

Measurement of Tryptase and CCI6/Albumin in Nasal Lavage Fluid as a Screening Tool of Allergic Rhinitis

Oh Eun Kwon, MD, PhD¹, Young Chan Lee, MD, PhD¹,
Jung Min Park, RN¹, Sung Wan Kim, MD, PhD¹,
Young-Gyu Eun, MD, PhD¹ , and Seong-Gyu Ko, MD, PhD, MPH²

Abstract

Background: There is no trial to make a diagnostic tool of allergic rhinitis (AR) utilizing biomarkers from nasal fluid. Base on previous studies, we selected following five biomarkers in nasal fluids that represent the characteristics of allergic reactions: tryptase, eosinophil cationic protein (ECP), interleukin 5 (IL-5), Clara cell protein 16 (CCI6) and CCI6-to-albumin ratio.

Objective: This study aimed to identify biomarkers in nasal discharge that may be used in biosensors to diagnose AR as an additional diagnostic tool.

Methods: Patients showed rhinorrhea and tested positive on allergic skin and specific immunoglobulin E (IgE) tests were included in the AR group. The non-AR group included individuals no dominant nasal symptoms and tested negative on allergy tests. Nasal lavage fluid samples were collected from all participants. Biomarkers in the samples were quantified using enzyme-linked immunosorbent assay.

Results: Forty-five patients with AR and 28 non-AR subjects were enrolled in this study. Comparing the concentrations of biomarkers, the concentrations of tryptase and IL-5 were significantly higher in the AR group than in the NAR group. And CCI6 level and CCI6-to-albumin ratio were significantly lower in the AR group. In the combination of tryptase or CCI6-to-albumin ratio, the sensitivity was 90.7% and the specificity was 64.3% ($p = 0.013$).

Conclusion: The combination of “tryptase or CCI6-to-albumin” could be used as a screening tool for AR. Although this diagnostic method could not replace conventional diagnostic tools, we could consider the method we proposed as an additional screening tool for patients who could not undergo allergy tests.

Keywords

allergic rhinitis, Clara cell 16, tryptase, interleukin 5, screening

Introduction

Allergic rhinitis (AR) is an immunoglobulin E (IgE)-mediated disease caused by aeroallergens. It causes troublesome symptoms such as nasal obstruction, watery rhinorrhea, sneezing, and nasal itching sensation, which affect patients' quality of life.¹ In the diagnosis of AR, patients' critical symptoms and physical examination findings with positive allergy test results are important. Skin tests are useful for identifying the cause of the allergen but have several limitations, including the risks of adverse reactions and effects of medication and patients' skin conditions. The specific IgE test, in vitro immunoassay method, is convenient, and the grade of the specific IgE shows a significant correlation

with the severity of AR symptoms.^{2,3} In previous studies, skin- and serum-specific IgE tests show various sensitivity and specificity for diagnosing AR. Usually, the skin

¹Department of Otolaryngology–Head & Neck Surgery, School of Medicine, Kyung Hee University, Seoul, Republic of Korea

²Department of Preventive Medicine, College of Korean Medicine, Kyung Hee University, Seoul, Korea

Corresponding Author:

Young-Gyu Eun, Department of Otorhinolaryngology–Head and Neck Surgery, Kyung Hee University, 26 Kyung Hee Dae-ro, Hoegi-dong, Dongdaemun-gu, Seoul 02447, Republic of Korea.

Email: ygeun@khu.ac.kr

prick test is more sensitive than the specific IgE test, which has higher specificity than the skin prick test.⁴

The pathogenesis of AR is divided into early and late phases, and depending on the phase, many mediators act and cause allergic symptoms.⁵ During the early phase, mast cells and basophils act mainly, and degranulate and release mediators such as histamine, tryptase, leukotrienes, and prostaglandin D₂. In the late phase, inflammatory cells such as T-helper (Th) 2 cells, eosinophils, and basophils infiltrate into the nasal mucosal tissue and conduct late-phase symptoms.⁵

According to the pathogenesis of AR, several studies have investigated biomarkers related with allergic diseases.⁶ Mast cell-derived markers include histamine, tryptase, prostaglandin D₂, and leukotrienes. The levels of these markers increase in nasal lavage fluid during AR and are restored after intranasal steroid therapy.⁷ Of the eosinophil-derived markers, eosinophil cationic proteins (ECP) can be used for the experiments. In addition, various cytokines such as interleukin (IL)-5 can be detected from nasal fluid or nasal mucosa in AR patients. Previous studies proposed the role of IL-5 in AR based on its secretion by allergen-specific Th2 cells and the presence of elevated IL-5 levels in nasal mucosa samples taken from AR patients.⁸ Albumin could be the biomarker that represents nasal mucosa permeability during AR reaction. In addition, Clara cell protein 16 (CC16), which is mainly produced by mucosal epithelial cells and has a protective role in airway inflammation, has been studied as a biomarker in upper and lower airway diseases.⁹ The CC16-to-albumin ratio could be a marker of nasal epithelial function.¹⁰

Until now, several studies have been conducted about biomarkers related with AR. However, even though many studies attempted to identify unique biomarkers of AR in nasal fluid, no study has used biomarkers as a diagnostic tool of AR.

On the basis of several reports of previous studies, we selected the following four biomarkers in nasal fluids that represent the characteristics of allergic reactions: tryptase, ECP, IL-5, and CC16. In addition, as a marker of nasal epithelial function, we selected the CC16-to-albumin ratio. Therefore, this study aimed to identify biomarkers in nasal discharge that may be used in biosensors to diagnose AR quickly and easily.

Materials and Methods

Study Design

Patients with nasal symptoms who tested positive in an allergy test were enrolled in this study and included in the AR group (n = 45). Patients aged <18 years or >75 years were excluded from the study, including those who were pregnant, had any other sinonasal disease such as

chronic rhinosinusitis and suspected as having cerebrospinal fluid rhinorrhea. Thus, the AR group included patients who satisfied both of the following conditions: 1) manifested allergy symptoms such as nasal obstruction, watery rhinorrhea, itching sensation, and sneezing, and 2) tested positive in the allergy skin test (score of >3+)¹¹ and multiple allergen simultaneous test (score of >2+).¹² It was included in the patient group only when the type of allergen was perennial allergen (*Dermatophagoides farinae*, *Deramtophagoides pteronyssinus*, and Cockroach) for allergic skin reaction tests. Among the patients who came to the ENT department, patients who did not complain of nasal symptoms and reported negative results in all the allergy tests were categorized in the non-AR (NAR) group (n = 28). Only patients who did not receive intranasal steroid or antihistamine treatment for more than 2 weeks before collecting sample were enrolled. For all the patients, we measured the total serum IgE level.

From all the individuals who participated in this study, the total nasal symptom score (TNSS) was collected. TNSS was self-assessed and recorded by all the participants (both in the AR and NAR groups). The TNSS consisted of four major symptoms (sneezing, nasal obstruction, nasal itching, and watery nasal discharge). The degree of severity of each symptom was based on the following scores: 0 = none; 1 = mild; 2 = moderate; and 3 = severe. The maximum total scores was 12 points.

Sampling Method for Nasal Fluid and Enzyme-Linked Immunosorbent Assay

In the AR group, nasal lavage fluid samples were collected from each patient. The lavage nasal fluid sample was collected after instilling 0.9% normal saline in both nostrils.¹³ In the NAR group, the nasal lavage fluid sample was collected using the same method as that used in the AR group. Samples were collected from the patient's sitting position, and 2ml of saline was sprayed into each nasal cavity, and 1ml of nasal fluid was collected using Juhn Tym-tap (Xomed Products, Jacksonville, FL). Collected samples were transferred to Eppendorf tubes, and stored at -70°C.

The levels of tryptase, ECP, IL-5, albumin, and CC16 in the nasal fluid samples were measured using human enzyme-linked immunosorbent assay kits (Cloud-Clone Corp, Katy, TX). Nasal fluid was added to the wells of a precoated 96-well plate followed by the addition of detection reagent A. The plate was then incubated for 1 hour at 37.8°C, and the wells were washed and incubated with detection reagent B for 30 minutes at 37.8°C. The wells were washed and incubated with a tetramethylbenzidine substrate solution for 15 minutes at 37.8°C. The reaction was stopped using Stop Solution,

and the samples were evaluated by measuring absorbance at 450 nm using a microplate reader. The assays were performed in triplicates.

Ethical Consideration

This study was conducted in accordance with the guidelines stipulated in the Declaration of Helsinki. From all the patients enrolled in the study, we received prior permission with written informed consent for using their samples and provided them with explanation of the purpose of the study. This study was approved by the institutional review board of Kyung Hee University Medical Center prior to its initiation (IRB No. KHUH 2015-07-102).

Statistical Analyses

Parametric grouped data were presented as mean values with their standard deviations (SDs) using the Student *t* test, and non-parametric grouped data were compared using the Mann-Whitney *U* test. Receiver-operating characteristic (ROC) curves were calculated. The area under the ROC curve was determined, and the significance of the differences was assessed. From the ROC curve, the appropriate cutoff values were obtained for each biomarker. Categorical variables were compared using the chi-square test. The sensitivity, specificity, diagnostic accuracy, positive predictive value (PPV), negative predictive value (NPV), and false-positive rate for each biomarker were calculated. In addition, the sensitivity, specificity, diagnostic accuracy, PPV, NPV, and false-positive rate of two or three biomarker combinations were calculated. Statistical significance was set at $p < 0.05$. The statistical analysis of data was performed with the SPSS v 20.0 statistical software (IBM Corporation, USA).

Results

Forty-five patients with AR (31 men and 14 women) and 28 NAR subjects (16 men and 12 women) were enrolled in this study (Table 1). In the AR group, the mean age of

the patients was 30.56 years, and the mean total IgE level was 329.2 IU/ml. For the investigation of the subjective symptoms in the AR group, we checked TNSS from all participants. The mean total TNSS was 8.2 ± 2.6 in the AR group and 4.5 ± 3.2 in the NAR group. In the AR group, the watery rhinorrhea, sneezing, and nasal itching sensation were more severe than the nasal obstruction symptom.

When we compared the concentrations of the biomarkers, the concentrations of tryptase and IL-5 were significantly higher in the AR group than in the NAR group. However, the CC16 level and CC16-to-albumin ratio were significantly lower in the AR group (Table 2).

From the ROC curves, we calculated the cutoff values of all the biomarkers. The cutoff values of tryptase, IL-5, CC16, and CC16-to-albumin ratio were 344.7 pg/ml (area under the curve [AUC]=0.69, $P=0.006$), 19.7 pg/ml (AUC=0.67, $P=0.025$), 906.3 ng/ml (AUC=0.71, $P=0.002$), and 0.68 (AUC=0.74, $P=0.001$), respectively. Finally, we calculated the sensitivity, specificity, PPV, NPV, and false-positive rates of tryptase, IL-5, CC16, and CC16-to-albumin ratio for the diagnosis of AR (Table 3).

From these results, we calculated the sensitivity and specificity again in combination of two or three biomarkers. Sensitivity and specificity were calculated for the following seven combinations: tryptase and IL-5, tryptase and CC16-to-albumin ratio, IL-5 and CC16-to-albumin ratio, tryptase or IL-5, tryptase or CC16-to-albumin ratio, IL-5 or CC16-to-albumin ratio, and all of three markers combined. In the combination of tryptase or CC16-to-albumin ratio, the sensitivity was 90.7% and the specificity was 64.3% ($P=0.013$). In the combination of tryptase and IL-5 and CC16-to-albumin, the sensitivity was 51.1% and the specificity was 92.8% ($P < 0.001$; Table 4).

Discussion

The use of biomarkers as diagnostic tools in AR was still unpopular owing to the difficulties involved in procuring

Table 1. Demographics.

Variables	AR (N=45)	Non-AR (N=28)	P-Value
Gender (male:female)	31 : 14	16 : 12	0.442
Age, yr	30.56 ± 11.5	50.75 ± 17.96	<0.01
Total Ig E, IU/ml	329.2 ± 624.5	109.89 ± 310.32	0.182
Total TNSS (0~12)	8.2 ± 2.6	4.5 ± 3.2	<0.01
Nasal obstruction	1.96 ± 1.04	—	
Watery rhinorrhea	2.13 ± 0.75	—	
Sneezing	2.2 ± 0.75	—	
Nasal itching sensation	2.0 ± 0.90	—	

AR = allergic rhinitis; Ig E = Immunoglobulin E; TNSS = total nasal symptom score.

Table 2. Comparison of Concentration of Biomarkers Between AR and Non-AR Group.

Variables	AR (N = 45)	Non-AR (N = 28)	P-Value ^a
Tryptase (pg/ml)	767.14 ± 872.7	334.65 ± 240.19	0.006
ECP (pg/ml)	9313.5 ± 18818.1	7414.79 ± 14019.84	0.49
IL-5 (pg/ml)	49.07 ± 51.35	27.11 ± 26.26	0.025
CC16 (ng/ml)	804.5 ± 586.2	1049.96 ± 402.81	0.002
CC16/Albumin	0.62 ± 0.58	1.54 ± 1.58	0.001

^aMann-Whitney U-test.

AR = allergic rhinitis; ECP = eosinophil cationic protein; CC16 = Clara cell 16.

Table 3. Sensitivity, Specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV), and False Positive Rate of Each Biomarker as a Diagnostic Marker of AR.

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	False Positive Rate (%)	P-Value
Tryptase	77.3	46.4	69.4	56.5	53.5	0.042
IL-5	70.7	53.8	70.7	53.8	46.0	0.071
CC16	69	71	79.5	58.8	28	0.001
CC16/Albumin	71.1	71.4	80	60.6	28.8	0.001

AR = allergic rhinitis; CC16 = Clara cell 16.

Table 4. Sensitivity, Specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV), and False Positive Rate According to Two or Three Biomarkers Combinations as a Screening Tool of AR.

		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	False Positive Rate (%)	P-Value
2 SET	Tryptase and IL-5	56.8	71.4	75.7	51.3	28.6	0.029
	Tryptase and CC16/albumin	57.7	85.7	86.6	55.8	14.3	<0.001
	IL-5 and CC16/albumin	62.2	89.3	90.3	59.5	10.7	<0.001
	Tryptase or IL-5	92.8	26.9	67.2	70	73.1	0.036
	Tryptase or CC16/albumin	90.7	64.3	68.4	71.4	64.2	0.013
	IL-5 or CC16/albumin	80.5	61.5	67.3	55.5	61.5	0.1
3SET	Tryptase and IL-5 and CC16/albumin	51.1	92.8	92	54.1	7.1	<0.001

AR = allergic rhinitis; CC16 = Clara cell 16.

samples for detection of biomarkers. In this study, a biomarker obtained from nasal lavage fluid was used to identify a biomarker that is useful for creating a diagnostic biosensor in a quick method of determining whether a patient with rhinitis symptoms is allergic or not. From the study result, the concentrations of tryptase, IL-5, CC16, and the CC16-to-albumin ratio showed significant differences between the AR and NAR groups. Finally, the sensitivity and specificity of the combination of tryptase or CC16-to-albumin ratio for the diagnosis of AR were 90.7% and 64.3%, respectively. This result demonstrates the potential of the combination as a screening tool for AR.

In a previous study, nasal tryptase level showed a significant positive correlation with nasal allergy.¹⁴ Another study reported that the IL-5 concentration in nasal secretion increased by fivefold in patients with AR.¹⁵ In studies that used CC16, the CC16

concentration was lower in patients with persistent AR than in healthy individuals,¹⁶ and the CC16 levels in nasal secretions and the severity of rhinitis symptoms negatively correlated.¹⁷ Our results corresponded to those of the previous studies. The other study team reported that nasal ECP level increased in patients with AR.¹⁵ However, in our study, the mean nasal ECP level was higher in the AR group but not significant statistically. Measurement of the ECP level, such as that in the serum, could be useful for the diagnosis of AR. However, on the basis of our study results, ECP was excluded from the analysis.

We obtained the cutoff values of the following biomarkers: tryptase, IL5, CC16, and CC16-to-albumin. From the cutoff values, we calculated the sensitivity, specificity, PPV, NPV, false-positive rate of each biomarker. Although the AUC values of tryptase and IL-5 level were not satisfactory, this could be solved if

the number of samples is increased and samples are collected from the healthy control group.

No previous report has evaluated the diagnostic values of biomarkers of AR. Sensitivity, specificity, PPV, NPV, and false-positive rate could be influenced by the quality of data. If the samples are contaminated, the false positive rate could increase.¹⁸ To increase the sensitivity and specificity, we could combine several biomarkers.¹⁹ Therefore, we combined selected biomarkers and calculated their sensitivity and specificity of the combinations. As the “AND” combination and the number of biomarker combinations increased, the specificity of the test increased, but the sensitivity decreased. In the “OR” combination of two biomarkers, the sensitivity of the test was increased. Ideally, when we used the test for disease screening, high-sensitivity and high-specificity tests were recommended but were difficult to find.^{18–21} In this study, the combination of tryptase or CC16-to-albumin ratio seemed likely to be a screening tool of AR.

The skin prick and specific IgE tests revealed various degrees of sensitivity and specificity, which differed depending on the type of allergen. The skin prick test is known to be more sensitive than the specific IgE test.^{22,23} For example, in a previous study performed skin prick and specific IgE tests in 2 species of house dust mites (*Dermatophagoides pteronyssinus* and *D. farina*). For the two allergens, the sensitivities of the skin test were 90% and 86%, and the specificities were 99% and 93%, respectively. The sensitivity and specificity of the specific IgE test for *D. pteronyssinus* and *D. farinae* were 96% and 89%, respectively.²⁴ In another study, when the sensitivity and specificity of the skin and radioimmunosorbent tests were compared among the 4 aeroallergens (*D. farinae*, cockroach, cat, and dog), the sensitivity and specificity differed depending on the type of allergens, but the skin prick test was more sensitive.²⁵ As such, the current diagnostic methods also showed varied degrees of sensitivity and specificity.

To assess the efficacy of the treatment of AR, the conventional diagnostic tests for AR must be performed, including skin prick and specific IgE tests. Therefore, if an allergy test could not be performed or while awaiting the result of the serum-specific IgE test, information about the patient's condition can be obtained from nasal discharge quickly and easily by using the screening tool suggested in this study. Although our proposed diagnostic tool cannot replace the previous diagnostic tools, this additional diagnostic tool could be used as a screening tool, patients could be assessed more easily.

This study has several limitations. First, the sample size was small. Second, the control group was not age-matched. Third, because the AUC in the ROC curve were not high, the cutoff values could change in a large group.

However, we believe that our study outweighs the limitations in that it confirmed that the tryptase, IL-5, and CC16 levels and CC16-to-albumin ratio could be used as effective biomarkers for use in the diagnosis of AR. Moreover, from the results, we identified the possibility of biomarkers in nasal lavage fluid as a diagnostic tool for AR. In particular, when investigating the sensitivity and specificity of the tests using biomarker combinations, the combination of tryptase or CC16-to-albumin ratio in nasal fluid seemed to be suitable for use as a screening tool for AR.

Conclusion

Tryptase, IL-5, CC16 and CC16-to-albumin ratio measured in nasal lavage fluids showed significant sensitivity and specificity. According to the result, the “tryptase or CC16-to-albumin” combination could be used as a screening tool for AR. Although this diagnostic method could not replace the skin prick or specific IgE test, we could consider the method we proposed as an additional screening tool for patients who could not undergo allergy tests. In addition, further study in a large pool of patients is needed to verify our finding.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This research was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF), funded by the Korean government (MSIP&MOHW NRF-2015M3A9E2029189, NRF-2018R1D1A1B07050154 and NRF- 2020R1A5A201941311). The study was also supported by a grant from Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health and Welfare, Republic of Korea (grant No. HI18C1039).

ORCID iD

Young-Gyu Eun  <https://orcid.org/0000-0003-4081-5207>

References

1. Greiner AN, Hellings PW, Rotiroti G, et al. Allergic rhinitis. *Lancet*. 2011;378:2112–2122.
2. Corsico AG, De Amici M, Ronzoni V, et al. Allergen-specific immunoglobulin E and allergic rhinitis severity. *Allergy Rhinol (Providence)*. 2017;8:1–4.
3. Chen ST, Sun HL, Lu KH, et al. Correlation of immunoglobulin E, eosinophil cationic protein, and eosinophil

- count with the severity of childhood perennial allergic rhinitis. *J Microbiol Immunol Infect.* 2006;39:212–218.
4. Emanuel IA. In vitro testing for allergy diagnosis. *Otolaryngol Clin North Am.* 2003;36:879–893.
 5. Eifan AO, Durham SR. Pathogenesis of rhinitis. *Clin Exp Allergy.* 2016;46:1139–1151.
 6. Diamant Z, Boot JD, Mantzouranis E, et al. Biomarkers in asthma and allergic rhinitis. *Pulm Pharmacol Ther.* 2010;23:468–481.
 7. de Graaf-in't Veld C, Garrelds IM, Jansen AP, et al. Effect of intranasal fluticasone propionate on the immediate and late allergic reaction and nasal hyperreactivity in patients with a house dust mite allergy. *Clin Exp Allergy.* 1995;25:966–973.
 8. Benson M, Strannegård IL, Wennergren G, et al. Interleukin-5 and interleukin-8 in relation to eosinophils and neutrophils in nasal fluids from school children with seasonal allergic rhinitis. *Pediatr Allergy Immunol.* 1999;10:178–185.
 9. Špadijer-Mirković C, Perić A, Belić B, et al. Clara cell protein 16 and eosinophil cationic protein production in chronically inflamed sinonasal mucosa. *Int Forum Allergy Rhinol.* 2016;6:529–536.
 10. Sardella A, Voisin C, Nickmilder M, et al. Nasal epithelium integrity, environmental stressors, and allergic sensitization: a biomarker study in adolescents. *Biomarkers.* 2012;17:309–318.
 11. Fatteh S, Rekkerth DJ, Hadley JA. Skin prick/puncture testing in North America: a call for standards and consistency. *Allergy Asthma Clin Immunol.* 2014;10:44.
 12. Ho TM, DeBruyne J, Ahamad M, et al. Evaluation of the MAST CLA allergy system for diagnosis of allergies to house dust mites and cats. *Asian Pac J Allergy Immunol.* 1997;15:123–126.
 13. Greiff L, Pipkorn U, Alkner U, et al. The 'nasal pool' device applies controlled concentrations of solutes on human nasal airway mucosa and samples its surface exudations/secretions. *Clin Exp Allergy.* 1990;20:253–259.
 14. De Prins S, Marcucci F, Sensi L, et al. Exhaled nitric oxide and nasal tryptase are associated with wheeze, rhinitis and nasal allergy in primary school children. *Biomarkers.* 2014;19:481–487.
 15. Kramer MF, Ostertag P, Pfrogner E, et al. Nasal interleukin-5, immunoglobulin E, eosinophilic cationic protein, and soluble intercellular adhesion molecule-1 in chronic sinusitis, allergic rhinitis, and nasal polyposis. *Laryngoscope.* 2000;110:1056–1062.
 16. Perić A, Mirković ČŠ, Đurđević BV, et al. Eosinophil chemokines and Clara cell protein 16 production in nasal mucosa of patients with persistent allergic rhinitis. *Eurasian J Med.* 2017;49:178–182.
 17. Johansson S, Keen C, Ståhl A, et al. Low levels of CC16 in nasal fluid of children with birch pollen-induced rhinitis. *Allergy.* 2005;60:638–642.
 18. Saah AJ, Hoover DR. "Sensitivity" and "specificity" reconsidered: the meaning of these terms in analytical and diagnostic settings. *Ann Intern Med.* 1997;126:91–94.
 19. Parikh R, Mathai A, Parikh S, et al. Understanding and using sensitivity, specificity and predictive values. *Indian J Ophthalmol.* 2008;56:45–50.
 20. Lalkhen AG, McCluskey A. Clinical tests: sensitivity and specificity. *Cont Educ Anaesth Crit Care Pain.* 2008;8:221–223.
 21. Simon R. Sensitivity, specificity, PPV, and NPV for predictive biomarkers. *J Natl Cancer Inst.* 2015;107:djv153.
 22. Ownby DR, Bailey J. Comparison of MAST with radioallergosorbent and skin tests for diagnosis of allergy in children. *Am J Dis Child.* 1986;140:45–48.
 23. Siles RI, Hsieh FH. Allergy blood testing: a practical guide for clinicians. *Cleve Clin J Med.* 2011;78:585–592.
 24. Pumhirun P, Jane-Trakoonroj S, Wasuwat P. Comparison of in vitro assay for specific IgE and skin prick test with intradermal test in patients with allergic rhinitis. *Asian Pac J Allergy Immunol.* 2000;18:157–160.
 25. Chinoy B, Yee E, Bahna SL. Skin testing versus radioallergosorbent testing for indoor allergens. *Clin Mol Allergy.* 2005;3:4–4.