sIgE in nasal secretions collected from all 51 patients and demonstrated that 43 patients, including all 40 AR patients,

had positive local nasal sIgE, the levels of which ranged from 0.35 kU/L to 17.5 kU/L (the total concentration of local nasal sIgE in undiluted secretions ranged from 0.5 kU/L to 26.2 kU/L). In accordance with the findings for serum sIgE, the most prevalent sensitizing aeroallergen detected according to nasal sIgE was also mugwort (62.7%), followed by *Dermatophagoides* (23.5%) and fungal allergens (5.9%). It is possible that this distribution spectrum for nasal sIgE was slightly different from that of the serum sIgE because about 50% of allergic rhinitis patients had serum IgE levels that were within the normal range.⁵ In the present study, the nasal secretion was diluted with 0.9% sodium chloride solution for

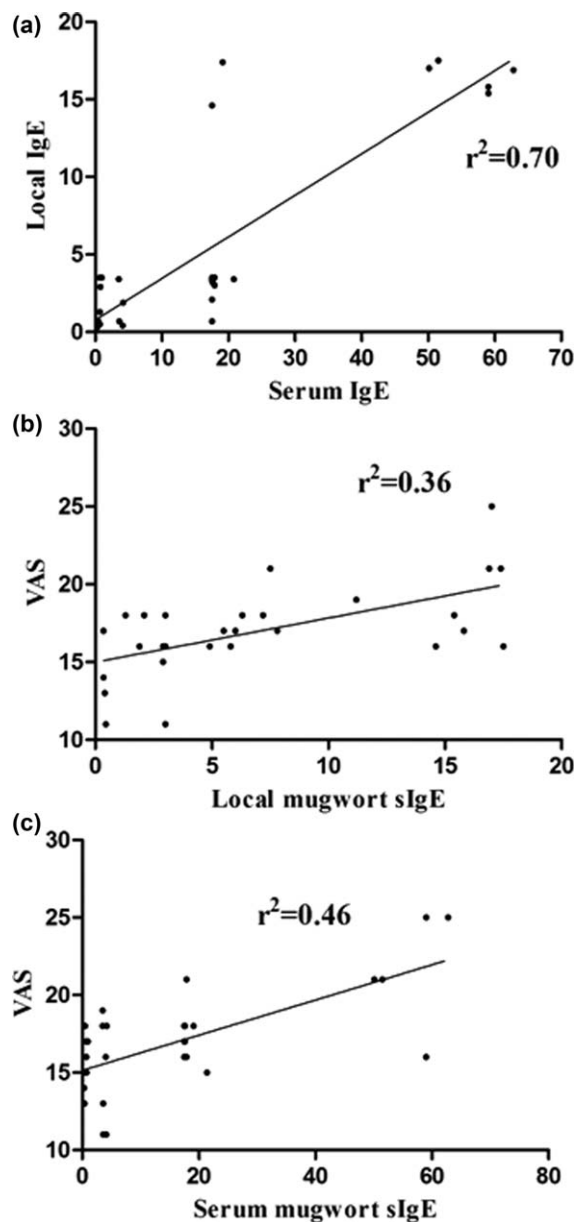


Fig. 2. (a) The correlation between local nasal mugwort sIgE and serum mugwort sIgE. (b) The correlation between local nasal mugwort sIgE and VAS scores. (c) The correlation between serum mugwort sIgE and VAS score. specific immunoglobulin = sIgE; VAS = visual analogue scale.

analysis for sIgE. In this regard, the observation that 43 (84.3%) patients could be detected with local nasal sIgE levels of at least 0.35 kU/L suggests that local nasal sIgE may be a more sensitive indicator than serum sIgE in the diagnosis of allergic rhinitis. Indeed, in the present study we found three patients with detectable local nasal sIgE but not serum sIgE who were classified as local allergic rhinitis (LAR) patients. Although the prevalence of LAR in the general population is not currently known, several studies have demonstrated the incidence of this comparatively new LAR phenotype.¹⁹ LAR is defined by the occurrence of symptoms of allergic rhinitis based on the presence of allergen-specific IgE in the nasal mucosa but with negative results to common allergy testing.¹⁹ In this regard, local production of sIgE in the nasal mucosa has actually been used as one of the diagnostic criteria for LAR in some previous studies.^{5,12} Moreover, in addition to being a sensitive indicator for allergic rhinitis, measurement of local nasal sIgE has an advantage over serum sIgE because the collection of nasal secretions is noninvasive, unlike the collection of the peripheral blood for serum sIgE examination. Similarly, local nasal sIgE measurement has an advantage over the skin prick test (SPT). In particular, despite SPT being the preferred technique for diagnosis of AR, and because it is relatively nontraumatic, cheap, and reproducible, this technique is prone to producing conflicting results between clinical symptoms and serum-sIgE. In this respect, although nasal allergen provocation tests (NAPT) can be used to reveal the existence of IgE-mediated nasal inflammation, and most studies investigating LAR were based on the development of symptoms following NAPT,¹⁹ to date there is no consensus on the specific criteria or method for performing NAPT. Furthermore, the technique is time-consuming, sophisticated to perform, and is not widely accepted as a recognized a routine diagnostic test for AR in clinical practice.

In the present study, the prevalence of mugwort was significantly higher than the prevalence of *Der f*, most likely because the study was conducted in August, the autumn season in Beijing during which the most prevalent aeroallergen in northern China is mugwort.¹⁸ Although the house dust mite allergen demonstrated high prevalence in the present study, this aeroallergen has the highest sensitization in southern China. Thus, because mugwort was the most prevalent aeroallergen in the present cohort, we selected out only the 40 mugwort-sensitized allergic rhinitis patients to perform any correlation analyses and demonstrated that the local nasal mugwort sIgE level was positively correlated with serum mugwort sIgE level, and both local nasal mugwort sIgE and serum mugwort sIgE levels were positively correlated with VAS scores.

The present study demonstrated that, despite having appropriate nasal symptoms indicative of rhinitis, many patients in this cohort had negative results of both serum sIgE and local nasal sIgE. According to Papadopoulos et al.,¹ these patients could be diagnosed

as idiopathic rhinitis patients. However, four of these patients were found to have asthma; and a total eosinophil count in the nasal smear of these patients indicated an eosinophil count $\geq 25\%$ of the cell count total (data not shown). These patients meet the diagnosis criterion of NARES.^{20,21}

CONCLUSION

The findings of the present study suggest that measurement of sIgE levels in nasal secretion, instead of in the serum, is an effective noninvasive indicator for a diagnosis allergic rhinitis. However, these findings are somewhat limited by the relatively small numbers of patients investigated, and therefore need to be confirmed in a much larger cohort in a multicenter study and in multi-ethnic patients.

BIBLIOGRAPHY

1. Papadopoulos NG, Bernstein JA, Demoly P, et al. Phenotypes and endotypes of rhinitis and their impact on management: a PRACTALL report. *Allergy* 2015;70:474–494.
2. Maurer M, Zuberbier T. Under-treatment of rhinitis symptoms in Europe: findings from a cross-sectional questionnaire survey. *Allergy* 2007;62:1057–1063.
3. Greiner AN, Hellings PW, Rotiroli G, et al. Allergic rhinitis. *Lancet* 2011;378:2112–2122.
4. Bousquet J, Van Cauwenberge P, Khaltaev N. Allergic rhinitis and its impact on asthma. *J Allergy Clin Immunol* 2001;108(suppl):S147–S334.
5. Powe DG, Jagger C, Kleinjanw A, et al. 'Entropy': localized mucosal allergic disease in the absence of systemic responses for atopy. *Clin Exp Allergy* 2003;33:1374–1379.
6. Burney P, Malmberg E, Chinn S, et al. The distribution of total and specific IgE in the European Community Respiratory Health Survey. *J Allergy Clin Immunol* 1997;99:314–322.
7. Sibbald B, Rink E. Epidemiology of seasonal and perennial rhinitis: clinical presentation and medical history. *Thorax* 1991;46:895–901.
8. Huggins K, Brostoff J. Local production of specific IgE antibodies in allergic rhinitis patients with negative skin tests. *Lancet* 1975;2:148.
9. Platts-Mills TA. Local production of IgG, IgA and IgE antibodies in grass pollen hay fever. *J Immunol* 1979;122:2218–2225.
10. Coker HA, Durham SR, Gould H. Local somatic hypermutation and class switch recombination in the nasal mucosa of allergic rhinitis. *J Immunol* 2003;171:5601–5610.
11. Smurthwaite L, Durham SR. Local IgE synthesis in allergic rhinitis and asthma. *Curr Allergy Asthma Rep* 2002;2:231–238.
12. Rondon C, Campo P, Togias A, et al. Local allergic rhinitis: concept, pathophysiology, and management. *J Allergy Clin Immunol* 2012;129:1460–1467.
13. Fokkens WJ, Lund VJ, Mullol J, et al. European position paper on rhinosinusitis and nasal polyps 2012. *Rhinol Suppl* 2012;23:1–298.
14. Watelet JB, Gevaert P, Holtappels G, et al. Collection of nasal secretions for immunological analysis. *Eur Arch Otorhinolaryngol* 2004;261:242–246.
15. Bousquet J, Khaltaev N, Cruzet AA, et al. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). *Allergy* 2008;63(suppl 86):8–160.
16. Zhang L, Han D, Huang D, et al. Prevalence of self-reported allergic rhinitis in eleven major cities in china. *Int Arch Allergy Immunol* 2009;149:47–57.
17. Wang X, Zheng M, Lou H, et al. An increased prevalence of self-reported allergic rhinitis in major Chinese cities from 2005 to 2011. *Allergy* 2016;71:1170–1180.
18. Lou H, Ma S, Zhao Y, et al. Sensitization patterns and minimum screening panels for aeroallergens in self-reported allergic rhinitis in China. *Sci Rep* 2017;7:9286.
19. Incorvaia C, Fuiano N, Martignago I, et al. Local allergic rhinitis: evolution of concepts. *Clin Transl Allergy* 2017;7:38.
20. Zambetti G, Ciofalo A, Romeo R, et al. Nasal histamine responses in non-allergic rhinitis with eosinophilic syndrome. *Allergy Rhinol* 2015;6:e94–e100.
21. Leone C, Teodoro C, Pelucchi A, et al. Bronchial responsiveness and airway inflammation in patients with nonallergic rhinitis with eosinophilia syndrome. *J Allergy Clin Immunol* 1997;100:775–780.